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Preface

Protein is an essential macronutrient for humans. It provides amino acids, which are the building blocks for growth and maintenance of health. Animal-based protein foods, such as those based on red meat, contain saturated fat and cholesterol. In contrast, plant-based protein foods contain fiber, are low in saturated fat, and have zero cholesterol. Several studies have shown that regular consumption of plant-based protein foods instead of animal-based protein foods can reduce the risk factors of cardiovascular diseases, diabetes, and certain cancers.

Apart from human health, the environmental impact of production of animal protein is higher than that of plant-based protein. For example, the greenhouse gas emission from the production of 1 pound of lamb meat is 30 times higher than 1 pound of lentils. Today, as consumers are better informed than before on the importance of healthy diet and environmental impact of food products they purchase, the demand for plant-based protein foods is increasing globally. This trend has prompted many large-scale projects to focus on products derived from plant-based protein in the industry, as well as fundamental research in academia. Nowadays, several established multinational meat companies are beginning to add plant-protein product lines to meet the current demand.

This book is the first of its kind on plant-based protein foods, covering a wide range of topics in 18 chapters, including processing, product development, nutritional value, consumer acceptance, and market opportunities for plant-based protein products. We humbly believe that this book will benefit academics, industry professionals, dieticians, and many others in utilizing the full potential of plant-based protein foods.

We are grateful to all the authors for contributing chapters to this book. We are also thankful to the staff of the editorial and production departments of Springer for their support and their efforts to bring this book to publication.

Guelph, ON, Canada

Annamalai Manickavasagan
Loong-Tak Lim
Amanat Ali

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About the Editors

Annamalai Manickavasagan, PhD, PEng, obtained his Doctorate in Biosystems Engineering from the University of Manitoba, Canada. At present, he is an associate professor at the University of Guelph, Canada. He has published 75 scientific papers in peer-reviewed journals and edited 8 books. He is a recipient of several research awards, including Research Excellence Award – University of Guelph (2021–2022); John Clark Food Engineering Award – Canadian Society for BioEngineering (2020); Distinguished Researcher Award – Sultan Qaboos University (2014); and Best Young Researcher Award – Sultan Qaboos University (2012).He is the founder and president of Whole Grains Research Foundation in India. Through this foundation, he is promoting whole grains research in Asia.

Loong-Tak Lim received his doctoral degree in food science from the University of Guelph, Canada, and BSc degree in food science from Acadia University, Canada. He is currently a tenured professor in the Department of Food Science and team leader of the Packaging and Biomaterial Group. He is also an adjunct professor in the School of Packaging at Michigan State University. His research is focused on exploiting ultrafine fibers and particles for encapsulation and advanced packaging applications, aiming to reduce food waste and improve food safety during distribution. He has authored/co-authored over 160 peer-reviewed journal articles and book chapters, as well as co-edited several books. Prior to joining the University of Guelph in 2005, he was with Husky Injection

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Amanat Ali received his BSc from the University of Punjab, Lahore; MSc from the University of Agriculture Faisalabad, Pakistan; and PhD (Dr. agr.) from the University of Bonn, Germany. He obtained a specialized Diploma in Higher Education and University Teaching from the University of Kassel, Germany. He has more than 45 years of teaching and research experience in the areas of animal nutrition, human nutrition, and food science. He has worked globally at various reputable teaching and research institutions as well as in industry in Pakistan, Germany, Saudi Arabia, Oman, and Canada. He is currently working as a visiting research professor in College of Engineering and Physical Sciences, University of Guelph, Ontario, Canada. He has published more than 150 scientific and research papers in various reputable journals and 16 book chapters and has made more than 125 presentations globally at various seminars, workshops, and conferences. He is editor, associate editor, and member of editorial boards of various scientific journals. He is member of a number of scientific and professional societies and also received the titles Professional Agrologist (P. Ag) and Certified Agricultural Consultant (CAC). He has supervised a number of graduate students for their MSc and PhD thesis research. He has expertise in the areas of animal nutrition, human nutrition, nutritional biochemistry, nutritional toxicology, food safety, and food products development. His current research interests focus on metabolic aspects of dietary carbohydrates, lipids, and lifestyle factors in health and disease, bioactive components, biomarkers of oxidative stress in the etiology of chronic diseases, nutritional quality and food safety, and development of value-added food products and functional foods.

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Chapter 1
High Protein Foods: A Comparison of Animal Origin vs Plant Origin

Sanaullah Iqbal

1 Introduction

The term "protein" was first introduced by Geradus Johannes Mulder in 1838 and derived from Greek word *proteos* meaning "of prime importance" (Sumner 1926). Proteins are an integral part of the human body, found in skeletal muscles, structural part of cells, body organs, skin and blood. Amino acids are building blocks of proteins and are determinant of their structure and ultimate predictor of protein's biological functions. Amino acids are arranged in a specific sequence through biochemical reactions leading to peptide bond formation which yields short chain peptides and longer polymers such as polypeptides forming a particular structure of proteins. The functional groups of each amino acid, the number and sequence of amino acids are determinants of protein biological functions and other characteristics attributed by them. All proteins found in living organisms are made of only 20 types of L-amino acids. These are classified on the basis of essentiality, structure, polarity and net-charge. With respect to essentiality, amino acids are divided into two categories, essential or indispensable amino acids which cannot be synthesized in human body, nine amino acids – histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine – and are always needed to be taken from exogenous sources and non-essential amino acids – alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine – which can be synthesized in the body from non-protein sources like carbohydrates and lipids or modifying other amino acids. Cellular protein synthesis in the body to perform various biological functions is always based on "all or none principle" showing that all amino acids are required to synthesize a protein.

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In case of deficiency of one essential amino acid in the body, the process of protein synthesis will not be completed.

Proteins being the most versatile macromolecule in living organisms are not only crucial for proper functioning of metabolic processes but are also structural elements as well as serve a vast range of functions. They work as catalysts to increase the rate of biological reactions occurring in the body, act as chemical messengers in the form of hormones and give immunity to the body in the form of immunoglobulins to fight against diseases. Other functions include the transport of substances, vitamins, minerals and other nutrients and acid base balance through buffering properties (Gropper et al. 2018). The bioactive peptides, produced during protein digestion in the body or due to enzymatic or microbial metabolism during fermentation process, can contribute as anti-hypertensive, hypolipidemic and hypocholestrolemic, anti-cancer, anti-microbial, anti-oxidants and in several other biological functions (Bhandari et al. 2020; López-Expósito et al. 2012). However, their physiological functions are mainly dependent on their bioavailability in intact form in the body (Bhandari et al. 2020).

As for as protein requirement is concerned, it is mainly dependent on age, physical activity and quality of protein source. The Recommended Dietary Allowance (RDA) of protein for healthy adults with sedentary life style, is 0.8 g protein per kg body weight (BW) per day (WHO 2007). The requirement will be increased to 1.0, 1.3 and 1.6 g protein/kg BW for minimal, moderate and intensive physically active individuals to maintain skeletal-muscle protein and physical strength. Chronic high protein intake i.e. more than 2 g/kg BW/day may result in vascular, digestive and renal abnormalities and should of avoided (Wu 2016). With respect to toxicity of either protein or individual amino acids, little information is available so the tolerable upper level is not established yet (Watford and Wu 2018) however, it is advised to not use single amino acid in supplements at levels significantly higher than found in normal foods.

2 Protein Quality

With respect to human nutrition point of view, the quantity and quality of protein are equally important to perform metabolic functions of the body. It is evident that protein quality varies among different protein sources due to the difference in ratio of amino acids present in specific protein sources. The quality of a protein in a food is dependent on its amino acid contents, especially essential amino acids (EAAs) and their bioavailability in circulation after digestion and absorption to maintain normal body composition and function throughout the life cycle. In case of cellular protein synthesis, protein quality has more importance as compared to protein quantity. So, the protein quality influences protein requirement of a person; higher the quality, lower the protein requirement. There are several methods for the assessment of protein quality based on clinical or metabolic studies in humans, nitrogen balance, and biological assays in laboratory animals, mainly growing rats, protein efficiency ratio (PER) or net-protein utilization (NPU). These methods have their own merits and demerits due to practical difficulties and poor sensitivity; however, they underestimate the quality of some plant and animal proteins for humans. The quality of protein in human nutrition is also expressed as Amino Acid Score (AAS); the content of EAAs and their capacity to fulfill body's requirements. The AAS is defined as "ratio of its content of EAA to the amount required". In 1990s, Food and Agriculture Organization (FAO) of the World Health Organization (WHO) introduced a new method i.e. Protein Digestibility-Corrected Amino Acid Score (PDCAAS) (FAO/WHO 1991a). As the PDCAAS depends on overall digestibility of protein and it may vary for individual amino acids, so a new method based on digestibility of individual EAAs was introduced by FAO i.e. Digestible Indispensable Amino Acid Score (DIAAS) (FAO/WHO 2013). A recent study has concluded that PDCAAS values overestimate the protein quality, especially in low quality protein sources due to inaccurate amino acid digestibility in the ileum as compared to DIAAS. So in clinically sensitive humans, DIAAS values should be used to meet better protein requirements (Mathai et al. 2017). Berrazaga et al. (2019) has reviewed the quality of proteins from different animal as well as plant sources on the basis of protein digestibility, biological value, PDCAAS and DIAAS. They have found that all animal source proteins have a remarkably more score for all parameters than plant proteins. Almost all animal sources have a PDCAAS value of near to 1 while in plant sources only soy and canola have values comparable to animal foods (Table 1.1) (Berrazaga et al. 2019).

On the basis of provision of the EAA, protein sources are divided into two categories, i.e., "high quality or complete proteins" which provide all EAAs in appropriate quantities and "low quality or incomplete proteins, in which one or more EAAs are deficient. In general, animal proteins are considered as high-quality proteins due to their amino acid pattern closer to human body requirements as well as good digestibility and provide all nine EAAs in adequate amounts. One exception is gelatin, an animal-based protein deficient in an EAA, tryptophan. In contrary, plant sources are considered as low-quality proteins due to one or more limiting amino acids, mainly lysine, methionine, threonine and tryptophan except soy bean, quinoa and Chia seeds (Gropper et al. 2018). Wheat, rice, corn, nuts and other grains and seeds are deficient in lysine and sometimes in threonine and tryptophan as well, whereas methionine is the limiting amino acid in legumes, peas, lentils and vegetables (Gropper et al. 2018). If planned poorly, a diet limited to only low-quality proteins may lead to EAAs deficiency resulting in the body's inability to produce cellular proteins which may lead to metabolic malfunctioning in the body. To provide all EAAs to the human body as per requirement is crucial and it can be achieved through increased protein intake, supplementation with limiting amino acids and blending with different plant as well as animal source proteins. For example; legumes have high content of lysine but deficient in Sulfur containing amino acid methionine, can be combined with cereals which are limited in lysine but have more than adequate amount of methionine and cysteine. Furthermore, unique recipes should be prepared like cereals may be combined with milk and other dairy products while legumes may be supplemented with meat products.

Table 1.1 Essential amino acids (EAA) requirement and protein content in different food sources

	Infant requirement[a]	Adult requirement[a]	Corn (white, raw)	Wheat (hard winter)	Rice (white, raw)	Barley (raw)	Sorghum (raw)	Quinoa (raw)	Soy (protein isolate)	Peas (cooked)	Kidney beans (cooked)	Canola[b]	Peanuts (roasted)	Beef	Hen egg[a]	Fish	Cow milk[a]	Chicken
Protein (g/100 g)	g/day		9	13	7	10	10	14	93	8	9		24	19	12.5	18	3.3	21
Essential amino acids (mg/g protein)																		
Tryptophan	18	6	7	13	12	17	51	12	14	11	12	13	9.7	2	16	2	14	3
Threonine	47	24	38	29	36	34	30	30	36	36	37	44	34	8	49	8	45	9
Isoleucine	57	23	36	36	43	37	38	36	47	41	47	23	35	8	63	9	60	11
Leucine	101	52	123	68	83	68	13	60	80	72	85	71	65	15	88	15	98	16
Lysine	69	47	28	27	36	37	20	54	61	73	70	56	36	16	70	17	79	18
SAA	38	23	39	42	44	41	31	36	24	26	22	45	25	7	56	7	34	9
AAA	87	41	90	78	87	85	79	61	88	75	82	70	88	14	98	13	96	16
Valine	56	56	51	44	61	49	47	42	48	47	57	55	42	9	72	10	67	11
Histidine	23	17	31	23	24	23	22	29	26	24	27	31	25	6	24	5	28	7
AAS	–	–	0.5	0.52	0.71	0.73	0.40	1	0.9	1	0.9	–	0.7	1	–	1	–	1
PDCAAS value	–	–	0.6	0.42	0.63	0.61	0.16	0.7	0.91	0.6	0.7	0.93	0.5	1	–	1	–	1
DIAAS (%)	–	–	0.5	0.43	0.64	0.51	0.29	–	0.9	0.6	0.6		0.4	1	–	1	–	1

Table is adopted from (Balandrán-Quintana et al. 2019) otherwise mentioned. [a] From (Michaels 2004); [b] From (Day 2013)

AAS Amino Acid Score, *PDCAAS* Protein Digestibility Corrected Amino Acid Score, *DIAAS* Digestible Indispensable Amino Acid Score, *SAA* Sulphur Amino Acids including Methionine + cysteine, *AAA* Aromatic Amino Acids, Phenylalanine + Tyrosine

Regular consumption of low-quality proteins may lead to the deficiency of EAAs. This deficiency and continuous increasing dietary intake of same incomplete proteins result in reduced or no cellular protein synthesis and increase in tissue specific degradation of amino acids. Syndrome and symptoms of EAAs deficiency include vomiting and low appetite, emotional disorders, insomnia, anemia, skeletal muscle wasting, physical fatigue and weakness, weight loss, metabolic disorders, cardiovascular abnormalities, hypertension, oxidative stress, advanced aging, impaired immune system, increased susceptibility to infections and peripheral edema (Hou and Wu 2018). If deficiency still persist, it may further increase tooth and bones decay, hair loss, scaly skin, very poor growth, dyslipidemia and hyperglycemia and may lead to death.

3 Protein Sources

Exogenous high protein sources are divided into two main categories i.e., plant protein sources and animal protein sources. Plant sources include cereals, pulses, legumes, seeds, nuts and their products while animal sources include milk and dairy products, meat and meat products, poultry, eggs and sea foods. As for as consumption is concerned, overall the consumption of animal proteins is more in America, Europe and Oceania as compared to Africa and Asia (Berrazaga et al. 2019). However, due to protein energy malnutrition around the globe, nutritional quality of protein from different food sources and their impacts on health, other protein sources have been investigated in the last few decades. After comprehensive analysis and detailed nutritional and biological studies, the algal and single cell proteins have found to be of high quality and comparable to several plant and animal proteins (Becker 2007; Mišurcová et al. 2014). The filamentous cyanobacterium *Arthrospira platensis* commonly known as "Spirulina" and various commercial species of the unicellular green alga *Chlorella* are best examples of edible fungi. Their dry mass may comprised of up to 70% high quality protein. Furthermore, these protein sources also contain ample amounts of lipids – mainly long chain polyunsaturated fatty acids including omega 3 and omega 6 fatty acids, dietary fibers and several colorful algal compounds including chlorophylls, carotenoids like α-carotene, β-carotene, lycopene and xanthophylls and phycobiliproteins which have proven health benefits (Chakdar and Pabbi 2017). However, the cost of production and presence of certain native toxic compounds are the limiting factors for their application in food industry (Mišurcová et al. 2014). Another source of protein is the "single cell protein" derived from cells of microorganisms. For this purpose, mainly yeast – Saccharomyces and Candida species, fungi – Aspergillus and Rhizopus species, Lactobacillus, Bacillus and Pseudomonas species are preferred for extraction (Goldberg 2013). Different microbes contain varied amount of single cell protein on dry weight basis i.e., fungi 30–45%, algae 40–60%, yeast 45–55% and bacteria 50–65%. Presence of nucleic acid, secondary toxic metabolites and high cost of production are the limiting factors for their application for human nutrition.

However, due to their good nutritional value and less processing, demand is increasing as a feed ingredient in livestock and aquaculture (Jones et al. 2020).

3.1 Proteins from Animal Sources

In general, proteins from animal sources are superior than plant proteins with respect to digestibility, bioavailability of nutrients, presence of all EAAs in enough quantities (Table 1.1) and the absence of any anti-nutritional factors. These properties make them more anabolic that help in tissue growth and maintenance of body and maintain skeletal muscle mass in comparison to plant proteins in same quantity (Berrazaga et al. 2019). The nutritional quality of animal proteins is not same but vary for different proteins, i.e. whey protein has higher amounts of arginine, lysine, leucine and sulfur amino acids as well as has "fast-release" properties as compared to casein (Pennings et al. 2011). Due to anabolic properties animal proteins, especially whey and egg proteins are preferred by athletes and body builders. Furthermore, animal-based proteins are a good source of some micronutrients such as vitamin B12, vitamin D, heme-iron, zinc, DHA (in fatty fish).

Detailed nutritional profile of red meat, including beef, veal, lamb and mutton (Table 1.2) shows that these are excellent sources of high biological value protein along with a wide range of vitamins, including vitamin B complex, vitamin A & D and minerals i.e., iron, zinc, phosphorus and selenium. The red meat is also rich in endogenous antioxidants and other bioactive compounds, including glutathione, creatine, carnitine and ubiquinone (Williams 2007). Recently the consumer demand for organic meat is also increasing in markets of developed countries and various strategies are being utilized to enhance nutritional value of red meat. Along with high quality protein, this organic meat also contains a good lipid profile with less fat and less cholesterol and higher contents of α-linolenic acid, α-tocopherol, β-carotene, coenzyme Q10, taurine, anserine and carnosine (Ribas-Agustí et al. 2019). Some of the bioactive compounds, mentioned in Table 1.4, are indispensable for cellular metabolism and other physiological functions. It is also evident that animal foods are lacking in different bioactive ingredients like phytochemicals and flavonoids which have proven health benefits.

Most of the animal-based foods/proteins are highly processed at high temperatures to produce value added foods as per need of consumer. However, at high temperature (115 °C) and prolonged heat treatment (20 to 180 min) can result in racemization of amino acids in poultry proteins and significantly reduce the protein quality in terms of digestibility (Bellagamba et al. 2015).

Animal protein sources on the basis of per unit weight or per unit energy, are considered as richer source of six micronutrients than plant sources with good digestibility and bioavailability; vitamin A, riboflavin (B2), vitamin B12, iron, calcium and zinc as compared to vegetarian diets. Thus, foods from animal sources fill multiple micronutrient gaps at a lower volume of intake than the plant source foods not only in children, but in all age groups as well (Bwibo and Neumann 2003;

Table 1.2 Nutritional composition of major protein foods of animal origin

Sr no.	Food Name	Energy	Protein	Fat	SFA	MUFA	PUFA	ω3	TFA	TC	Ca	Fe	Zn	Vit A	B_{12}	DFE
1	Goat meat mixed	208	44	3.5	–	–	–	–	–	145	17	4.9	7.8	3	1.1	23
2	Beef boneless	182	37	3.7	–	–	–	–	–	72	5	2.3	7.6	2	0.9	18
3	Pork (Fried)	200	36	5.8	–	–	–	–	–	66	5	1.0	2.5	1	0.3	43
4	Veal boneless (grilled)	157	34	1.9	30.7	43.9	20.3	5.97	4.9	85	6	1.7	4.9	2	3	0
5	Tuna (baked)	142	32	1.4	–	–	–	–	–	62	22	1.0	0.7	21	0.6	2
6	Rabbit (flesh)	170	29	5.7	44.7	24.6	30.6	0.3	0.6	92	17	1.3	2.1	7	8	9
7	Cheese, edam	361	28	27.2	66.9	30.3	3.0	0.2	3.1	78	810	0.3	4.2	219	1.9	28
8	Milk powder (cow)	508	25	27.3	70.8	23.4	2.6	0.1	2	110	850	–	2.3	370	0.7	117
9	Cheese, cheddar, (regular fat)	397	25	32.8	69.9	24.9	4.07	0.2	3.1	108	763	0.1	3.6	173	1.9	52
10	Ostrich meat (raw)	109	24	1.4	35.0	39.4	25.3	2.2	0.5	53	3	2.8	3.8	0	5	8
11	Rabbit, whole, raw	113	23	2.1	40.0	31.3	27.7	1.3	0.6	67	9	1.0	1.6	10	5.3	8
12	Beef, mince, regular fat, raw	170	22	8.9	45.8	45.4	3.04	0.3	3.4	76	7	1.5	2.3	0	3	21
13	Chicken, breast, lean, raw	105	22	1.6	32.9	44.0	19.7	1.5	0.5	59	12	0.4	0.7	8	0.2	0
14	Cheese, mozzarella	290	22	22.1	68.8	24.9	2.5	0.2	3.2	71	685	0.1	3.1	146	1	48
15	Goat, meat, all cuts, lean, raw	105	22	1.8	–	–	–	–	–	73	9	2.6	4.2	2	1	21
16	Turkey, breast, lean flesh, raw	117	22	3.3	28.6	29.6	41.9	0.4	0.5	45	7	0.4	1.3	21	0.6	0
17	Prawn, flesh, raw (green)	91	21	0.8	–	–	–	–	–	406	72	0.2	1.4	2	1	16
18	Camel meat, raw	151	20	7.7	59.6	33.2	5.8	0.4	3.8	42	4	1.9	1.9	2	1.7	8
19	Chicken, mince, raw	136	19	6	31.9	50.8	17.1	0.5	0.6	79	10	0.7	1.5	19	0.7	14
20	Quail, lean flesh & skin, raw	173	18	11	31.7	43.5	24.7	0.3	0.6	99	6	1.3	0.7	20	1.2	0
21	Fish, eel, raw	178	18	11.7	22.5	68.5	9.0	2.11	0	126	20	0.5	1.6	1043	3	15
22	Duck, lean flesh, raw	121	18	5.5	32.9	52.4	14.6	0.1	0.6	110	7	1.8	2	18	0.7	0
23	Pigeon, whole, raw	291	16	25.5	28.4	58.9	12.4	0	0.5	84	13	2.4	2.2	73	0.4	6
24	Cheese, cottage	126	15	5.7	64.3	26.2	4.9	0.1	4.7	22	89	0.1	0.2	38	0.5	3
25	Egg, chicken, whole, raw	127	13	8.5	32.6	50.4	16.9	1.0	0.1	488	47	1.9	1.1	130	1.4	110

(continued)

Table 1.2 (continued)

Sr no.	Food Name	Energy	Protein	Fat	SFA	MUFA	PUFA	ω3	TFA	TC	Ca	Fe	Zn	Vit A	B_{12}	DFE
26	Egg, duck Raw[a]	179	12	13.6	25.0	45.1	12.5	10.8	–	747	52	2.5	1.5	188	5.4	80
27	Milk Sheep, fresh[a]	100	6	6.4	64.2	25.1	0.5	0.25	–	6	164	0.1	0.6	46	0.5	5
28	Yoghurt, natural, 3% fat	73	5	3.2	71.9	22.8	2.7	–	2	15	175	0	0.6	50	0.5	25
29	Milk goat, fresh[a]	83	4	5.3	60.7	27.3	0.06	0.04	–	6	159	0.1	0.4	38	0.1	1
30	Milk, cow, (3.5% fat)	67	3	3.4	67.8	27.5	2.8	0.1	3.5	10	104	0.1	0.3	51	0.6	14
31	Milk, camel, fresh[a]	58	3	4.0	43.5	26.7	0.04	0.02	–	9	116	0.2	0.4	13	–	–

Source: Food Standards Australia New Zealand, [a] FAO Database – 2019. Composition is mentioned in solids or liquids per 100 g of food. Energy (Kcal) is given without dietary fiber. No added fat in all foods/recipes. All foods are unfortified. SFA, MUFA, PUFA, ω3, TFA (Trans FA) are presented as percentage of total fats in the mentioned food. TC (Cholesterol), Ca, Fe, Zn are given in "mg" per 100 grams of food. Vitamin A, B12 and DFE (Dietary Folate Equivalent) are given in "µg" per 100 grams of food

Table 1.3 Nutritional composition of major protein foods of plant origin

	Food name	Energy	Protein	Fat	DF	SFA	MUFA	PUFA	C18:2 ω6	C18:3 ω3	Ca	Mg	Fe	Zn	VIT A	DFE
1	Soy Flour	338	50	8.9	16	18.5	20.9	60.5	52.5	8	285	285	8.2	4.1	4	289
2	Yellow pea, whole	301	26	2.3	17.7	17.4	26.1	60.9	50.6	10.3	59	113	4.8	2.5	42	274
3	Lentil, hulled	290	25	1.9	14.4	–	–	–	–	–	60	93	6.7	1.8	4	80
4	Bean, mung, whole	287	24	2	13.7	40	5.0	55	51.1	3.9	89	184	4	2.8	11	625
5	Breakfast cereals	365	23	2.5	2.5	18.7	26.9	53.8	51.6	2.1	284	41	11.2	1.5	0	314
6	Fenugreek seed	167	23	6.4	47.6	16.8	14.7	68.5	44.8	23.7	176	191	33.5	2.5	24	57
7	Sunflower seeds	552	23	51	10.8	8.8	20.2	70.8	70.8	0	100	370	4.6	5.8	1	229
8	Bean, red kidney	254	22	1.8	21.5	18.7	10.0	71.3	27.7	43.6	95	140	5.6	3	1	394
9	Bean, haricot	252	22	2.2	18.5	0.22	0.2	0.8	0.17	0.5	150	160	6.4	2.4	0	1
10	Chickpea flour	285	20	5.5	20.8	12.7	23.6	57.9	55.8	2.2	159	134	4.6	3.1	11	178
11	Almond, with skin	549	20	50.5	10.9	7.9	63.5	26.6	26.6	0	265	266	3.8	3.6	0	37
12	Pistachio nuts	590	20	50.6	9	12	55.2	33.4	32.7	0.7	90	100	3.9	2.3	22	51
13	Cumin seed	406	18	22.3	10.5	8.1	74.5	17.4	16.5	0.9	931	366	66.4	4.8	127	10
14	Amaranth, grain, whole	343	15	6.4	11.1	28.1	23.4	50	49.2	0.8	147	237	7	2.7	0	82
15	Hazelnut	623	15	61.4	10.4	4.6	83.1	12.2	12	0.2	86	160	3.2	2.2	3	113
16	Walnut	682	14	69.2	6.4	6.7	18.3	75	65.5	9.5	89	150	2.5	2.5	4	70
17	Quinoa, red	340	14	6.9	13.8	13	31.9	55.1	50	4.4	41	168	4.3	3.6	1	184
18	Chia Seed	360	14	30.7	34.4	11.3	7.8	80.4	19.8	60.6	631	335	7.7	4.6	0	49
19	Coriander seed	241	12	17.8	41.9	6.1	83.2	10.7	10.7	0	709	330	16.3	4.7	20	0
20	Whole wheat Flour	306	12	2.1	11.2	19.6	15.9	63.9	60	3.9	30	102	3	1.3	0	47
21	Oats, hulled	359	12	9.8	8.6	19.4	46.9	34.7	33.6	1.1	37	117	3.9	2.3	0	17
22	Rye flour	289	11	2.3	17.3	17.5	17.6	65	55.9	9.1	44	123	4	2.1	1	38
23	Millet group	350	11	3.5	3.7	11.4	20	68.6	64.8	3.8	8	104	5.2	2.7	0	85
24	Cardamom seed	242	11	6.7	28	34.3	43.9	21.7	15.7	6.1	383	229	14.0	7.5	4	3

(continued)

Table 1.3 (continued)

	Food name	Energy	Protein	Fat	DF	SFA	MUFA	PUFA	C18:2 ω6	C18:3 ω3	Ca	Mg	Fe	Zn	VIT A	DFE
25	Semolina	304	11	1.2	3.2	21.5	9.7	68.8	65.4	3.4	16	23	0.6	0.5	0	22
26	Barley	315	10	3.1	13.1	22.6	16.1	61.3	57.8	3.5	28	95	2.2	1.2	0	23
27	Wheat Flour, white	349	10	1.3	2.2	20.2	13.8	64.1	59.9	4	21	36	1.2	0.8	0	11
28	Turmeric, ground	248	10	3.2	22.7	60.4	14.8	24.8	22.1	2.8	168	208	55	4.5	0	20
29	Sorghum, grain	331	8	3.6	8.6	14.1	33.8	50.8	48.6	2.1	10	124	2.3	1.4	0	20
30	Rice, white	338	7	0.9	0.8	22.2	33.3	44.4	36.6	7.7	4	20	0.2	1.2	0	20
31	Bread, gluten free	205	5	3.9	3.9	14.7	47.5	36.6	30.9	5.7	50	23	1	0.5	0	63
32	Broccoli, Fresh	24	5	0.3	3.7	0	0	0	0	0	32	21	0.8	0.6	48	158
33	Apricot, dried	196	4	0.2	8.4	0	0	0	0	0	67	57	3.1	0.8	397	0
34	Kale, raw	39	4	0.1	3.6	18.9	10.8	70.3	28.7	37.4	150	47	1.5	0.6	992	141

Source: Food Standards Australia New Zealand

1. Composition is mentioned in solids or liquids per 100 g of food. Energy (Kcal) is given without dietary fiber. No added fat in all foods/recipes. All foods are unfortified while Breakfast cereals are mixed grain and fortified. DF, Dietary Fiber; SFA, saturated fatty acids; MUFA, Monosaturated fatty acids; PUFA, Polyunsaturated fatty acids; DFE, Dietary folate equivalent
2. SFA, MUFA, PUFA, ω3 and ω6 are presented as percentage of total fats in the mentioned food. Some plants have traces of TFA (Trans FA)
3. The amount of cholesterol in all foods is "zero"
4. Ca, Fe, Zn are given in "mg" per 100 grams of food.
5. Vitamin A and DFE are given in "µg" per 100 grams of food. All mentioned plant foods are very poor sources of vitamin B_{12}

Table 1.4 Unique bioactive compounds and protein content in plant and animal origin foods

	Food name	Protein (g/100 g)	Unique bioactive compounds
Plant origin foods			
1	Soy Flour	50	Soy Saponins, Soy Isoflavones, β-conglycinin, glycinin (Chatterjee et al. 2018; Silva and Perrone 2015)
2	Yellow pea, whole	26	Inulin, RS, OS, Polyphenols, phytosterols (Singh et al. 2017)
3	Lentil	25	Inulin, RS, Lectins and protease inhibitors (Roy et al. 2010)
4	Bean, mung, whole	24	Inulin, RS, phenolic acids, flavonoids, tannins, PS, peptides (Hou et al. 2019)
5	Breakfast cereals	23	PS, phenols, phytosterols, tocols, dietary fibers (mainly beta-glucan), lignans, alkyl resorcinols, phytic acid, γ-oryzanols, cinamic acid, ferulic acid, inositol, betaine (Gani et al. 2012)
6	Fenugreek seed	23	Polyphenols, galactomannan, diosgenin, quercetin, trigonelline and 4-hydroxyisoleucine (Chatterjee et al. 2010)
7	Sunflower seeds	23	ω3 FA, tocopherols, phenols xanthophyll, lycopene, (Kiczorowska et al. 2019)
8	Bean, red kidney	22	RS, PS, Ferulic acid, flavonol glycosides, anthocyanins, tannins (Singh et al. 2017)
9	Chickpea flour	20	Inulin, RS, Bioactive peptides (Xue et al. 2015), Lectins, protease inhibitors (Roy et al. 2010)
10	Almond, with skin	20	Flavonoids, triterpenoids, betulinic, urosolic, oleanolic acids, flavonol glycosides, phenolic acids (Esfahlan et al. 2010)
11	Pistachio nuts	20	Healthy lipid profile, vitamin mineral dense, zeaxanthin, polyphenols, xanthophylls, tocopherols, stigmasterol, campesterol, resveratrol, lutein, catechins (Bulló et al. 2015)
12	Cumin seed	18	Bioactive peptides, phenols, thymoquinone, flavonoids, p-cymene, dithymoquinone, carvacrol, thymol, thymohydroquinone, 4-terpineol, t-anethole, sesquiterpene longifolene, α-pinene, cuminaldehyde, cymene and terpenoids (Srinivasan 2018)
13	Amaranth, grain, whole	15	Inulin, anti-carcinogenic peptide lunasin (Silva-Sánchez et al. 2008), Phenolic acids including salicylic acid, syringic acid, gallic acid, vanilic acid, ferulic acid, p-coumaric acid and sinapic acid (Khanam and Oba 2013)
14	Hazelnut	15	soluble free, conjugated soluble, insoluble bound and total phenolic compounds (Gorji et al. 2018; Taş and Gökmen 2015)
15	Walnut	14	Healthy lipid profile with MUFA and PUFA, antimicrobial and antioxidants (Gorji et al. 2018; Pereira et al. 2008)
16	Quinoa, red	14	ω3 FA, ACE inhibitor activity (Chen et al. 2019)
17	Chia Seed	14	ω3 FA, dipeptidyl peptidase-IV inhibitors, ACE inhibitors, and antioxidant capacity (Grancieri et al. 2019)
18	Coriander seed	12	High quality lipids, polyphenols, sterols, more than 50 bioactive compounds (Laribi et al. 2015)
19	Whole wheat Flour	12	Dietary fiber, phenolic acids, carotenoids, tocopherols, phytosterols, alkyl resorcinols, benzoxazinoids, and lignans (Luthria et al. 2015)

(continued)

Table 1.4 (continued)

	Food name	Protein (g/100 g)	Unique bioactive compounds
20	Oats, hulled	12	Dietary fibers and β-glucan (Wolever et al. 2011)
21	Rye flour	11	Alkyl resorcinols, lignans, benzoxazinoids, phenolic acids, phytosterols (Andersson et al. 2014)
22	Millet group	11	Antioxidants and DNA damage protection compounds (Salar et al. 2017), albumin, globulin, glutelin, cross-linked prolamin, β-prolamin, hydroxycinnamic acids, hydroxybenzoic acids and derivatives (Okwudili et al. 2017)
23	Cardamom seed	11	Flavonoids (Catechin, Myricetin, Quercetin, Kaempferol), terpenoids, 1,8-cineole, α-terpinyl acetate, α-terpineol, Sabinene, anthocyanins, alkaloids and other phenolic constituents (Ashokkumar et al. 2020)
24	Barley	10	Inulin, RS, anti-cancer lunasin peptide (Jeong et al. 2010), β-glucan, phenolic acids, flavonoids, lignans, tocols, phytosterols, and folate (Idehen et al. 2017)
25	Turmeric, ground	10	Curcumin, α-phellandrene, sabinene, cineol, borneol, zingiberene and sesquiterpenes (Gan et al. 2017)
26	Sorghum, grain	8	Inulin, phenolic compounds, especially 3-deoxyanthocyanidins and tannins (de Morais et al. 2017), polyphenols mainly ferulic acid, flavonoids and carotenoids, gluten-free (Przybylska-Balcerek et al. 2019; Vanamala et al. 2018)
27	Broccoli, Fresh	5	Glucosinolates, phylloquinone, kaempferol and quercetin glucosides derivatives (Moreno et al. 2006)
28	Apricot, dried	4	Seventeen Phenolic acids, eight flavonoids, apigenin quercetin and catechin (Hussain et al. 2013) anthocyanins, carotenoids (Čanadanović-Brunet et al. 2013)
29	Kale, raw	4	Chlorophylls, carotenoids, ascorbic acid, flavonols, phenolic acids (Akdaş and Bakkalbaşı 2017)
Animal origin foods			
1	Goat meat mixed	44	L-carnitine (Kim et al. 2019)
2	Beef boneless	37	Good lipid profile, α-linolenic acid, α-tocopherol, β-carotene, coenzyme Q10, taurine, anserine and carnosine in organic beef (Ribas-Agustí et al. 2019)
3	Veal boneless	34	Bioactive peptides (Vongsawasdi and Noomhorm 2014)
4	Cheese, edam	28	Bioactive peptides (Anti-hypertensive, Anti-oxidant) (Akuzawa et al. 2009)
5	Cheese, cheddar	25	Bioactive peptides (Pritchard et al. 2010)
6	Cheese, mozzarella	22	Antihypertensive peptides, Phosphopeptides, antimicrobial, antioxidant peptides (López-Expósito et al. 2012)
7	Turkey, breast	22	taurine, l-carnitine, choline, alpha-lipoic acid, conjugated linoleic acid, glutathione, creatine, coenzyme Q10, bioactive peptides (Kulczyński et al. 2019)

(continued)

Table 1.4 (continued)

	Food name	Protein (g/100 g)	Unique bioactive compounds
8	Egg, chicken, whole, raw	13	protease inhibitors including ovostatin, ovomucoid, ovoinhibitor, cystatin and defensins (Gautron et al. 2019; Huopalahti et al. 2007)
9	Yoghurt, 3% fat	5	Probiotics
10	Camel milk, fresh	3	Insulin like factor, lactoferrin (El-Agamy 2009)

Calloway et al. 1992) as identified by the Nutrition Collaborative Research Support Program in rural Egypt, Kenya and Mexico. For example, entire day's recommended intake of protein, vitamin B-12 and zinc can be provided by only 100 g of cooked beef while the major portion of the RDA of riboflavin and iron can be met. Fish, poultry and dairy products also have similar nutritive value.

With respect to efficiency and yield, the highest amounts per kilogram body weight are produced by growing broiler chicken followed by laying hens and dairy cows whereas lowest yields are produced by beef cattle. However, the environmental cost and resource efficiency, use of fertilizer, pesticides and water as well as production of waste/manure and emission of greenhouse gases, to produce plant proteins is much lower than to produce equal quantities of beef, chicken and eggs. For example, to produce 1 kg of protein from beef, it requires approximately eighteen times more land, ten times more water, nine times more fuel, twelve times more fertilizer and ten times more pesticide in comparison to produce the same quantity of protein from kidney beans. The beef generates five to six times more manure to produce 1 kg of protein in comparison to chicken and eggs (Sabaté et al. 2015). Due to these factors, recently some companies as well as research organizations not only working on production of animal proteins, fats and other ingredients but are also producing "animal free milk" and "slaughter free/cultured meat" through in vitro cell culture. The greater purchasing power of consumer encourages animal protein consumption in developed countries is the driving force for intensive production of livestock and poultry which has more environmental cost comparing plant origin foods (Balandrán-Quintana et al. 2019). Furthermore, plant-based foods are more sustainable than animal foods and are best solution to solve issues of food insecurity in the world with fewer natural resources and less adverse impacts on environment (Sabaté and Soret 2014).

3.2 Proteins from Plant Sources

Plant foods are cheaper and abundant sources of proteins available for human consumption than any other food source. Most of the plant proteins remain in seeds and grains and are known as cereals or legumes, including oilseed legumes.

Majority of the plant foods are comprised of protein, starch, lipids, soluble and insoluble dietary fibers and non-starch polysaccharides (Day 2013). Important nutritional composition of plant foods has been mentioned in the Table 1.3. Furthermore, almost all plant foods contain several bioactive compounds in smaller quantities ranging from polyphenols, organic acids, flavonoids, resistant starch, quinones, and many more (Table 1.4). Some of them are specific for specific foods while most of them are widely present in all plant foods. Plant proteins, on the basis of solubility and extractability in water, are divided into four major classes known as "Osborne classification" (Osborne 1924). Glutelins is the major storage protein followed by prolamins, globulins and albumins respectively. In general, plant proteins are considered as poor-quality proteins due to lesser amounts of lysine, tryptophan, methionine and cysteine EAAs than animal proteins (Table 1.1). Limiting amino acids is the one factor for protein quality, others include protein digestibility and bioavailability. As for as quantity of protein is concerned, cereals, legumes, pulses and nuts are good source of plant proteins and nutritional values are comparable to animal proteins except limiting some EAAs while fruits and vegetables are poor source of proteins. When EAAs are compared, plant foods like quinoa, soybean, sorghum and corn are good source of indispensable amino acids and fulfill requirements of adults (Table 1.1). Furthermore, the best protein composition, comparable to animal proteins, can be achieved through mixing of various foods of plant origin (Day 2013).

Cereals are converted into flour after milling, resulting separation of various grain fractions. While legumes are mostly consumed directly after soaking and cooking. Due to increasing demand of plant proteins, several protein ingredients like protein concentrates, protein isolates and hydrolysed proteins are also produced from different plant proteins like soybean, wheat, rice, peas, canola (Day 2013).

The proximate composition, mineral analysis and amino acid profile of chickpea, lentil, cowpea and green pea show that these are better suppliers of minerals, particularly iron, zinc, calcium potassium and phosphorus (Table 1.3). Furthermore, they are rich sources of two EAAs lysine and leucine to fulfill their requirement of human diet while lacking in S-containing amino acids and tryptophan. To make balanced diet, it is required to supplement legumes with other foods of animal origin like milk, meat or poultry (Iqbal et al. 2006).

Although legume seeds are considered as low-quality protein sources due to limiting EAAs, however, they also contain several comparatively minor proteins, sometime also termed as antinutritional factors including protease and amylase inhibitors, lectins, lipoxygenase, defense proteins and others, which have shown potential in the treatment or prevention of various types of cancers, obesity and hypertension (Roy et al. 2010). Furthermore, the pulses are not consumed raw, but after soaking, sprouting, heating or fermentation which inactivates the antinutritional factors. Some legumes like lupines and peas have good digestibility ranging from 89% to 96% and PDCAAS values from 81 to 96. These values are comparable to most animal proteins (Erbersdobler et al. 2017).

Some plant proteins like gluten present in wheat and other cereals are not digested by 1–2% population and cause some allergic reactions commonly known as celiac disease. For these individuals "gluten-free" products are available in market.

4 Protein and Human Health

4.1 Animal Proteins and Health

High quality animal proteins are very important for good health however, it's important to pay attention that a diet containing low quality animal protein like processed red meat and high fat cuts may become harmful to health. Processed meats often contain nitrates as preservative which can damage blood vessels and contribute to hardening of arteries while sodium in these diets can lead to high blood pressure. Although high animal proteins are linked with type 2 diabetes mellitus risk, but sea foods and milk especially low fat are good sources of branched chain amino acids and taurine which are beneficial in glucose metabolism and in the management of blood pressure (Elmadfa and Meyer 2017).

Generally, the amino acid requirements of healthy adults can be fulfilled from all protein sources, animal as well as plants due to consumption of higher protein quantities, however, it might not be possible for vulnerable groups to fulfill the needs from only plant source. The infants and young children require animal protein sources because of increased requirement of EAAs compared to adults (Table 1.1). For example, infants up to 6 months require 484 mg of EAAs per gram of protein required as compared to adolescents and adults 286 and 277 mg/gram protein, respectively (Elmadfa and Meyer 2017).

Normally cereal as well as sugar-based energy dense with low quality protein foods are selected for complementary feeding at the age of 5 to 12 months that can have significant impact on baby's health and can be the major reason for overweight and obesity related metabolic disorders. Anemia and iron deficiency issues in children can be prevented using animal-based foods. Furthermore, the dairy, poultry, meat and fish based complementary foods along with breastfeeding may be helpful in the development of the healthy gut microbiota which have their own importance throughout life (Tang 2019). To be successful in life, a person's physical as well as mental health is crucial and these cannot be maintained with micronutrient deficiencies like B_{12}, folate, riboflavin, vitamin A, vitamin D, iron and zinc as well as protein energy malnutrition. Choosing only plant-based foods for these nutrients without any supplementation or fortification may create problems in life due to low quality plant proteins and anti-nutritional factors present in plant foods (Balehegn et al. 2019) whereas animal source foods can positively contribute in cognitive development, school performance and lifelong achievements as proven by evidenced based studies.

Animal based proteins have high digestibility and have amino acid pattern required for good health, however, the risk of noncommunicable diseases can also be increased with consumption of more protein-rich animal foods especially if consumed alone, due to the presence of saturated fatty acids and potential carcinogens in processed meat but also the atherogenic methionine metabolite homocysteine (Elmadfa and Meyer 2017). A European prospective study found a positive correlation between prostate cancer in men with the consumption of animal foods, protein and even calcium from dairy products but calcium not from other foods. The possible mechanism behind is the increased level of insulin-like growth-factor-I (IGF-1) due to dairy proteins which in turn may promote prostate cancer development (Allen et al. 2008). High quality and sufficient quantity of protein is required throughout life for the synthesis of IGF-1, necessary for proper growth, development, bone mineralization in children as well as maintenance of healthy bones and lean body mass in old ages, however higher protein intake than needed by body lead to over expression of IGF-1 (Elmadfa and Meyer 2017) which result in cell proliferation and increased risk of some types of cancer. Furthermore, several heterocyclic amines and polycyclic aromatic hydrocarbons are produced in red meat products prepared directly at flame or at high temperature, are responsible for various types of cancers mainly colorectal cancer (Adeyeye 2018; Diallo et al. 2018). Two health professional follow-up studies find out that red meat and processed red meat are strongly associated with total, CVD and cancer mortality. Further explained that every additional serving of red and processed red meat can increase 10% and 16% higher risk of cancer death, respectively (Pan et al. 2012).

Multiple issues including milk allergy, lactose intolerance, dietary restrictions, calorie concern, other potential health risks related to dairy products as well as more preference to vegetarian diets has influenced consumers towards choosing milk alternatives. The soy, almond, oat, coconut, rice, cashew nuts, hemp and quinoa as plant foods to get milk have received much attention. However, consumer taste, nutrition and cost of production of plant milk sources are major limiting factors (Sethi et al. 2016).

Using multiple regression analysis, data of more than eighty-nine thousand men and women from five countries participating in European Prospective Investigation into Cancer and Nutrition in six and half year's duration was investigated to find association between total protein intake, protein source (animal/plants) and changes in weight and waist circumference per year. A high protein intake was not found associated with measured markers. In contrast, animal origin protein, especially meat and poultry, seemed to be positively associated with long-term weight gain (Halkjær et al. 2011).

Many meat bioactive compounds and peptides produced by enzymatic hydrolysis or fermentation exhibit antihypertensive effects as well as the protective effects on cardiovascular disease (Vongsawasdi and Noomhorm 2014). The dairy products have high biological value proteins as well as peptides, which are produced during fermentation process especially in ripened cheese like cheddar cheese and they exhibit antimicrobial properties against various pathogens including *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*, antioxidant activity through inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and antihypertensive properties as determined by inhibition of the angiotensin-converting enzyme (ACE) (Pritchard et al. 2010).

The animal proteins contain higher ratios of sulfur amino acids i.e., methionine and cysteine. While catabolism of these amino acids in body result in increased metabolic acid load which promote the desorption of calcium from bones and increase calcium urinary excretion in elderly women in China as compared to plant proteins (Hu et al. 1993). A prospective cohort study shows that a high ratio of dietary animal to plant proteins increases the rate of bone loss and nearly 4 times higher risk of fracture in postmenopausal women (Sellmeyer et al. 2001). There are also studies which does not show any relation between higher intake of animal proteins and decrease in bone mineral density in elderly men and women (Hannan et al. 2000) and demand further studies for final conclusion (Jesudason and Clifton 2011) showing that there are several factors other than protein source which can affect bone mineral density like alcohol consumption, calcium intake, age, gender as well as smoking and estrogen use.

Plant proteins contain more branched chain amino acids which make protein structure more globular and easier to digest while animal proteins consist of higher number of unbranched amino acids and sulfur amino acids making animal proteins straight and compact. This complex structure of animal proteins needs more acid for digestion which may lead to acidity. To neutralize it, more calcium is needed from bones resulting detrimental effect on bone health especially in old age (Adeva and Souto 2011). It is also evident that for good bone health, high protein intake than RDA including red meat, along with appropriate calcium, fruits and vegetables intake is more important in prevention of osteoporosis (Cao and Nielsen 2010).

Several epidemiological studies show that higher consumption of animal foods mainly red meats and their processed products are associated with several health problems (Montonen et al. 2013; Rohrmann et al. 2013) however no clear association has been found between animal proteins and CVD, diabetes, cancer and obesity (Pedersen et al. 2013; Richter et al. 2015) clearly showing that adverse health impacts associated with animal foods are only due to high amount of cholesterol, trans fats and other ingredients but not proteins.

4.2 Plant Proteins and Health

Plant based diet, due to their unique and complex nutritional composition with dietary fibers, appropriate fat composition, high levels of anti-oxidants, high levels of certain micronutrients, chlorogenic acids, certain amino acids, phytochemicals, and low levels of certain dietary factors (Table 1.4), are digested slowly in the gastrointestinal tract and exhibit several health benefits. Furthermore, plant foods have low glycemic index and glycemic load as well as influence the appetite sensation, increase satiety level and reduce energy intake of the consumer than animal based meals (Kristensen et al. 2016). These beneficial components and properties can help to maintain a healthy weight, reduce the risk of increased waist size, enhance glycemic control, improve lipid profile, improve vascular health, decrease inflammation, increase growth of healthy microbiome and all these potential mechanisms lower the risk of type-2 diabetes, CVD and various types of cancers as well (Richter et al. 2015; Chen et al. 2018; Lin et al. 2015; Satija and Hu 2018). Regardless of benefits of plant-based diets, animal foods consumption is more in the world due to several factors including social and psychological influences. The European Union, adolescents are consuming 96 g of protein per day in which 59% are from animal sources as revealed in the HELENA cross-sectional study of more than 1800 participants. The study concluded that intake of plant proteins may prevent obesity in adolescents (Lin et al. 2015). However, now days trend of "plant-based diet" is increasing with different names and believes. Some of them are strict vegetarian like vegetarian and vegan diets, where as some consume dairy products as well i.e., lacto-vegetarian diet. Some groups also consume dairy and egg products called lacto-ovo-vegetarian diet and in Pesco-vegetarian diet, people also consume fish in addition to egg and milk products but no other animal foods like red meat (Satija and Hu 2018).

A Japanese cohort including more than seventy thousand participants found that consumption of more plant proteins is associated with lower total and CVD-related mortality. While replacement of red and processed meat with plant proteins is strongly associated with total, cancer and CVD-related mortality (Budhathoki et al. 2019). Although the potential mechanism responsible for contribution of plant as well as animal-based proteins in the prevention or development of CVD are multifaceted involving nutritional composition of whole foods, protein and non-protein compounds, their metabolites, interaction with other nutrients and microbiome. Yet it is evidenced that dietary pattern with more foods from plant origin compared to processed animal foods, reduce the risk of CVD (Richter et al. 2015; Satija and Hu 2018). According to physicians report, plant-based diets are cost-effective and consumption of variety of fruits, vegetables, nuts, cereals and legumes in appropriate combination may help in ideal weight management and blood pressure, reduce HbA1C, and cholesterol levels (Tuso et al. 2013). It is also to mention that only healthy plant-based diets containing plant super foods and minimally processed foods can reduce the risk of coronary heart diseases whereas no association was found for less healthy i.e. refined and processed plant foods (Kim et al. 2019; Satija and Hu 2018; Satija et al. 2017). Furthermore, the healthy plant foods not only reduce disease risk through prevention but can also be used for treatment of various chronic diseases.

An analytic prospective cohort spread on 16 years data from 1995 to 2011 including more than 0.416 million participants of both genders revealed that higher plant proteins intake is associated with small risk reduction in overall and CVD linked mortality. The cohort further elaborated that replacement of 3% energy from animal to plant proteins can reduce 10% risk of overall mortality in both men and women. Furthermore, the men have 11%, while women have 12% lower risk of CVD mortality with this protein replacement (Huang et al. 2020).

Not only the proteins from animal sources, but various plant-based proteins have also been used to produce bioactive peptides of size ranging from 2–20 amino acids.

They do not only exhibit anti-oxidant properties (Sarmadi and Ismail 2010) but are also effective in a wide range of efficacy studies. The studies show that peptides from soybean (Chatterjee et al. 2018), Chia seed (Grancieri et al. 2019), mung beans (Hou et al. 2019), pulses (Roy et al. 2010) and amaranth exhibit one or more beneficial properties including anti-cholesterolemic, anti-oxidant, anti-hypertensive, anthropometrics and hypoglycemic when used in adequate amounts for few weeks.

With respect to physician's point of view, plant-based nutrition gives several health benefits in comparison to animal foods like (a) vegetarian diet give only 5–6% saturated fats which is compulsory for healthy heart diet, (b) provide very low dietary cholesterol and dietary fibers in food further reduce the cholesterol level through enterohepatic circulation, (c) contribute less in antibiotic load of consumer as compared to animal foods, (d) less contribution in production of IGF-1 which result in prevention of cancer proliferation, and (e) no toxic compounds are produced when cooked at high temperature like carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons are produced in meat (Hever 2016).

5 Summary

To answer the question, which protein source is better, animal or plants, is not black and white as it depends on several factors. When we consume a food for its protein, we also eat other nutrients present in the food i.e., fats, fiber, carbohydrates, vitamins, minerals and other bioactive compounds. We always take protein in a "package" form. So, it's not only the protein, but the whole food that exhibits health benefits, good or bad. The good quality, highly digestible and complete animal proteins also provide cholesterol, trans fats and are deficient in very important dietary fibers, phytochemicals and flavonoids. While plant proteins provide a wide range of complex carbohydrates, dietary fibers, vitamins and minerals along with bioactive compounds, but we have to compromise for limiting EAAs and the presence of antinutritional factors. Major pros and cons of plant and animal protein sources have been presented in Table 1.5. The deficiency in plant proteins can be minimized through combination of various plant sources to fulfill consumer needs of all EAAs. The protein source should be chosen wisely depending upon age, gender, physical activity and body requirements for protein and EAAs. During growing age, it is important to have proteins from animal sources to fulfill increased demand of EAAs for proper growth and development while in adult and old age it is better to consume variety of plant-based proteins and moderate intake of animal proteins as their larger consumption may contribute to chronic and non-communicable diseases. In conclusion, a dietary transition from purely animal based foods to healthy plant-based foods is required at global level along with active lifestyle to have better health and to decrease burden of non-communicable diseases.

Table 1.5 Comparison of animal and plant protein foods

	
More than 97% digestible	70–90% digestible (Tomé 2013)
90% bioavailability	60–70% bioavailability (FAO/WHO 1991b)
More anabolic, help in tissue growth and maintenance	Less anabolic (Berrazaga et al. 2019)
High in vitamin B12, vitamin D, heme-iron, zinc, DHA (in fatty fish)	High in magnesium, dietary folate, and vitamin A
Contain all EAAs	Deficient in EAAs (Lysine, tryptophan and SAA) (Schaafsma 2000)
Digestion produces a large number of bioactive peptides	Contain less bioactive peptides
Higher amounts of saturated fatty acids (German and Dillard 2004)	Higher amounts of MUFA and PUFA especially Omega 3 fatty acids (German and Dillard 2004; Coulston 1999)
Natural cholesterol level is high (Table 1.2)	Low levels of dietary cholesterol
Naturally, several animal foods have trans fats (Table 1.2)	Some plant foods have traces of trans fats
Lack of fiber	Contain higher amounts of soluble and insoluble fiber as well as prebiotics (Table 1.3)
Only few products have bioactive compounds like taurine, choline, lactoferrin	Wide range of bioactive compounds are present including, polyphenols, Isoprenoids, chlorophylls, organic acids, flavonoids and many more (Table 1.4)
Processed meat products may contain carcinogens (Diallo et al. 2018; Rohrmann et al. 2013)	Processed products may loss some nutritional value
Cost of production is high (Sabaté et al. 2015)	Cost of production is low
Consumption for a longer period may lead to a considerable risk of heart disease, cancer, diabetes, high cholesterol, weight gain (Wu 2016; Pan et al. 2012)	Decrease the risk factors for all mentioned con-communicable diseases (Day 2013; Satija and Hu 2018; Hever 2016)

EAAs Essential Amino Acids, *SAA* Sulfur Amino Acids, *MUFA* Monounsaturated Fatty Acids, *PUFA* Polyunsaturated Fatty Acids, *DHA* Docosahexaenoic acid

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Chapter 2
An Overview of Plant-Based Protein Rich Products

Saeed Akhtar, Tariq Ismail, Anam Layla, Majid Hussain, and Muhammad Qamar

1 Introduction

Plant proteins have received much attention as highly nutritious and sustainable source of essential amino acids in the past two decades (Lonnie and Johnstone 2020; Sá et al. 2020). Plant-based proteins are consumed as alternative sources of protein among underdeveloped nations where animal-based proteins are either expensive or scarce. In developed countries, plant protein sources represent a core component of routine diet particularly in vegan foods. Moreover, plant protein sources are naturally embraced with biologically active food components and generally low in saturated fats (Hever and Cronise 2017) making them popular among health-conscious consumer groups.

The worldwide market value of plant proteins was estimated around US$ 12.1 billion in 2019 and this consumption pattern is predicted to surpass US$35.54 billion in 2024 (Wood 2020). The continued increasing demand and drive towards plant proteins consumption have been influenced by different factors including health-related problems such as animal protein sensitivities, saturated fats, trans

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fats, milk hormones, ethical and environmental concerns from some consumer groups, and beneficial health claims linked to plant-protein based diets (Lonnie et al. 2020; Malekinejad and Rezabakhsh 2015; Rangel et al. 2016; Rotz et al. 2010).

Several plant proteins sources such as wheat, rice, sorghum, millets, quinoa, soybean, pulses, seeds and nuts have been extensively explored and utilized as cost-effective and sustainable sources of protein – energy. Protein is an essential macronutrient and its regular supply through a variety of dietary sources is mandated for proper growth, development, and health maintenance (Burd et al. 2019). The nutritive value of dietary proteins varies depending upon their source, extraction methods, proteins purity, amino acid profile, digestibility, bioavailability and anti-nutritional factors (Mattila et al. 2018; Herreman et al. 2020). Proteins from plant origin are often regarded as nutritionally incomplete or inferior to animal proteins. However, existing set of knowledge provokes intelligent exploitation of plant proteins by consuming a variety of plant protein sources. Such a combination of varied plant protein sources in daily diet ensures a balanced supply of essential and non-essential amino acids to meet human physiological needs (López et al. 2018; Naghshi et al. 2020; Sá et al. 2020).

Proteins' structure represents their functional and technological properties under different set of food processing conditions (Loveday 2019). Animal proteins are more organized and form fibrillar or fibrous structure while plants contain less organized globular proteins. Such a structural variation results in differences in functional properties such as gelation, viscosity, water/fat retention, foaming capacity, emulsion stability, and matrix formation (Ismail et al. 2020). Moreover, cost-effective plant protein such as soy, chickpea, wheat, millet, barley proteins are also used as a base ingredient in formulating dairy alternatives (Dupont et al. 2020), meat analogues (Kyriakopoulou et al. 2019) and comminuted meat products (Youssef and Barbut 2011). In addition, textural attributes of plant proteins enable them to be shaped in a variety of products like tofu, tempeh, edamame, hummus, seitan, Ezekiel bread, cereal flakes and snacks etc. (Reynaud et al. 2021; Lambrecht et al. 2018; Fukushima 2011). Plant proteins not merely attribute textural and functional properties to the edible goods but also improve protein contents and overall nutritional quality.

A plethora of research has been performed in the last two decades on extraction of plant proteins and their food features in different food formulations (Fasolin et al. 2019; Sá et al. 2020). This chapter provides a comprehensive overview of plant protein – based products, traditional and commercial applications of plant proteins, novel applications of plant proteins in developing edible packaging, and plant proteins isolates and concentrates as potential therapeutic solutions for protein – energy malnutrition.

2 Plant-Based Protein Rich Products

2.1 Grains Protein-Based Products

2.1.1 Wheat Protein

Wheat (*Triticum aestivum*) is a cereal grain widely cultivated as a staple food over the globe. Worldwide, annual consumption of wheat was recorded 751.5 million metric tons in 2019 with an increasing demand driven by population growth, urbanization, economic development and changing lifestyles (Statista 2020). Wheat is a dominant and ubiquitous cereal crop used in several food products formulation such as bread, pasta, cake, nutritional bars and as meat analogs. A major proportion of wheat is annually converted into wheat starch and protein with approximately 1,270,000 tons production of wheat gluten (Markets and Research 2020). Wheat grain holds a variety of proteins which are differentiated by their solubility and structure such as monomeric or polymeric. The monomeric proteins include albumins (water soluble), globulins (salt soluble), and gliadins (alcohol soluble) while polymeric proteins are mainly insoluble glutenins (Thierry and Larbi 2018).

Wheat gluten is an insoluble functional protein extracted from wheat flour in wet form (gum gluten), which is dried into free-flowing bland tasting protein powder. Gluten proteins, which include gliadins and glutenins constitute 75–80% of the wheat proteins. When gluten protein is isolated from wheat flour, albumins and globulins (soluble proteins) are mostly washed out with starch (MacRitchie and Lafiandra 1997). Gluten exhibits tremendous structural and functional properties including extensibility, strong hygroscopicity, liposuction emulsification and thermosetting adhesion (Ortolan and Steel 2017). These functional characteristics make wheat gluten a valuable ingredient for the baking industry and extruded products.

Viscoelastic properties of gluten and their ability to bind water mandate gluten as a core ingredient for the baking industry. Vital wheat gluten supplementation (protein contents ≥80%) is recommended in bread to improve softness, elastic texture, yield and shelf life of the bread. Gluten enables bread dough to form continuous viscoelastic film to entrap gas produced during fermentation and baking of bread that favorably increase final bread volume and brings soft texture to the crumb (Rathnayake et al. 2018; Flambeau et al. 2017).

Gluten supplementation in products like pasta and spaghetti reduces product stickiness. Gluten protein is also used in formulating extruded breakfast products as well as 30% wheat protein enriched cereal flakes (Delcour et al. 2012). Furthermore, gluten protein is used as a binding material in coating batters of fried food products such as crumbed meat and nuggets (Dogan et al. 2005; Kumar et al. 2012). Other than its uses in bakery products, wheat gluten has limited applications due to its insolubility and viscoelastic nature. Product hydrolysis catalyzed by enzymes or acids results in loss of viscoelastic property of the protein. Hydrolyzed wheat gluten is used in the preparation of various liquid foods for protein enrichment such as dairy products (milk, yogurt and ice cream), high-protein sports beverages, soups and many other foods. In addition, hydrolyzed wheat protein present foaming and emulsification properties and is used as alternative to caseinates in numerous food applications such as in confectionary and non-dairy creamers (Flambeau et al. 2017) (Table 2.1).

Texturized Wheat Protein

A variety of plant-based meat alternatives have been developed that represent modest form of pure plant proteins products e.g. seitan, meat extender, flakes, snacks. Seitan is a traditional meat substitute formulated by monks in China as an alternate to duck meat. Seitan is composed of wheat protein extracted from wheat flour and then cooked in a soup or fried in oil. The main limitation to widen seitan consumption is linked to its slightly soft and sticky texture. However, the processing of seitan in vegetable soup or oil allows incorporation of spices and flavoring compounds which improve product taste (Jacobs 1994; Marcincakova et al. 2004; Mal'a et al. 2010).

Texturized wheat protein is developed at high shear, high temperature and low moisture conditions in an extruder. Treatment of wheat protein at high shear and temperature dissociate and uncoil the macromolecules of wheat protein which bring them to rearrange and crosslink through specific linkages in an oriented pattern. On exiting from the extruder dye, surface water of extrudate evaporates which renders the structure of product somewhat spongy. Akdogan (1999) reviewed a high moisture (above 60%) extrusion process to avoid spongy structure of the texturized wheat protein. During cooling step (below 100 °C), a shear flow of melted protein in dye converts the product into a thick-layered fibrous structure that is quite identical to meat analogs. Texturized wheat proteins are used in ready to serve meals applications as meat extenders (Samard et al. 2019) or in combination with heat-gelling proteins like egg white and soy isolates (Lambrecht et al. 2017).

2.1.2 Rice Protein

Rice (*Oryza sativa*) was lauded as "gold of the Orient" in ancient times and is the most common daily staple consumed by nearly half of the world population (Roy and Shil 2020). The worldwide average per capita rice consumption was close to ~54 kg/year in 2017, while its consumption exceeded 100 kg/year per capita in many Asian countries (FAO 2017). Rice has around 20% share in worldwide human caloric intake and anticipate upto 16% of the daily protein requirements making it most important cereal crop for human nutrition next to wheat (Awika 2011; Hoogenkamp et al. 2017). A major segment of global population consume rice in the form of whole or broken kernel. However, the known nutritional benefits and hypoallergenic properties of rice proteins have fueled the demand for formulating a range of rice protein-based products. Rice proteins mostly finds applications in infant formulas and gluten free value-added products (Amagliani et al. 2017).

Table 2.1 Application of wheat proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Vital wheat gluten	Above 80%	4–6%	White pan bread enriched with 10% resistance starch	Addition of vital wheat gluten improved loaf volume of the bread, reduced crust hardness and baking loss.	Kim et al. (2013)
Vital wheat gluten	Above 80%	8%	Brown rice pasta	Vital gluten incorporation improved palatability and cooking quality of brown rice pasta.	Kaur et al. (2017)
Wheat gluten	75–82%	10–18%	analogue meat nuggets	Cooking yield, general appearance, texture binding and overall acceptability of analogue meat nuggets enhanced with subsequent increase in levels of gluten protein.	Kumar et al. (2012)
Hydrolyzed gluten protein (HGP)	72–86%	5 g	Meringue	As foaming agent hydrolyzed gluten protein produced meringue batter with superior qualities (density and apparent viscosity) and after baking had greater specific volume than egg white protein containing meringue.	Wouters et al. (2018)
Wheat flour	12–13%	24–34%	Seitan	Wheat dough was washed repeatedly to remove starch and some bran. Obtained wheat gluten (seitan) was soaked in flavoring ingredients, coated with chickpea flour and fried in oil for 20 min. Overall nutritional quality and taste enhanced while cooking loss of seitan lowered after frying process.	Anwar and Ghadir (2019)
Wheat gluten	75–82%	8–14%	Noodle	Higher gluten content greatly reduced starch digestion rate and stickiness of cooked noodles while other texture attributes were comparable to control.	Yao et al. (2020)

(continued)

Table 2.1 (continued)

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Wheat gluten	75%	4%	Low fat beef sausages	Addition of wheat protein increased water holding capacity and emulsion stability of low-fat sausages without posing any detrimental effect on sensorial properties.	Serdaroglu and Ozsumer (2003)
Wheat gluten	78%	10%	Textured wheat protein	Gluten extruded under alkaline pH environment had improved textural attributes (elasticity, hardness and chewiness) and developed compact fibrous microstructure.	Li et al. (2018)

Rice kernel is rich in glutelin (60–80% of the seed protein) while considerable amount of albumin (4–22%), globulin (5–13%), and prolamin (1–5%) have also been reported by various researchers (Ju et al. 2001; Wang et al. 2014). Rice proteins are mostly crystalline and water-insoluble, however a substantial amount of protein found in rice bran are soluble in water and salted solutions.

The aleurone and sub-aleurone layers of rice grain stores most of the rice protein whereas more protein are expressed in the sub-aleurone layer. Rohrer and Siebenmorgen (2004) reported that longer milling time remove most of the protein bound to rice bran and results in a low level of protein contents in milled rice. Rice bran that contains germ and aleurone fractions deliver up to 15% (fat-rich form) and 18% (oil-free form) more protein contents as compared to the endosperm (Kahlon 2009; Fabian and Ju 2011). Since the rice kernel (endosperm) is low in protein contents, therefore, it may be regarded as an expensive starting material for protein rich products formulation. Contrarily, rice coproducts such as broken rice kernels, rice bran and the residues of rice starch extraction which have less economic value can serve as potential candidates for rice protein extraction (Hoogenkamp et al. 2017).

Broken rice being a plausible carrier of proteins (~8%) has also been used as the substrate to obtain rice protein by enzymatic degradation and removal of starch, and generating protein dense concentrates (~25% protein) and isolates (~90% protein) (Euber et al. 1991; Ahmadifard et al. 2016). During the process of rice syrup manufacturing, α-amylases help to liquefy starch granules and proteases enable protein to get released from fiber and starch granules. This process generates soluble rice protein concentrates with varying levels of protein contents and insoluble rice residue as coproducts of rice syrup. In a study by Shih and Daigle (2000), researchers used protein-containing coproduct (50% protein rich-residues) and formulated a rice protein-based product with 85% protein contents. Notably, protein bodies are tightly bonded on the surface of the starch granules; therefore, protease treatment or high-alkaline conditions are needed to release and purify rice proteins (Puchongkavarin et al. 2005). Rice proteins possess hypoallergenic properties; therefore, rice protein-based infant formulas (RPIF) are proposed for the dietary management of cow's milk protein allergy in infants (Vandenplas et al. 2014). Rice protein-based infant formulas are more specifically developed from rice protein concentrates, isolates or hydrolysates. As RPIF are strictly plant-based recipes, optimum nutritional composition of the food is ensured by fortification with limiting nutrients like lysine and threonine (Dupont et al. 2020).

Rice bran incorporation in meat batters anticipates good emulsifying and gelling properties to the product. In addition, fiber and protein components of bran bind and stabilize moisture and oil contents of products, thus providing textural stability to meat batters during freezing and thawing (Alauddina et al. 2017). Rice proteins have several commercial applications such as in nutritional supplements, confectionary, beverages and as flavor ingredient in savory products (Phongthai et al. 2017). Germinated brown-rice derived protein has Generally Recognized as Safe (GRAS) status, and has whey proteins bearing functional properties such as building and repairing of muscles (FDA 2015). Hypoallergenic and nutritional properties of rice protein products balance any shortcomings of the functional properties, thus making them competitive alternative plant-based protein products (Table 2.2).

2.1.3 Sorghum and Millets Protein

Sorghum (*Sorghum bicolor*) and millets are tropical cereals generally cultivated in Africa, Asia and in some regions of central America. Millets represents a group of several small cereal-grains species including finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*) and teff (*Eragrostis tef*). Sorghum and millets are often considered together due to having same geographical distribution and matchable agronomic practices for cultivation (Taylor 2019). According to the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), around 600 million people in more than 30 countries depend on sorghum and millets as staple food crops (ICRISAT 2018). Sorghum and millet are gluten-free, rich in proteins, fibers and micronutrients (i.e., calcium and iron), and are indexed lower than wheat, rice and maize for glycemic response. Sorghum and millet based low to medium glycemic indexed foods are recommended for the dietary management of diabetes and celiac diseases (Kumar et al. 2018; Ciacci et al. 2007).

Sorghum and millets are mostly considered as "poor people's staple foods" contributing 8–15% of the daily protein requirements of the farming communities. The protein contents of these cereal grains vary considerably between species for example pearl millet contains protein-rich germ and deliver 14.5% protein. Contrarily, finger millet has relatively low (~8%) protein contents (Taylor and Taylor 2017). The major storage proteins of sorghum and millets are the endosperm-specific prolamins named kafirin and pennisetin, respectively. The other protein fractions are germ bound albumin and globulins while glutelins are concentrated in endosperm

Table 2.2 Application of rice proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Rice protein isolates (RPI)	91%	10%	Gluten free rice noodle	Combined application of RPI and transglutaminase (1%) increased viscosity, decreased cooking loss, cracks and water turbidity of rice noodle during cooking.	Kim et al. (2014)
Rice flour	6–8%	1.2–1.6%	Sponge and layer cakes	The finest rice flour (100 μm) gave viscous batter with lower specific volume and produced cakes having lower firmness and higher volume.	de la Hera et al. (2013)
Rice protein hydrolysates	78.2%	1.7–2%	Infant formula	Hydrolyzed rice protein formula enriched with lysine and threonine provided complete nutrition and well tolerated by infant allergic to cow milk protein.	Reche et al. (2010)
Broken rice protein	56–65%	2–9%	Sausages	Higher water holding, emulsifying and foaming capacity of broken rice protein significantly ($p < 0.05$) increased yield of sausages.	Hou et al. (2010)
Rice protein	79%	4% and 8%	Restructured beef steaks	Rice protein supplementation reassembled meat structure and increased availability of free amino acids with improved digestibility.	Baugreet et al. (2019)
Rice protein isolates	91%	3–12%	Meat extenders	Addition of RPI decreased cooking loss and increased water holding capacity of extended nuggets compared to control. All treatments involving RPI liked by panelists and showed high sensorial score.	Shoaib et al. (2018)

(continued)

Table 2.2 (continued)

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Brown rice protein isolates	80–90%	80–90%	Oryzatein	Protein sourced from whole rice grain providing all essential amino acids being used in multiple food grade products such as allergen free formulations, protein substitution in beverages and baked goods, confectionary, protein bars and as binder in savory meats beside cosmetics and nutraceuticals.	Janow (2018)
Rice protein isolates	92%	2.4 g	Maillard products	RPI glycated with glucose improved solubility, emulsification activity and emulsification stability of the Maillard reaction products	Li et al. (2009)
Rice protein extracts	–	0.5–10%	Brown rice beverage	Addition of rice protein extracts improved pasting properties, suspension and storage stability of brown rice beverage.	Nitisuk et al. (2019)

(Shewry 2002). Alike other cereals, sorghum and millets are deficient in lysine; however, they hold considerable amount of other essential amino acids (Joint FAO and WHO 2007). Millet storage protein (pennisetin) is far less studied than sorghum storage proteins (kafirin) but it is evident that both have similar amino acid composition regardless of differences in degree of polymerization or hydrophobicity (Taylor and Taylor 2017).

Sorghum and millets storage proteins (prolamins) differ substantially from those of wheat and other cereals and hence considered safe foods for individuals suffering from wheat allergy (Pontieri et al. 2013). Consumer food market has now been familiarized with gluten free food formulations. A variety of sorghum and millet based value-added baked goods, beverages and fermented products are now available in consumer market (Gull et al. 2014; Adebiyi et al. 2018). However, potential applications of pure proteins extracted from sorghum and millets are less documented with exception of the sorghum storage protein, kafirin (Taylor and Taylor 2017). Despite of its poor nutritional quality, sorghum kafirin has some unique functional attributes like high hydrophobicity, good alcoholic solubility, controlled morphology and the ability to self-assemble into biomaterials that give it commercial potential (Xiao et al. 2017) (Table 2.3).

Table 2.3 Application of Sorghum and millets proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Sorghum flour	11%	–	Pasta	Sorghum flour containing gluten-free pasta showed slower rate of starch digestion with high level of polyphenol contents and protein hydrolysis in simulated digestion.	Palavecino et al. (2019)
Sorghum Kafirin	83.6%	2%	Edible film	Application of kafirin containing edible coating on pears as a post-harvest treatment retarded respiration rate and progression of senescence during storage; however, was unable to prolong shelf life after 14 days of storage.	Buchner et al. (2011)
Sorghum Kafirin	–	750 mg	Encapsulating material	Catechin or sorghum condensed tannins (400 mg) containing kafirin microparticles showed no protein digestion but effectively released (50–70%) dietary antioxidants.	Taylor et al. (2009)
Pearl millet extruudates	9.6%	6.72%	Extruded supplementary foods	Millet flour (70%) blended with legumes flour (30%) to prepare extruded ready-to-eat foods. The high caloric supplement paste had viscous texture and improved carbohydrates digestibility being suitable for children and mothers.	Sumathi et al. (2007)
Germinated finger millet (finger millet malt)	7.81%	–	*Togwa* (Non-alcoholic beverage)	Addition of finger millet malt (source of amylase) in maize flour slurry (source of starch) cleared gel like consistency to viscous liquid and contributed sweet flavor.	Ndabikunze et al. (2001), Kitabatake et al. (2003)

(continued)

Table 2.3 (continued)

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Minor millets flour blends (barnyard, foxtail and kodo millets)	9–14%	–	Cookies	Gluten free cookies prepared from germinated minor millets flour blends were highly nutritious and were having acceptable textural properties.	Sharma et al. (2016)
Proso millet flour	12%	–	Gluten free pasta	Gluten free pasta was developed using proso millet as sole ingredient. Additionally, rheological and textural properties of pasta were improved by supplementing (1–2%) guar gum and xanthan gum.	Romero et al. (2017)
Pennisetin (Millet Protein)	95%	1.68 g	Casting biofilm	Pennisetin films casted from different plasticizers showed favorable mechanical and barrier properties comparable to other cereal proteins. However, their application as biofilm yet to be known.	Gillgren et al. (2011)

Kafirin Protein and Its Intended Uses

Kafirin protein is mainly extracted from industrial byproducts of milling, brewing, and bioethanol industry which reduce product cost, increase product demand and its consumption. Updates on use of kafirin at industrial scale promote its application as a functional protein in gluten-free formulations and value-added biomaterials (Husnain-Raza et al. 2017). Sorghum kafirin lacks viscoelastic properties like wheat-gluten which is a major drawback that limits its application in volume rising products (Schober et al. 2011). However, the inertness and hydrophobic nature of kafirin in aqueous environment benefits its application in high-value biodegradable biofilms synthesis. Giteru and his colleagues (2015) formulated kafirin based bioactive film loaded with plant essential oil citral and the polyphenol quercetin as bioactive packaging to maintain food quality and safety. It was found that Kafirin-citral films reduced the total viable microbial count and Kafirin-quercetin films inhibited lipid oxidation in fresh-chilled chicken fillets (Giteru et al. 2017). Kafirin could also be used as encapsulating agent for micronutrients and nutraceuticals. Condensed tannins from sorghum encapsulated with kafirin presented strong inhibitory effect against α-amylase and withstand simulated digestion showing potential to attenuate hyperglycaemia and control type 2 diabetes (Links et al. 2015).

2.1.4 Quinoa Proteins

Quinoa (*Chenopodium quinoa* wild) native to the Andes, is an annual herbaceous flowering plant primarily grown for its edible seeds (Jancurová et al. 2009). Quinoa is not a true cereal, but rather a dicotyledonous plant unlike most monocotyledonous cereals e.g., wheat, rice and barley (Mir et al. 2018). Quinoa is mainly cultivated in Peru, Bolivia, Chile and Ecuador representing 97% of world quinoa production (Balandrán-Quintana et al. 2019). Today, this plant has been introduced into different climatic regions because of its great ability to resist different agro-ecological conditions (Bazile et al. 2016). Quinoa is considered a complete food which deliver nutritionally well-balanced protein in terms of essential amino acids (López et al. 2018) and healthy lipids containing poly unsaturated fatty acids (PUFA i.e., linoleic and linolenic acids) (Altuna et al. 2018), desirable levels of vitamins and minerals, and other phytochemicals (Graf et al. 2015). Furthermore, quinoa protein is free from prolamin epitopes making it a safe candidate for formulating gluten free diets of celiac patients and those with wheat allergies (Vilcacundo and Hernández-Ledesma 2017).

Quinoa grains contain 14–18% protein contents encompassing a well-balanced amount of essential amino acids such as lysine, threonine and methionine (Gorinstein et al. 2002). Quinoa proteins mainly contain albumins and globulins, with little or no availability of prolamin proteins (Wang and Zhu 2016). Such a composition of quinoa proteins favors utilization of quinoa grains in developing protein-rich and gluten-free health advantageous products. Quinoa proteins have also good foaming capacity and hence can be used as functional ingredient in gluten-free baked and dairy recipes including bread, biscuits, pasta, crackers and milk (Deželak et al. 2014; Montemurro et al. 2019). Quinoa proteins lack viscoelastic properties which limit volume retention of the product during proofing and baking. Despite limited volume retention property, the addition of quinoa protein delivers quality attributes and mouth feel to gluten-free milk and other beverages (Abugoch et al. 2008). Furthermore, provided sufficient levels of essential amino acids meeting the criterion of FAO/WHO recommendations, quinoa proteins can also be suggested to formulate infant formulas (Vilcacundo and Hernández-Ledesma 2017). Some important functional properties like emulsification and structural gel matrix formation allow the use of quinoa protein in edible films packaging purposes. Quinoa protein-based films are reported to attribute antifungal properties which anticipate improved shelf stability of the consumer goods (Dakhili et al. 2019; Abugoch et al. 2011). Moreover, quinoa proteins are capable to hold flavors, add nutriture and bioactive components to foods and hence can be utilized in the fabrication of encapsulating material (Quintero et al. 2017; Zhu 2017) (Table 2.4).

Table 2.4 Application of quinoa proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Quinoa flour	16–18%	3.6%	Spaghetti	Quinoa addition increased net protein utilization, decreased true digestibility of starch and improved nutritional quality of corn-based spaghetti.	Giménez et al. (2016)
Quinoa grains	12.6%	5.0%	Cereal bar	Quinoa enriched cereal bar had high nutritional contents and acceptable functional parameters	Kaur et al. (2018)
Quinoa flour	16%	16%	Infant food	Feeding of quinoa-based supplementary food improved weight and plasma level of insulin-like growth factor-1 (IGF-1) in undernourished children.	Ruales et al. (2002)
Quinoa grains	16–18%	1.7%	Quinoa milk	Novel quinoa milk presented low glycemic index (52) and acceptable sensorial properties.	Pineli et al. (2015)
Whole or malted quinoa flour	16–18%	4.8–5.4%	Gluten free muffins	Addition of whole or malted quinoa flour to rice flour improved sensorial, textural and nutritive qualities of gluten free muffins.	Miranda-Villa et al. (2019)
Quinoa flour	16–18%	7.5 mg	Edible film	Strawberries coated with quinoa edible film inhibited yeast and mold growth and retained sensorial qualities.	Valenzuela et al. (2015)
Quinoa flour	16–18%	0.62%	Edible film	Coating of fresh blueberries with quinoa protein/chitosan/sunflower oil based edible film delayed fruit ripening and controlled growth of molds and yeasts during storage period of 32 days.	Abugoch et al. (2016)
Quinoa protein	–	10%	Encapsulating material	Use of quinoa protein as encapsulating agent of bioactive compounds (polyphenol and bixin) provided thermal stability and inhibited degradation of these compounds at high temperature.	Quiroz et al. (2020)

2.2 *Legume's Protein*

2.2.1 Soy Protein

Soybean (*Glycine max*) belongs to Fabaceae, the legume or pea family. Soybean is native to East Asia, widely distributed and grown in other continents for producing edible oil and protein rich products (Leamy et al. 2016). Soybean is naturally rich source of macronutrients such as high-quality protein (36%), soluble and insoluble carbohydrates (30%), fats (18%) and other plant nutrients including micronutrients (16%) (Thrane et al. 2017). Soy proteins deliver multiple nutritional and functional properties and have been used in the processing of many food products. In food industry, soy protein is supplemented with animal-based proteins such as eggs, poultry, meat, and dairy to enhance the protein quality of products. Moreover, soy proteins replacement with animal-based proteins reduces expense of food formulations and meets criterion of sustainable provision of good-quality protein in food supply chain (Riaz 2005; Singh et al. 2008). Soy proteins have multiple food applications in human nutrition such as processing of meat extenders (Carvalho et al. 2017), developing casein free infant formulas (Bhatia and Greer 2008), protein fortification (Rachman et al. 2019) and affordable plant-based milk alternatives development (Sethi et al. 2016) (Table 2.5).

Textured Soy Protein

Textured soy protein is used as a meat substitute in many meat products. Soy protein is replaced by 30–40% with meat in food products such as chicken nuggets and beef patties (Yeater et al. 2017). The soy protein substituted products have appearance and texture matchable to meat and meat products and provides high quality protein analog to that of lean meat. Textured soy protein has ability to be dyed using spices and malt extracts, and absorbs natural or synthetic flavors to increase sensorial properties of products. Notably, soy protein has excellent water holding capacity, therefore, soy protein substituted products remain soft by retaining more moisture during cooking, freezing, thawing and tolerate high temperature as compared to meat products made without supplementation of plant-based proteins (Thrane et al. 2017). Texturized soy protein can also be used in conventional food recipes as a substitute of meat-based protein. Omwamba et al. (2014) isolated texturized soy proteins from defatted soy flour and replaced meat at 25–100% levels as a protein source in samosa stuffing. Soy proteins supplementation in samosa significantly ($p < 0.05$) increased crude protein level and reduced fats contents and total calories (~24%) without damaging sensorial properties of product in comparison with 100% meat stuffed samosa (control). Additionally, oxidation of frying oil was also reduced in textured soy proteins stuffed samosa.

Table 2.5 Application of soy proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein content				
Soy protein isolates	90.07%	5–30%	Sponge Cake	SPI addition in cake batter at 20% improved nutritional quality and preserved quality of sponge cake.	Majzoobi et al. (2014)
Soy flour	38%	15.5–22.7%	Breakfast cereal	Soy-based high-protein breakfast cereal had increased nutritional contents and comparable acceptance ratings.	Yeu et al. (2008)
Soaked soybean	35.8%	1.2–3.2%	Soy milk	Soymilk displayed higher protein content and suits well as dairy alternative.	Kundu et al. (2018)
Soy protein isolates	91.5–92.2%	15%	Chiba tofu	Tofu prepared from SPI showed high hardness, springiness, and excellent quality.	Zheng et al. (2020)
Soy protein isolates	≥90%	15–25%	Sausages	Incorporation of SPI in buffalo meat improved texture, juiciness, and shelf-life of buffalo meat emulsion sausage	Ahmad et al. (2010)
Textured Soy protein concentrate	≥70%	10–40%	Nuggets (Meat extender)	Soy protein substituted nuggets had appearance and texture like meat-based nuggets.	Yeater et al. (2017)
Soy protein isolates	≥90%	4%	Coating powder	Coating powder formulated by using SPI had better water retention activity and solubility as compared to whey protein-based coating powder	Erdem and Kaya (2020)
Soy protein isolates	92%	2.5%	Encapsulating material	Encapsulation of fish oil in SPI/inulin composite film masked off-flavor and unpleasant odor of oil and showed stability against pH and thermal treatment.	Rios-Mera et al. (2019)
Soy protein isolates	90%	0.6 g	Biodegradable film	The biodegradable film prepared from SPI and poly lactic acid showed high transparency, strong adhesion, and markedly reduced water vapor permeability. Further this bilayer film loaded with natamycin, and thymol presented antifungal and antibacterial in *in-vitro* microbiological assays.	González and Igarzabal (2013)

Soy Protein Isolate

Soy protein isolate (SPI) is a highly purified form of soy protein extracted from defatted soy flour. SPI contains ~90% protein content on a dry weight basis and produces less flatulence than raw soy flour (Singh et al. 2008). SPI are mainly used in the food sector to enhance water retention, improve texture and protein contents of meat products, and as an emulsifier (Niu et al. 2017; Youssef and Barbut 2011). In a recent study, soy protein or whey protein isolates were mixed with sunflower oil using freeze drying technology to formulate coating powders (Erdem and Kaya 2020). The interaction between protein isolates and sunflower oil produced strong networks in formulated powders via hydrogen bonding. The coating powder formulated by using SPI had better water retention activity and solubility as compared to whey protein-based coating powder. It was worth noting that SPI supplemented coating powder was used in baked products and protected textural structure of sliced cake by its water retaining potential.

It has been observed that plant-based protein supplementation in meat emulsions prevent excessive tosses and deliver homogenous appearance to the product. Supporting this argument Youssef and Barbut (2011) reported that SPI supplementation (@12–14%) in meat protein batters improves emulsification by reducing meat protein aggregation and fat globules' agglomeration. Results from textural studies of SPI supplemented batters presented improved hardness and cohesiveness of the batters.

2.2.2 Pulse Proteins

Pulses are Leguminosae crops harvested solely as dry seeds. Pulses play an important role in sustainable and cost-effective supply of plant proteins. Because of their cost-effectiveness to meat and meat-based products, pulses are also claimed as "poor man's meat" in some countries (Shevkani et al. 2019). Pulses are a category of superfoods that include chickpeas (garbanzo beans), lentils (green, red, black, small, brown and French green), dry peas (split and whole) and beans (adzuki, black, kidney, pinto, fava, mung and lima beans). Pulses are nutrient dense foods characterized with high protein and fiber contents and relatively low fats contrary to the legumes like peanuts and soy. Protein contents vary considerably between different pulses based on genotypes, germination, fertilizers application and environmental stress during growth and development (Powers and Thavarajah 2019). Generally, pulses contain 20–30% protein contents concentrated as small spherical protein bodies in the seed cotyledons (Singh 2017). Pulse proteins are primarily composed of globulins (70–80%) and albumin (10–20%) while prolamin and glutelins are minor proteins accounting less than 5% (Gupta and Dhillon 1993). Pulse albumins are the most nutritious proteins in terms of amino acids profile. However, they may contain some anti-nutritional components like trypsin, hemagglutinins and amylase inhibitors which adversely affect their bioavailability (Bessada et al. 2019). The main functional proteins in pulses are globulins stored in the pulse seeds in the form of legumins and vicilins protein bodies. Based on amino acids composition of pulse proteins, legumins contain more sulfur-containing amino acids (cysteine and methionine) than vicilins (Robinson et al. 2019). The main advantage of pulse proteins over cereal proteins is that they are gluten free and rich in essential amino acids like lysine, thus, considered suitable in the diets of people living with gluten intolerance and celiac diseases (Mlyneková et al. 2014).

Pulse proteins find applications in multiple value-added and industrial products due to their low cost, wide acceptability, comparative functionality, high nutritional and nutraceutical properties (Klupšaitė and Juodeikienė 2015; Shevkani et al. 2019) (Table 2.6).

Pulse Protein Concentrates and Isolates

The purified pulse proteins as concentrates or isolates are typically used as nutritional additives and ingredients in novel and traditional food products. In addition to improving dietary protein quality, pulse proteins also contribute to improving sensorial properties of cereal-based foods. The addition of thermally modified i.e. denatured and glycated cowpea proteins in wheat flour improved water absorption during dough/batter formation and imparted soft texture to wheat bread and sponge cake. Additionally, it was also reported that replacement of whole egg with glycated cowpea protein @ 20% during batter formation did not affect sensorial acceptability of sponge cake (Campbell et al. 2016). In an attempt to develop B-saponins rich composite flour bread (Serventi et al. 2018), chickpea protein concentrate was partially substituted (one third) with soy blend for bread formulation. Incorporating chickpea in bread increased B-saponins levels as well as preserved bread loaf quality. Pulse proteins also improve textural properties of gluten-free products by the formation of viscoelastic protein networks in batters and dough systems (Shevkani et al. 2019). Protein isolates recovered from cowpeas, field peas, and kidney beans are being exploited for their possible application in gluten-free cupcakes and muffins. Kidney beans and pea protein isolates incorporation @10% level increased viscoelastic properties of corn starch-based batter and resulted in muffins with improved quality characteristics which include appearance, crust color, firmness, specific volume, cohesiveness, springiness and porosity (Shevkani and Singh 2014). The effect of chickpea protein isolates, transglutaminase and xanthan gum supplementation at various supplementation levels on quality and rheological attributes of millet muffins was evaluated by Shaabani et al. (2018) using the response surface methodology. Findings of the study suggested that textural qualities and formation of protein networks in gluten-free batter and muffins are possible to attain with the addition of chickpea proteins and transglutaminase.

Table 2.6 Application of pulse proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Broad-bean, yellow-pea and green-pea	11.5–18%	4.6–7.2%	Crackers	Pulse flour supplementation improved nutritional profile and eating quality of baked crackers.	Millar et al. (2017)
Chickpea protein concentrate (CPC)	64%	5–15%	Cereal foods (Cookies, pasta and fried corn snacks)	Quality and sensory attributes of cereal foods fortified with chickpea protein concentrate were not affected with subsequent increase in CPC content.	Yanez-Farias et al. (1999)
Pea protein isolates	86.99%	1–6%	Gluten free bread	Addition of pea protein positively influenced rheological and structural properties of gluten free dough.	Mariotti et al. (2009)
Pulse protein concentrate (chickpea, lentil and pea)	66.8%, 82.5% and 81.4%	3–9%	Bread	Wheat flour substitution with chickpea protein gave highest mass volume to bread and increased protein content.	Aider et al. (2012)
Lentil and white bean (protein extracts)	50.3% and 49.2%	3%	Sponge and pond cake	Lentil and white bean protein extracts application in baked products showed excellent foaming/emulsifying capacities, heat stability and gelling properties having potential to replace protein from animal origin.	Bildstein et al. (2008)
Chickpea, lentil, faba bean, mung bean, winged bean, pea and smooth pea (protein extracts)	~90%	2.3–3%	Bean curd (Dairy alternative)	Bean curds developed from chickpea and faba bean milk extracts had comparable quality and sensorial properties to soybean curd.	Cai et al. (2001)

(continued)

Table 2.6 (continued)

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Chickpea and lupin	19–36%	1–2.4%	Beverage (Milk alternative)	Chickpea and lupin protein addition optimized sensorial feature and provided stability to non-dairy alternative beverages	Lopes et al. (2020)
Chickpea protein concentrate	76.06%	1.5–5%	Merguez (Cooked sausages)	Chickpea protein improved process yield, protein content and color stability of sausage and also reduced cooking loss and lipid oxidation.	Ghribi et al. (2018)
Pea protein isolates	85.3%	3–12%	Meat extenders	Addition of pea protein isolates decreased cooking loss and increased water holding capacity of extended nuggets compared to control (100% chicken nuggets).	Shoaib et al. (2018)
Pea protein isolates	84.4%	0.5%	Encapsulating material	Microencapsulation of PUFA-rich oil in pea protein and pectin-based emulsion provided oxidative stability to oil.	Aberkane et al. (2014)
Chickpea protein isolates	88.1%	0.1 g	Encapsulating material	Chickpea protein showed effective loading capacity and provided stability to folate at various ranges of pH (2 to 8).	Ariyarathna and Karunaratne (2015)

Pulse Proteins Based Imitation Milk Products

Pulse proteins as concentrates or isolates are also explored in the preparation of imitation milks, beverages and bean curds. Protein isolates recovered from different pulses were used as protein source in the formulation of imitation milk and beverages (Sosulski et al. 1978). The pulses protein-based imitation milks were like as cow milk in color and viscosity but lower in taste and odor. The pulses were rated in the following increasing order of preferences for imitation milk formulation: faba bean<field pea<chickpea<lentils<lupine = northern peas<lima bean = mung bean = pea bean. Similarly, in another study by Cai et al. (2001) protein extracts from several pulses were applied in the development of bean curds. The authors reported that bean curds developed from chickpea and faba bean milk extracts (2.3–3% protein contents) using 1.5% CaSO4 as coagulant were best in terms of

quality and sensorial properties. Recently, Lopes et al. (2020) optimized sensorial features and stability of pulse beverages using different processing technologies which involved seed soaking, cooking with water, milling, sieving and beverage pasteurization. The milk beverages prepared by following these processing steps masked characteristic beany flavour and yielded protein contents 1.8–2.4% (w/v) in lupin beverage and 1.0–1.5% (w/v) in chickpea beverage. Marketing of such products target people allergic to cow and soy milk, older adults with poor appetite and difficulty in chewing and those prone to suffer from protein malnutrition.

Pulses Proteins-Based Comminuted Meat and Meat Analogs

Among various plant protein sources, pulses are famous candidates in meat products formulations as meat substitutes and binders to increase nutritional and textural properties (Pintado and Delgado-Pando 2020). In comminuted meat products, the starch, fiber and protein contents of pulses help to form complex gel networks and bind meat proteins. These protein networks further form strong bonds and entrap water or other compounds, thus facilitate to retain moisture in the meat matrix and prevent tosses during processing (Bassett et al. 2010). Additionally, the type and quantity of the pulse used, and the type of product also determine overall stability and water retention capacity of product. In this regard, Nagamallika et al. (2005) used Bengal gram and pea flour (levels of 5% and 10%) as meat substitute in patties. They reported that pea flour was more acceptable and yielded patties with higher emulsion stability and water holding capacity as compared to Bengal flour. Ghribi et al. (2018) investigated effect of chickpea protein concentrates supplementation at different levels (1.5–5% (w/w)) on the textural properties of raw and cooked sausages. Authors reported that chickpea protein not only improved process yield, protein contents and color stability of sausages but also reduced cooking loss and degree of lipid oxidation.

2.2.3 Peanut Protein

Peanut (*Arachis hypogaea* L.) is a leguminous crop of tropical and sub-tropical region that contains 16–36% high biological value protein contents. Peanuts are being accepted as functional food and extensively consumed as raw roasted, peanut butter, baked products, soup, confectionary and extender in meat analogue (Singh et al. 2021). Peanut proteins play an important role in developing various food products due to their nutritional value and for contributing special texture to food products (Shafiquer et al. 2018). Partially defatted flour of peanut is rich in protein contents and has been used in combination with other conventional flours to improve the nutritional value of the composite bread. Various other forms including peanut milk, peanut bar are also consumed in developing countries to combat protein energy malnutrition (Bansal and Kochhar 2013; Arya et al. 2016)

2.3 Nuts Protein-Based Products

Nuts are thick dried fruits and often contain hard shells that cover their edible kernel (Bewley et al. 2006). Nuts are healthier and nutrient-rich plant protein sources. Most nuts fulfil ~17% of daily protein requirements typically with 2–3 tablespoons (Freitas and Naves 2010). The best-known edible nuts include almond, walnut, Brazil nut, cashew nut, pistachio, hazelnut, chestnut and peanuts. Considerable scientific literature has elucidated consumption of nuts to hold cholesterol lowering, antioxidative, cardio-protective, anti-diabetic and anti-proliferative effects (Alasalvar et al. 2020). Nuts are mostly consumed as whole, raw or toasted; however, nuts protein based value-added products are also available in market.

2.3.1 Almond Protein

Almond is a popular tree nut consumed as a part of healthy diet due to its wide availability and health significance. Almond is also considered as a common culinary ingredient for formulating dairy desserts in many cultures and is used in many forms e.g., whole, gritted, slivered and as nibs for decorating finished goods. Almond protein powder is utilized in value addition of multiple food categories such as ready-to-drink protein shakes/smoothies, breakfast cereals, high-protein nutrition bars and food service products (de Carvalho et al. 2011; Hashemi et al. 2017). Almond protein is preferred over whey, soy, or pea protein and has significantly higher purchase intent due to its nutty flavor and extra-fine smooth texture. Almond meal-based confection "*Marzipan*" is a protein-rich original fondant traditionally has been prepared in the Middle East and Mediterranean regions for making decorative shapes. To develop *Marzipan*, almond meal is cooked with sugar or honey followed by cooling and crystallizing the mixture. In addition, a binding agent such as egg white, gelatin or starch syrup is added to improve binding and moulding properties (Romero et al. 2001; Eby 2020). Use of almond in the formulation of plant-protein based beverages and imitation milks is much popular. Almond milk is naturally cholesterol and lactose free dairy alternative for vegans and individuals suffering with lactose intolerance (Salpietro et al. 2005; Kundu et al. 2018). Commercial almond milk is usually flavored (vanilla or chocolate) and fortified with micronutrients such as calcium and vitamin D. It is reported that global market of almond milk was 5.8 billion US dollar in 2018 and estimated to reach 13 billion US dollar by 2025 (Coppola 2020).

2.3.2 Walnut Protein

Walnut is another member of nut family recognized to contain significant amounts of protein (18–24%), heart friendly polyunsaturated fatty acids (PUFA) and phytonutrients (Sze-Tao and Sathe 2000). Walnut protein concentrates and isolates are added as potential functional food ingredients in multiple food formulations such as fudge, cakes, soups, sauces and salad dressings (Mao and Hua 2012; Barber and Obinna-Echem 2016). In an attempt to develop protein-rich bread, walnut flour was supplemented in wheat flour at different levels (20–50%). Results revealed that walnut flour substitution at 30% gave best overall quality attributes to bread and enhanced ~46% protein content as compared to control (Almoraie 2019). Recently, a walnut protein based-edible coating was prepared by Grosso et al. (2020) and applied on the surface of walnut kernel to increase shelf life. On day 84, kernels covered in walnut protein based-coating preserved walnut flavor, inhibited genesis of oxidized and cardboard flavors, improved γ-tocopherol (306.78 mg/kg) and carotenoid contents (2.01 mg/kg) and prevented PUFA deterioration. Furthermore, walnut protein-based coating was preferred by consumers over methylcellulose coating and could be used as a natural alternative to prolong the shelf life of nuts.

2.3.3 Pistachio Protein

Pistachio is a nutrient dense food cultivated in Mediterranean, central and southwest Asia. They are good source of vegetable protein constituting 20% essential amino acids which exert antiplatelet and antioxidative effects (Terzo et al. 2019). A study by Shakerardekani et al. (2013) reported that pistachio nut could be a good source of plant proteins to develop dairy alternative imitation milk. The best processing conditions to develop pistachio milk include milling of roasted kernels and blending the pistachio slurry at pH 8.5 for 30 min. Moreover, sensorial properties of pistachio milk could be enhanced by the addition of 5.0% sugar and 0.02% vanilla flavor.

2.4 Edible Seeds Protein-Based Products

Inclusion of edible seeds in the diet is a worldwide growing demand of consumers for the consumption of plant-based healthy ingredients (Sá et al. 2020). Flaxseed is amongst richest sources of high-quality protein, soluble fiber and phytonutrients. Apart from lysine, flaxseed proteins deliver good concentration of essential amino acids mandated for human nutrition requirements (Panaite et al. 2017). Giacomino et al. (2013) suggested that extrusion of flaxseed meal increases its nutritional quality without disturbing stability of amino acids. These authors incorporated extruded flaxseed meal in flour mixes and cereal-based bars and recorded significant increase in protein levels (20% and 17%, respectively) with improved protein digestibility and biological value. Similarly, in another study by Hussain et al. (2012) flaxseed flour (raw and defatted) supplementation in unleavened flat breads improved levels of essential amino acids and total dietary fiber.

Pumpkin seed is another rich source of protein (24.5–36.0%) used for the enrichment of multiple products such as cereal bars, breads and cookies (Costa et al. 2018). Quanhong and Caili (2005) proposed various extraction procedures of protein from germinated pumpkin seeds. Pumpkin protein based edible films are reported to have strong mechanical and barrier properties thus have potential to be utilized for packaging purpose (Lalnunthari et al. 2019).

Oil seed crops such as rapeseed and sunflower seeds are considered enriched sources of plant proteins. The extracted fractions of rapeseed and sunflower seed proteins have been incorporated in many baked goods to improve nutritional profile of finished products (González-Pérez and Vereijken 2007; Tan et al. 2011).

Rapeseed (*Brassicaceae* family) a herbaceous annual plant cultivated as oil seed crop contains approximately 17–26% of protein (Li et al. 2012; Lim 2012). The protein isolates of rapeseed contain at least 90% protein and may be considered as good alternatives to other plant proteins resources. On account of availability of a balanced amount of amino acids, rapeseed proteins can also be used in formulating gluten free bakery products, biscuits and sausage like preparations which may serve functional and therapeutic properties to the consumers (Wanasundara et al. 2016; Ostrowska et al. 2018). Sunflower seeds are used for oil purpose but the dehulled sunflower seeds contain higher amount of protein (20–40%) as well. Sunflower protein application has been suggested to fortify variety of edible goods including infants formulas, milk, meat and bakery products (González-Pérez and Vereijken 2007) (Table 2.7).

3 Conclusion

The findings of the recent research advocate plant proteins as cost-effective, nutritionally enriched and environment friendly sources of protein to meet the global protein supply needs. The utilization of plants-derived proteins in novel product formulations might serve as a desirable vehicle to fulfil the dietary demands of globally escalating population. The various food product with variable protein concentrations impart desired qualitative features and attracts the consumers depending on their desired eating preferences and possess specific nutritional, functional and therapeutic properties. Conclusively, it has been declared that food products incorporated with plant protein concentrates are good alternatives in provision of health benefits for the people who are allergic and for those who can't afford the high cost of protein from animal resources. Therefore, these resources have been declared as poor man's meat. Incorporation of protein concentrates in the development of various products may enhance the utility of plant based protein products and would be of human health significance in maintaining their regular essential body requirements.

Table 2.7 Application of nuts and seeds proteins in food products

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Soaked almond	21.15%	1.38%	Almond milk	Almond milk (60%) substitution with soymilk masked beany note and recorded highest sensory scores in terms of overall acceptability.	Kundu et al. (2018)
Almond paste	26–50%	26–50%	*Marzipan* (Confectionary)	An original fondant glazed on multiple sweet products (chocolates and icing cake) for decorative purpose and shaped into small imitations of fruits and vegetables.	Sinclair (2011)
African walnut flour	26.3%	3%	Cookies	The level of likeliness and sensorial attributes of cookies were acceptable at 5–15% walnut flour substitution.	Barber and Obinna-Echem (2016)
Walnut flour	49.36%	2.96%	Edible coating	Walnut kernels covered in walnut protein based coating preserved walnut flavor, inhibited genesis of oxidized and cardboard flavors, improved nutritional profile and prevented PUFA deterioration.	Grosso et al. (2020)
Brazil nut	14%	3.31%	Symbiotic drink	Brazil nut drink proved to be viable dairy alternative with outstanding nutritional quality.	Cunha Júnior et al. (2021)
Roasted pistachio nuts paste	3–4%	3–4%	Pistachio milk	Sensorial properties of pistachio milk were liked by panelist in terms of overall acceptability.	Shakerardekani et al. (2013)

(continued)

Table 2.7 (continued)

Raw material		Percentage added in the food preparation	Product/ enriched product	Remarks	Reference
Protein Source	Protein Content				
Flaxseed meal	26.35%	7.12%	Cereal-based products	Extruded flaxseed meal incorporation in flour mixes and cereal-based bars significantly increased protein contents and improved protein digestibility.	Giacomino et al. (2013)
Fluted pumpkin seed flour	28.88%	2.8–14.4%	Bread	Fluted pumpkin flour showed acceptability at 20% substitution level in wheat flour for bread making and gave desired loaf volume quality.	Agu et al. (2010)
Watermelon seed protein concentrates	72.26%	2.5–10%	Cookies	Watermelon seed protein addition increased dough stability, mixing tolerance index and cookies spread facto at 5–7.5% substitution level and produced cookies with improved protein quality.	Wani et al. (2015)

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Chapter 3
Processing Technologies to Produce Plant Protein Concentrates and Isolates

Martin Mondor and Alan Javier Hernández-Álvarez

1 Introduction

The demand for new healthy food ingredients and products is on the rise. The global plant-based protein market is projected to grow from US$10.3 billion in 2020 to US$14.5 billion by 2025, recording a compound annual growth rate of 7.1% during the forecast period (Markets and Markets n.d.). Increasing consumer awareness of the health benefits associated with the consumption of plant-based protein products continues to open up new marketing opportunities for the food industry. Plant protein ingredients, namely concentrate (65–90% w/w protein dry basis) and isolate (90%+ w/w protein dry basis), are increasingly finding their way into a broad range of food products, not only due to their nutritional value but also given that such ingredients interact well with other food ingredients (Lam et al. 2018; Tiwari and Singh 2012). However, their functional properties differ depending on the protein source and on the way the ingredients are processed. Key functional properties include solubility, water- and fat-adsorption capacities, emulsifying properties, foam-forming capacity and stability, and gelling properties (Lam et al. 2018; Stone et al. 2015a; Toews and Wang 2013). The main plant protein sources are oilseeds (soybean, canola, flax, etc.), pulses (pea, chickpea, bean, lentil, etc.), and cereals (wheat, corn, barley, etc.). Oilseeds are characterized by the high oil content of their seeds (10–50%), from which the oil is usually extracted for food, energy, or

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industrial use through a process called trituration during which the seeds are crushed and pressed, leaving a meal by-product, which can be used as animal feed or valorized for its proteins. Pulses constitute the dried seeds of non-oleaginous legume crops. They are characterized by their low oil content (<5%) and a crude protein content typically ranging between 21% and 26% by weight. Cereals are a rich source of carbohydrates (70–80%), which are mainly present in the endosperm and are consumed as a good energy source. Cereal protein content (7–15%) is lower than for pulses. However, the majority of cereal proteins can be found in the bran and germ. Thus, it is possible to separate the bran and the germ from the endosperm and to process them to isolate and purify the proteins. Soybean, wheat, and corn are the main commercial plant protein sources (Sari et al. 2015). However, new alternative sources are finding their way into the market, including barley, bean, camelina, canola, chickpea, flax, hemp, lentil, mustard, peanut, pea, quinoa, rice, sesame, sorghum, and sunflower (Ozbek and Bilek 2018). Plant proteins can be extracted directly from the oilseeds, pulses and cereals or from their by-products. Several plant proteins are derived from industrial waste materials (Aiking 2011). The redirection of by-products, which are usually used as animal feed livestock, to human consumption helps to preserve the environment, ensure food security and support the sustainability of food systems (Pojić et al. 2018). Many technologies are involved in the production of plant protein concentrate and isolate, including milling to obtain flour, and drying to convert the protein extracts into a powder when wet extraction is carried out. However, the core of the process is protein extraction and separation. Protein extraction and separation processes can be classified into two categories: dry fractionation and wet extraction processes. Wet extraction processes are the most common methods used to produce plant protein ingredients and include conventional processes such as alkaline extraction–isoelectric precipitation (AE-IP), salt extraction–dialysis (SED), and micellar precipitation (MP) (Lam et al. 2018). They also include emerging processes such as enzyme-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, and membrane technologies (Ozbek and Bilek 2018). The selection of the most appropriate plant protein extraction/separation process depends on many factors, such as the composition of the oilseeds, pulses, and cereals (fiber-rich content, polysaccharides, and fat), the part that is used, the targeted level of proteins in the ingredients, and so on (Ozbek and Bilek 2018). This chapter provides a review of the processing technologies used to produce plant protein concentrate and isolate, and discusses their impact on the main plant protein sources.

2 Processing for Protein Extraction and Separation

2.1 Milling

Plant proteins can be extracted directly from the oilseeds, pulses and cereals or from their by-products. When the proteins are extracted from the oilseeds, pulses or cereals, the starting material is usually a flour. Flour production involves grinding the oilseeds, pulses or cereals into small particles. The main milling methods that are applied for the production of flour include those using hammer, pin, roller, and stone mills. A hammer mill consists of a steel drum containing a rotating shaft or drum on which hammers are mounted. The oilseeds, pulses or cereals are fed into a feed hopper while the rotor is spun at high speed inside the drum. Being exposed to the hammers, they are fractured into small particles that are expelled through screens in the drum of a selected size. The basic principle of the pin mill is similar to that of the hammer mill since the fracturing is performed by impact and shearing. However, it is characterized by a faster tip speed rotor-stator configuration. In practice, it consists of two horizontal steel plates with vertical projections arranged in concentric circles on opposing faces and becomes more closely spaced towards the periphery. Instead of hammers, a series of pin breakers are attached to discs to fracture the oilseeds, pulses or cereals. In contrast, roller mills and stone mills fracture the oilseeds, pulses or cereals by compressing them between two hardened surfaces (Maskus et al. 2016). Roller mills use a single, double, or triple cylindrical heavy wheel mounted horizontally and rotated about their long axis either in opposing pairs or against flat plates, to perform the fracturing. The particle size of the fractured oilseeds, pulses or cereals is a function of the feed rate, as well as the gap and speed differential between the wheels. A stone mill consists of a bottom stone, which is stationary. Above the stationary stone, another rotating stone fractures the oilseeds, pulses or cereals that are fed to the mill. The milled material can then be separated with different sieves to obtain fractions of various particle sizes.

2.2 *Dry Fractionation*

2.2.1 Air Classification

In the air classification process, a finely milled flour is split into two size fractions (fraction enriched with large starch granules vs. fraction enriched with small proteins) using air flow to modify the particle size distribution (Rempel et al. 2019). An overview of air classification/sieving processes for the isolation of plant proteins from various sources is presented in Table 3.1. The air classification process is characterized by a cut diameter, which is the diameter of the particle that has an equal chance to end up in either the fine or the coarse fraction (Pelgrom et al. 2013). Operating parameters that impact the cut diameter are the classifier wheel speed, the air inlet flow rate, and the flour feeding rate. Air classification is an interesting alternative to wet fractionation since it does not result in protein denaturation or loss of insoluble proteins, water/chemicals are not used, and it requires less energy (Pelgrom et al. 2013; Schutyser et al. 2015; Rempel et al. 2019). The main limitation of air classification is that it results in a relatively low level of protein enrichment (Schutyser et al. 2015). Consequently, it is very difficult to obtain a protein concentrate (65–90% w/w protein dry basis) using air classification. However, the protein-enriched fraction can be subsequently used as starting material for wet extraction processes.

Table 3.1 Overview of air classification/sieving processes for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of protein-enriched fraction	Protein-enriched fraction yield / protein yield	Remarks	Reference
Canola (partly defatted)	Classifier wheel speeds: 9000, 6000 and 4000 rpm	32.5%	9000 rpm fraction: 37.6% 6000 rpm fraction: 37.8% 4000 rpm fraction: 34.7%	PEFY 9000 rpm fraction: 27.2% PEFY 6000 rpm fraction: 12.1% PEFY 4000 rpm fraction: 15.2%	The coarse fraction from the classification at 9000 rpm was classified again at 6000 rpm. The coarse fraction from the 6000 rpm was further classified at 4000 rpm.	Hansen et al. (2017)
Faba bean	Classifier wheel speed: 5800 rpm	n.a.	64.1% d.w.	n.a.	Faba bean protein-enriched fraction showed higher protein solubility (85%) when compared to Faba bean protein isolate (32%) at pH 7.	Vogelsang-O'Dwyer et al. (2020)
Pea	Classifier wheel speeds: 5000, 6000, 8000, 10,000 and 12,000 rpm Air flow: 52 m^3/h Screw feeder speed: 1 kg/h	23%	51–55% d.w.	PY: Up to 77%	Protein yield decreased with an increase in the classifier speed.	Pelgrom et al. (2013)
Pea	Classifier wheel speed: 2700 rpm	16–21%	42–50% (first fine fraction)	PY (total of the 7 fine fractions): 84.7–87.3%	Air classification was repeated on the resulting coarse fractions 7 times.	Rempel et al. (2019)

(continued)

Table 3.1 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of protein-enriched fraction	Protein-enriched fraction yield / protein yield	Remarks	Reference
Quinoa–Atlas	Air jet sieving with different sieves (0.800, 0.630 and 0.315 mm) at 1500 Pa for 2.5 min	15.6% d.w.	Fraction >0.800 mm: 6.1% Fraction 0.800–0.630 mm: 7.3% Fraction 0.630–0.315 mm: 32.7% Fraction <0.315 mm: 21.0%	PEFY Fraction >0.800 mm: 50.5% PEFY Fraction 0.800–0.630 mm: 7.4% PEFY Fraction 0.630–0.315 mm: 27.2% PEFY Fraction <0.315 mm: 14.4%	The unheated protein-enriched fractions showed high water retention capacity and solubility.	Opazo-Navarrete et al. (2018)
Quinoa–Riobamba	Air jet sieving with different sieves (0.800, 0.630 and 0.315 mm) at 1500 Pa for 2.5 min	14.1% d.w.	Fraction >0.800 mm: 4.9% Fraction 0.800–0.630 mm: 10.5% Fraction 0.630–0.315 mm: 32.0% Fraction <0.315 mm: 12.2%	PEFY Fraction >0.800 mm: 51.6% PEFY Fraction 0.800–0.630 mm: 6.6% PEFY Fraction 0.630–0.315 mm: 29.6% PEFY Fraction <0.315 mm: 11.0%	The unheated protein-enriched fractions showed high water retention capacity and solubility.	Opazo-Navarrete et al. (2018)
Rice bran	Classifier wheel speeds: 21000 rpm Air flow: 50 m^3/h Screw feeder speed: 0.5 kg/h	18.5% d.w.	25.7% d.w.	PEFY: 27.2%	When compared to the raw rice bran, the protein-enriched fraction showed improved protein solubility and colloidal stability.	Silventoinen et al. (2019)

(continued)

Table 3.1 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of protein-enriched fraction	Protein-enriched fraction yield / protein yield	Remarks	Reference
Sunflower	Air flow: 5, 8.7 and 12.5 m^3/h	35.99% d.w.	33.36–50.90% d.w.	n.a.	The decrease in sieve opening diameter of the hammer mill sieve increased protein content in coarse fractions of sunflower meal obtained with the same air flow, and at the same time decreased matching fraction yield. An increase in air flow led to an increase in protein content along the same hammer mill sieve.	Banjac et al. (2017)

Abbreviations: *d.w.* dry weight basis, *n.a.* not available, *PEFY* Protein-enriched fraction yield, *PY* protein yield

2.2.2 Electrostatic Separation

As is the case with air classification, the starting material for electrostatic separation is a finely milled flour. However, for electrostatic separation, the separation is based on the charges of the different fractions. Electrostatic separation results in protein enrichment due to the removal of lignin from the protein fraction. In practice, the flour is conveyed by compressed air into a charging line, and it is charged by tribo-electric effects due to the impact of particles on each other and with the charging walls. The separation chamber consists of a chamber with two high-voltage electrodes (10,000 V) with one of the electrodes being positively charged and the other electrode being negatively charged. When the charged particles are introduced into the separation chamber, the positively charged particles are attracted to the negative electrode and the negatively charged particles to the positive electrode. The two fractions are then recovered with a system equipped with two cyclones. Efficiency of the electrostatic separation for protein enrichment will depend on parameters such as the milling mode, electrode voltage, charge, and particle size (Kdidi et al. 2019). Advantages and disadvantages of the electrostatic separation are the same as for air classification. This process has been applied to fractionate bean (Tabtabaei et al. 2019), canola (Laguna et al. 2018; Kdidi et al. 2019), soybean (Xing et al. 2018), sunflower (Laguna et al. 2018) and wheat fractions (Hemery et al. 2011).

Xing et al. (2018) applied an electrostatic separation process to prepare protein-enriched fractions from defatted soy flour. A protein enrichment of 15% was achieved while 62% of the protein, from the defatted soy flour, was recovered. In another work, Laguna et al. (2018) processed canola and sunflower meals using an electrostatic separation. Electrostatic separation increased the protein content by 50–55%, while the overall recovery yield of the most enriched fractions was varying between 31–32%. In another electrostatic separation work aiming the production of protein-enriched fractions from canola meal Kdidi et al. (2019) compared the impact of the milling process on the efficiency of the separation. They concluded that impact grinding was the most suitable milling process since it resulted in a significantly higher protein yield (74.20%) than for ball milling (66.82%) and jet milling (55.01%). Tabtabaei et al. (2019) applied an electrostatic separation process to fractionate navy bean flour. They obtained protein-enriched fractions with 36–38% protein (dry basis) compared to 25.4% protein (dry basis) in the starting flour. Approximately 43% of the total available protein from the flour was found in the protein-enriched fractions. Functional properties of these fractions were compared to the ones of a navy bean protein isolate obtained by isoelectric precipitation. Results indicated that the electrostatically separated protein fractions exhibited superior solubility, emulsion stability, foam expansion and foam volume stability than the isolate produced by isoelectric precipitation. The aforementioned results suggest that electrostatic separation is a promising process to prepare functional protein concentrates that could be used for the formulation of food products enriched in proteins.

2.3 Wet Extraction Processes

2.3.1 Alkaline Extraction–Isoelectric Precipitation (AE-IP)

The most widely used process for the production of plant protein concentrate and isolate is the AE-IP process (Fig. 3.1). The first step consists of the extraction of proteins at an alkaline pH (usually between 9 and 11) under agitation for 30 to 180 min. Extraction at pH values above 11 is not recommended since it may result in the formation of toxic compounds such as lysinoalanine (Fabian and Ju 2011) and will also increase starch swelling, resulting in starch contamination in the concentrate or isolate. The starting material can be flakes, flour, protein-enriched fraction obtained by dry fractionation, or a by-product containing the proteins of interest. Usually, defatted material is used since protein–lipid interactions limit protein solubility and reduce the yield of the extraction step. The mass ratio between the starting material containing the proteins and the extraction solvent varies between 1/5 and 1/20, while the temperature is between 50 °C and 60 °C. A higher temperature could

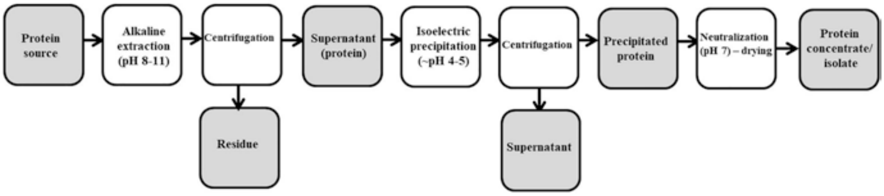

Fig. 3.1 Alkaline extraction–isoelectric precipitation

result in a higher extraction yield, but would at the same time denature the proteins and negatively impact their functional properties. The extraction step is followed by a centrifugation step to remove the insoluble residue, which makes it possible to recover the supernatant containing the proteins. This step is followed by isoelectric precipitation of the proteins by adjusting the pH of the supernatant to the isoelectric point of the proteins by adding a strong acid, such as HCl or H_2SO_4. The isoelectric point of the proteins varies as a function of the protein type but is around 4.5 for most plant proteins. The precipitated proteins are then recuperated by centrifugation and they are resolubilized in water at pH 7 before being dried to obtain a protein concentrate or isolate (Lam et al. 2018). The main advantage of this process is its high productivity and its easy scalability. On the other hand, the use of harsh chemicals for both the extraction and the isoelectric precipitation steps may have a negative impact on the functional properties of the resulting ingredients and makes this process not so friendly from an environmental point of view.

Over the past 30 years, this process has been applied to extract and separate barley (Bilgi and Celik 2004; Houde et al. 2018; Liu and Barrows 2017; Mohamed et al. 2007; Wang et al. 2010), bean (Adebowale et al. 2007; Arogundade et al. 2009; Du et al. 2018; Hernández-Álvarez et al. 2013; Langton et al. 2020; McCurdy and Knipfel 1990; Mune Mune and Singh Sogi 2015; Mune Mune et al. 2016; Otegui et al. 1997; Piñuel et al. 2019; Rui et al. 2011; Tan et al. 2014; Vogelsang-O'Dwyer et al. 2020; Wang et al. 2011; Yin et al. 2010), camelina (Boyle et al. 2018; Sarv et al. 2017), canola (Aider and Barbana 2011; Dong et al. 2011; Fetzer et al. 2019; Karaca et al. 2011b; Östbring et al. 2020; Rodrigues et al. 2017; Tan et al. 2011; Von Der Haar et al. 2014; Wanasundara et al. 2017; Xu and Diosady 2002), chickpea (Boye et al. 2010; Mondor et al. 2009; Papalamprou et al. 2010; Sanchez-Vioque et al. 1999), corn (Kongo-Dia-Moukala and Zhang 2011), flax (Kaushik et al. 2016; Krause et al. 2002; Lan et al. 2020; Loginov et al. 2013; Marambe and Wanasundara 2017; Martinez-Flores et al. 2006; Tirgar et al. 2017; Wanasundara and Shahidi 1996), hemp (Dapčević-Hadnađev et al. 2019; Malomo et al. 2014; Tang et al. 2006; Teh et al. 2014; Wang et al. 2008a; Yin et al. 2010), lentil (Barbana and Boye 2013; Boye et al. 2010; Jarpa-Parra et al. 2014; Karaca et al. 2011a), mustard (Sarker et al. 2015; Talati et al. 2004), pea (Boye et al. 2010; Gao et al. 2020; Pazmino et al. 2018; Soetrisno and Holmes 1992; Taherian et al. 2011; Tömösközi et al. 2001; Zeidanloo et al. 2019), peanuts (Liu et al. 2011), quinoa (Dakhili et al. 2019; Elsohaimy et al. 2015; Föste et al. 2015; Steffolani et al. 2016), rice (Agboola et al. 2005; Amagliani et al. 2017; de Souza et al. 2016; Fabian and Ju 2011; Hou et al. 2017; Kumagai et al. 2006; Paraman et al. 2008; Piotrowicz and Salas-Mellado 2017), sesame (Fasuan et al. 2018), sorghum (Bean et al. 2006; de Mesa-Stonestreet et al. 2010; Pontieri et al. 2019), soybean (Brasil et al. 2015; de Moura et al. 2011; L'Hocine et al. 2006; Luthria et al. 2018; Rosenthal et al. 1998; Russin et al. 2007), and sunflower proteins (Lovatto et al. 2017; Ordonez et al. 2001; Salgado et al. 2011; Shchekoldina and Aider 2014).

The protein content of protein concentrates/isolates prepared by isoelectric precipitation vary depending on the starting material, and as a function of the experimental conditions. However, under optimal conditions the protein content of the resulting ingredients is usually between 75% and 95% (Adebowale et al. 2007; Boye et al. 2010, 2018; de Souza et al. 2016; Kaushik et al. 2016; Malomo et al. 2014; Sarker et al. 2015; Steffolani et al. 2016), while values ranging between 60% and 95% are most of the time reported for the protein recovery yield (Du et al. 2018; Lam et al. 2018; Liu and Barrows 2017; Sanchez-Vioque et al. 1999). The experimental parameters which impact mostly the protein content and the protein recovery yield are pH of extraction and the starting material to water ratio (Lam et al. 2018). Concerning the functional properties of protein concentrates/isolates prepared by isoelectric precipitation, they are also greatly affected by experimental parameters including the pH of extraction. In general, when the pH of extraction is lower than 10, the functional properties of protein concentrates/isolates prepared by isoelectric precipitation are acceptable (Boye et al. 2010). However, increasing the extraction pH to 10 or higher can denature the proteins which generally has a negative impact on the functional properties of the protein concentrates/isolates (Jarpa-Parra et al. 2014). In general, protein concentrates/isolates prepared by isoelectric precipitation have similar or less desirable functional properties than protein concentrates/isolates prepared by applying alternative processes such as ultrafiltration and salt extraction (Boye et al. 2010; Taherian et al. 2011; Tan et al. 2011; Papalamprou et al. 2010; Zeidanloo et al. 2019).

The isoelectric precipitation process can also have an impact on the level of antinutritional factors in the resulting ingredients. Despite the fact that the literature on the topic is scarce, a few works have been carried out (Barbana and Boye 2013; Mondor et al. 2009; Otegui et al. 1997; Talati et al. 2004). In general, the level of antinutritional factors found in protein concentrates/isolates prepared by isoelectric precipitation is similar to or lower than the one found in the raw material. For example, Otegui et al. (1997) reported a trypsin inhibitor activity of 4.51 TIA, a tannins content of 0.59 mg catechin eq/g protein, and a phytate content of 29.83 mg/g protein, in protein concentrate of Faba bean prepared by isoelectric precipitation compared to 4.62 TIA, 6.60 mg catechin eq/g protein, and 54.80 mg/g protein, in Faba bean seeds, respectively. In another work, Talati et al. (2004) observed that glucosinolates were completely removed by the isoelectric precipitation process whereas phytic acid was reduced by 86.3% compared to the levels in the mustard seed meal. Mondor et al. (2009) observed that the production of chickpea protein concentrates by isoelectric precipitation resulted in a decrease of the total phenolic content in the concentrate when compared to the flours, while the trypsin inhibitor content was barely affected. Finally, Barbana and Boye (2013) reported trypsin inhibitor activity ranging between 0.94 and 1.94 TIU/mg for the flours, but only between 0.17 and 0.66 TIU/mg for the corresponding protein concentrate produced by isoelectric precipitation.

2.3.2 Membrane Technologies

The basic principle of ultrafiltration is illustrated in Fig. 3.2. Separation is based on the difference between particle size and membrane pore size. The molecular weight cut-off of the membrane is selected to retain the proteins in the retentate, while allowing water and low molecular weight solutes to pass through the membrane in the permeate. A pressure gradient between both sides of the membrane is the driving force for the separation. In the diafiltration mode, water is added at the same rate as the permeate is removed, which gradually dilutes the retentate and helps in removing the low molecular weight solutes. The ultrafiltration and/or diafiltration step can replace the isoelectric precipitation step in the conventional AE-IP process.

Over the past 30 years, this variant has been applied to concentrate and purify canola (Dong et al. 2011; Fetzer et al. 2019; Ghodslavali et al. 2005; Tan et al. 2011; Von Der Haar et al. 2014; Xu and Diosady 1994), chickpea (Boye et al. 2010; Mondor et al. 2009; Papalamprou et al. 2010), corn (Hojilla-Evangelista 2002; Espinosa-Pardo et al. 2020), flax (Loginov et al. 2013), lentil (Boye et al. 2010), mustard (Dendukuri and Diosady 2003; Marnoch and Diosady 2006; Tabtabaei et al. 2017), pea (Boye et al. 2010; Taherian et al. 2011; Zeidanloo et al. 2019), quinoa (Navarro-Lisboa et al. 2017), rice (Paraman et al. 2008), soybean (Ali et al. 2010, 2011; Alibhai et al. 2006; Batt et al. 2003; de Moura et al. 2011; Hojilla-Evangelista et al. 2004; Kumar et al. 2003; Mondor et al. 2004, 2010; Noordman et al. 2003; Rao et al. 2002; Skorepova and Moresoli 2007; Vishwanathan et al. 2011; Zhang et al. 2019a), and sunflower proteins (Albe Slabi et al. 2020; Gonzalez-Perez et al. 2002).

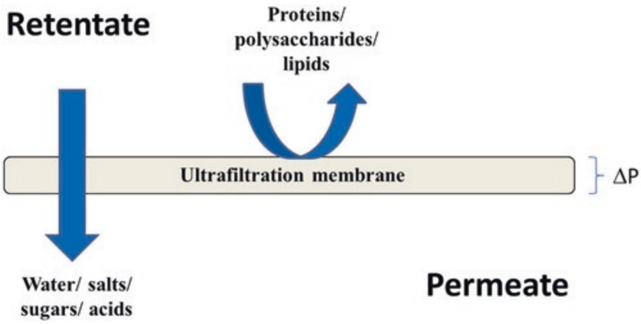

Fig. 3.2 Ultrafiltration process

The advantage of replacing the isoelectric precipitation step by a membrane filtration step is that fewer chemicals are used, which minimizes the denaturation of the proteins and may result in ingredients with better functional properties (Boye et al. 2010; Taherian et al. 2011; Tan et al. 2011; Zeidanloo et al. 2019). For example, Boye et al. (2010) observed that the solubility and the gelling properties of pea, chickpea and lentil protein concentrates prepared by ultrafiltration were generally superior to the concentrates prepared by isoelectric precipitation. Taherian et al. (2011) reported similar observations when the solubility and the gelling properties of a pea protein isolate prepared by ultrafiltration were compared with the ones of a commercial pea protein isolate. In another work, Zeidanloo et al. (2019) reported that the solubility, water binding capacity and the oil binding capacity of grass pea protein concentrates prepared by ultrafiltration/diafiltration were superior to the concentrates prepared by salt extraction and by isoelectric precipitation.

In addition, replacing the isoelectric precipitation step by a membrane filtration step also results in a global process that is more environmentally friendly, allowing the recovery of the whey-like proteins that otherwise cannot be precipitated (Ali et al. 2010). Under the right conditions, the membrane process may also remove a significant percentage of the antinutritional factors that are present in the extract (Mondor et al. 2009; Taherian et al. 2011; Xu and Diosady 2002). Mondor et al. (2009) reported that a combination of ultrafiltration and diafiltration resulted in a reduction of the trypsin inhibitor content in protein isolates prepared from Kabuli defatted chickpea flour, when compared to the isolates prepared by isoelectric precipitation. However, this was not the case when full fat Kabuli chickpea flour or Desi chickpea flours were used as the starting material suggesting that this may have a significant impact on the trypsin inhibitor content of the isolates and impact the ability of the membrane process to remove trypsin inhibitors. Taherian et al. (2011) reported that a pea protein isolate prepared by a combination of ultrafiltration and diafiltration had a phosphorus from phytic acid content of only 2.45 mg phytic acid-P/g protein compared to 7.71 mg phytic acid-P/g protein for a commercial pea protein isolate. Phytic acid is considered as an antinutritional factors since it may reduce the protein digestibility. In another work, Xu and Diosady (2002) applied a diafiltration step prior to isoelectric precipitation of canola proteins to decrease the phenolic acids and condensed tannins from the alkaline extract by 17–22% and 24–32%, respectively. All these studies suggest that the membrane process can be of interest when the objective is to prepare a protein concentrate/isolate with a low level of antinutritional factors.

However, the membrane process is less productive than its isoelectric counterpart due to membrane fouling, which is responsible for flux decline as a function of time (Navarro-Lisboa et al. 2017; Skorepova and Moresoli 2007). Navarro-Lisboa et al. (2017) used a 300 kDa ultrafiltration membrane to produce quinoa protein concentrate. The membrane treatment was carried out at pH 7 or pH 9.5, and a volume concentration factor of 3 was applied. For the experiment at pH 7, they observed a permeate flux decline from an initial value of 83 $L/m^2{\cdot}h$ to 42 $L/m^2{\cdot}h$ after 600 min, which represents a decrease of approximately 50%. The flux at pH 9.5 was higher than at 7 but a significant decline was still observed from 183 $L/m^2 \cdot h$ to 118 $L/m^2 \cdot h$ within 300 min. In another work, Skorepova and Moresoli (2007) used a 100 kDa hollow fiber membrane to produce soy protein isolate. The ultrafiltration was performed at pH 6 or 9, and a volume concentration ratio of 4.5 was applied. Again, a significant permeate flux decline was observed at both pH's. For the experiment at pH 6, the final flux was only 6 $L/m^2 \cdot h$ while it was around 12 $L/m^2 \cdot h$ for pH 9. These studies clearly demonstrated the great interest and potential of using membrane technologies for the production of plant protein concentrates/isolates. However, there are still problems related to membrane fouling, slowing down the growth of large-scale industrial use.

2.3.3 Salt Extraction–Dialysis (SED)

In the SED process, the plant proteins are first extracted in a salt solution, followed by a centrifugation step to remove the insoluble matter, and by dialysis to remove the salt before drying. An overview of the salt extraction-dialysis process for the isolation of plant proteins from various sources is presented in Table 3.2. This process generally results in an ingredient containing both globulins and albumins. The type of salt and its concentration are selected according to the salting-in characteristics of the protein to be isolated as well as the salting-out characteristics of any unwanted proteins (Lam et al. 2018). An advantage of the SED process is that mild extraction conditions (pH and temperature) can be applied. On the other hand, adverse interactions between the salt and the sample components can be considered one drawback of this process (Lam et al. 2018).

2.3.4 Micellar Precipitation (MP)

The first steps of the MP process are the same as for the SED process. These consist of the extraction of the plant proteins in a salt solution, followed by the removal of the insoluble residue (Krause et al. 2002; Stone et al. 2015b). However, the proteins are recovered by adding cold water (or a buffer solution of known pH and salt concentration) at a ratio of high-salt protein extract to water of 1:3 to 1:10 (v/v), resulting in the formation of micelles, which will be later on precipitated. The diluted solution may be left to stand for a duration to maximize micelle formation. It is then centrifuged to recover the precipitated micelles before being dried. The resulting ingredient, which has a micelle-like form and is stabilized by hydrogen bonds, contains both globulins and albumins. An overview of the micellar precipitation process for the isolation of plant proteins from various sources is presented in Table 3.3. The efficiency of micellar precipitation is highly affected by the electrostatic interactions between the micelles and proteins, the molar ratio of water to protein extract, pH, and ionic strength (Dapcevic-Hadnadev et al. 2019). Advantages of the MP process include the possible recovery of the solvent for repeated uses, which lowers costs. Also, since the protein is inside the micelle structure, it is protected from denaturation (Dapčević-Hadnađev et al. 2019). One disadvantage of this process is

Table 3.2 Overview of salt extraction-dialysis process for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Black bean meal (defatted)	10% w/v; 0.5 M aqueous NaCl; pH 6.13; 45 °C; 3 h. Residue went through a second extraction under the same conditions. Combined supernatants were partially freeze-dried and then dialyzed against deionized water using a 3.5 kDa membrane.	25.0% d.w.	81.8% d.w.	64.8%	Protein concentrate showed solubility varying between 84–95% at pH 2, 7, and 10.	Hojilla-Evangelista et al. (2018)
Camelina meal (defatted)	5% w/v; 0.05 M Potassium phosphate buffer with 1 M NaCl; pH 8; 50 °C; 1 h. Ammonium sulfate was added to supernatant to reach 85% saturation. Followed by dialysis of precipitated protein in DDW and freeze-drying.	29.2%	79.5–82.2%	35.1–42.4%	Concentrate produced by salt extraction had a higher solubility (~70%) at pH 3.4 than that (~50%) of soy protein isolate. It also had higher emulsification capacity and foaming capacity.	Boyle et al. (2018)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Canola meal (defatted)	10% w/v; 0.05 M Tris–HCl buffer with 0.1 M NaCl; pH 7; room temperature; 2 h. Dialysis of supernatant against water using 6–8 kDa membrane. Precipitated salt soluble proteins were freeze-dried.	31.93%	93.10%	n.a.	Isolate produced by salt extraction showed higher solubility and interfacial activity compared to those produced by isoelectric precipitation.	Karaca et al. (2011b)
Chickpea	10% w/v; 5% potassium sulphate aqueous solution; pH 7; room temperature; 1 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	16.71%	81.63%	n.a.	When compared to the isolates obtained by isoelectric precipitation, those produced by salt extraction resulted in isolates with lower surface charge and solubility.	Karaca et al. (2011a)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Dark red kidney bean meal (defatted)	10% w/v; 0.5 M aqueous NaCl; pH 6.13; 45 °C; 3 h Residue went through a second extraction under the same conditions. Combined supernatants were partially freeze-dried and then dialyzed against deionized water using a 3.5 kDa membrane.	26.6% d.w.	79.1% d.w.	69.2%	Protein concentrate showed solubility varying between 70–85% at pH 2, 7, and 10.	Hojilla-Evangelista et al. (2018)
Faba bean	10% w/v; 5% potassium sulphate aqueous solution; pH 7; room temperature; 1 h Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	23.94%	81.98%	n.a.	When compared to the isolates obtained by isoelectric precipitation, those produced by salt extraction resulted in isolates with lower surface charge and solubility.	Karaca et al. (2011a)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Flax meal (defatted)	10% w/v; 50 mM Na_3PO_4 buffer with 0.8 M NaCl; pH 8; room temperature; 30 m Dialysis of supernatant against water using 6–8 kDa membrane. Precipitated salt soluble proteins were freeze-dried.	25.41%	87.39%	n.a.	Isolate produced by salt extraction showed higher solubility and interfacial activity compared to those produced by isoelectric precipitation.	Karaca et al. (2011b)
Great Northern bean meal (defatted)	10% w/v; 0.5 M aqueous NaCl; pH 6.13; 45 °C; 3 h Residue went through a second extraction under the same conditions. Combined supernatants were partially freeze-dried and then dialyzed against deionized water using a 3.5 kDa membrane.	25.4% d.w.	80.8% d.w.	74.2%	Protein concentrate showed solubility varying between 70–85% at pH 2, 7, and 10.	Hojilla-Evangelista et al. (2018)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Lentil	20% w/v; 50 mM potassium phosphate buffer with 0.5 M NaCl; pH 7.2; room temperature; 1 h Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	18.43%	74.71%	n.a.	When compared to the isolates obtained by isoelectric precipitation, those produced by salt extraction resulted in isolates with lower surface charge and solubility.	Karaca et al. (2011a)
Pea	10% w/v; 0.1 M phosphate buffer with 6.4% KCl; pH 8; room temperature; 24 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	18.76%	81.09%	n.a.	Salt extraction resulted in isolates with lower surface charge and solubility compared to those produced via isoelectric precipitation.	Karaca et al. (2011a)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Pea	10% w/v; 0.1 M sodium phosphate buffer with 6.4% KCl; pH 8; room temperature; 24 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	19.17%	77.87%	42.49%	WBC (g/g): 2.39 ± 0.12; OBC (g/g) 2.16 ± 0.09; FC (%) 106.23 ± 0.64; FS (%) 118.34 ± 0.68; EC (%) 36.21 ± 0.23; EAI (m^2/g) 31.09 ± 0.33; ESI (min) 12.90 ± 0.19	Zeidanloo et al. (2019)
Pea–CDC Striker	10% w/v; 0.1 M sodium phosphate buffer with 6.4% KCl; pH 8; room temperature; 24 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	n.a.	76.1%	68.2%	WBC (g/g): 0.3 ± 0.0; OBC (g/g): 5.4 ± 0.1; Solubility (%): 91.1 ± 2.2; FC (%): 258.3 ± 11.8; FS (%): 48.9 ± 2.0; EC (%): 193.7 ± 0.0; ESI (%) : 97.6 ± 1.7.	Stone et al. (2015b)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Pea–Meadow	10% w/v; 0.1 M sodium phosphate buffer with 6.4% KCl; pH 8; room temperature; 24 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	n.a.	71.5%	74.8%	WBC (g/g): 2.6 ± 0.3; OBC (g/g): 5.2 ± 0.0; Solubility (%): 85.7 ± 2.5; FC (%): 163.3 ± 4.7; FS (%): 69.6 ± 1.2; EC (%): 193.7 ± 0.0; ESI (%) : 97.0 ± 1.4.	Stone et al. (2015b)
Pea–Dakota	10% w/v; 0.1 M sodium phosphate buffer with 6.4% KCl; pH 8; room temperature; 24 h. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	n.a.	79.3%	72.6%	WBC (g/g): 1.5 ± 0.2; OBC (g/g): 5.2 ± 0.1; Solubility (%): 85.8 ± 0.7; FC (%): 263.3 ± 4.7; FS (%): 56.3 ± 1.7; EC (%): 243.7 ± 0.0; ESI (%) : 99.6 ± 0.0.	Stone et al. (2015b)

(continued)

Table 3.2 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Pinto bean meal (defatted)	10% w/v; 0.5 M aqueous NaCl; pH 6.13; 45 °C; 3 h Residue went through a second extraction under the same conditions. Combined supernatants were partially freeze-dried and then dialyzed against deionized water using a 3.5 kDa membrane.	23.7% d.w.	82.4% d.w.	77.2%	Protein concentrate showed solubility varying between 70–85% at pH 2, 7, and 10.	Hojilla-Evangelista et al. (2018)
Soy	10% w/v; 50 mM sodium phosphate buffer with 0.8 M NaCl; pH 8; room temperature; 30 min. Dialysis of supernatant against Milli-Q™ water using 6–8 kDa membrane followed by freeze-drying.	45.41%	72.64%	n.a.	When compared to the isolates obtained by isoelectric precipitation, those produced by salt extraction resulted in isolates with lower surface charge and solubility.	Karaca et al. (2011a)

Abbreviations: *DDW* Double distilled water, *d.w.* dry weight basis, *EAI* emulsifying activity index, *EC* emulsifying capacity, *ESI* emulsifying stability index, *FC* foam capacity, *FS* foam stability, *n.a.* not available, *OBC* oil binding capacity, *WBC* water binding capacity

Table 3.3 Overview of micellar precipitation process for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Flax	10% w/v; 0.5 M NaCl; room temperature. Supernatant was concentrated 5 times by UF and then diluted fivefold with water, and precipitation in cold storage (5 °C) overnight, followed by freeze-drying of the pellet.	50%	93%	n.a.	Functional properties of native micelle protein are superior to the ones of isoelectric-precipitated protein isolate.	Krause et al. (2002)
Pea – CDC Striker	10% w/v; 1 N NaCl; room temperature; 2 h. Supernatant was diluted tenfold with deionized water (4 °C), and then left for 18 h at 4 °C, followed by centrifugation and freeze-drying of the pellet.	n.a.	87.8%	31.1%	WBC (g/g): 3.5 ± 0.1; OBC (g/g): 3.6 ± 0.2; Solubility (%): 42.8 ± 0.1; FC (%): 133.3 ± 0.0; FS (%): 77.8 ± 3.2; ESI (%): 99.7 ± 0.4.	Stone et al. (2015b)
Pea – CDC Meadow	10% w/v; 1 N NaCl; room temperature; 2 h. Supernatant was diluted tenfold with deionized water (4 °C), and then left for 18 h at 4 °C, followed by centrifugation and freeze-drying of the pellet.	n.a.	81.9%	30.9%	WBC (g/g): 3.2 ± 0.0; OBC (g/g): 3.6 ± 0.1; Solubility (%): 48.9 ± 1.0; FC (%): 161.6 ± 2.3; FS (%): 62.7 ± 0.9; ESI (%): 99.5 ± 0.1.	Stone et al. (2015b)

(continued)

Table 3.3 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Pea – CDC Dakota	10% w/v; 1 N NaCl; room temperature; 2 h. Supernatant was diluted tenfold with deionized water (4 °C), and then left for 18 h at 4 °C, followed by centrifugation and freeze-drying of the pellet.	n.a.	85.9%	30.7%	WBC (g/g): 3.6 ± 0.2; OBC (g/g): 3.6 ± 0.1; Solubility (%): 46.0 ± 1.6; FC (%): 193.3 ± 4.7; FS (%): 52.8 ± 2.7; ESI (%): 99.5 ± 0.1.	Stone et al. (2015b)

Abbreviations: *d.w.* dry weight basis, *ESI* emulsifying stability index, *FC* foam capacity, *FS* foam stability, *n.a.* not available, *OBC* oil binding capacity, *WBC* water binding capacity

its scalability from laboratory scale to the industrial scale (Dapcevic-Hadnadev et al. 2019).

2.4 Technologies to Improve Cell Disruption and Protein Extraction

Protein extraction can be limited by the interactions between the proteins and other components, such as lipids and polysaccharides present in the cells. For that reason, cell disruption is needed to release protein from the cells. Traditionally, this is achieved by mechanical methods such as grinding and milling. However, novel processing technologies are emerging to improve cell disruption and, consequently, protein extraction. The most promising technologies in terms of improved protein extraction yield, extraction time and costs, and environmental impact are enzymatic-, ultrasound-, and microwave-assisted extraction (Pojić et al. 2018).

2.4.1 Enzyme-Assisted Extraction

Different types of enzyme can be used to aid in the extraction of proteins from oilseeds, pulses or cereals. Carbohydrases, which include cellulase, hemicellulase, xylanase, and combinations of cell wall-hydrolyzing enzyme, may increase protein extraction yield by hydrolyzing the polysaccharide matrix (Fig. 3.3) (Fischer et al. 2001; Görgüç et al. 2019; Hanmoungjai et al. 2002; Preece et al. 2017c; Rommi

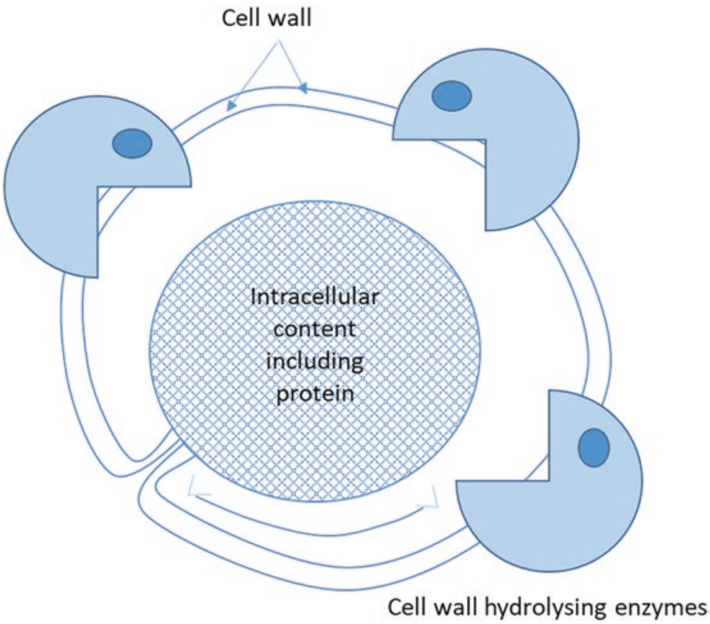

Fig. 3.3 Enzyme-assisted extraction

et al. 2015a; Tang et al. 2003). Phytase and α-amylase may also enhance protein extraction by attacking the interaction of proteins with phytate and starch, respectively (Tang et al. 2002, 2003). For plant protein sources containing lipids, lipase can be used to hydrolyze the interaction between the proteins and the lipids. Proteases, which hydrolyze the proteins, can also be used to improve protein extractability. However, in that particular case, the final ingredient will be a mixture of intact and hydrolyzed proteins (peptides and amino acids) known as a hydrolysate (Apinunjarupong et al. 2009; Campbell and Glatz 2010; Fabian and Ju 2011; Latif and Anwar 2011; Latif et al. 2013; Sari et al. 2013; Rosenthal et al. 2001; Tirgarian et al. 2019; Wang et al. 2008b; Zhang et al. 2011). The ratio between the intact proteins and the hydrolyzed proteins will depend on the degree of hydrolysis. Hydrolysates may behave differently than concentrates and isolates in terms of their functional properties, depending on the degree of hydrolysis. Since enzyme-assisted extraction is performed in mild conditions, it can be considered more environmentally friendly than conventional methods, which use harsh chemicals to perform the protein extraction (Ozbek and Bilek 2018). An overview of the enzyme-assisted extraction process for the isolation of plant proteins from various sources is presented in Table 3.4.

2.4.2 Ultrasound-Assisted Extraction

An emerging technique to improve plant protein extractability is ultrasound (sonication) processing. An overview of the ultrasound-assisted extraction process for the isolation of plant proteins from various sources is presented in Table 3.5. The basic

Table 3.4 Overview of enzyme-assisted extraction for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Barley flour (defatted)	Sequential enzymatic treatment with α-amylase (10,000 U/g, pH 6.5, 65 °C, 1 h), amyloglucosidase (660 U/g, pH 6.5, 40 °C, 16 h), and β-1,3,4-glucanase (8 U/g, pH 5.0, 37 °C, 1 h). Followed by centrifugation, dialysis and freeze-drying. Protein was then extracted by isoelectric precipitation (pH 3.7).	n.a.	41.4%	78.3%	Emulsifying capacity of protein concentrate was comparable to that of whey protein isolate.	Houde et al. (2018)
Canola	Phytase (0.4 and 0.8 U/g), pH (10 and 12.5), solid/liquid ratio (5 and 10) and temperature (55, 65, and 75 °C). Alkaline extraction was then carried out at pH 10 or 12.5 and temperatures of 55, 65 and 75 °C.	38.8% d.w.	60.77–81.36%	5.61–72.1%	The highest protein extraction yield attained was 72.1%, for 2 h at 55 °C, with a phytase dosage of 0.8 U/g when the alkaline extraction was performed at 75 °C, pH 12.5 and 60 min for a solid/liquid ratio of 10 g/100 mL water.	Rodrigues et al. (2017)
Canola press cake	Pectinex (10 mg protein/g d.w.) at 30 °C for 48 h.	Intact: 35.9% Dehulled: 40.1%	Intact: 39% Dehulled: 54%	Intact: 56% Dehulled: 74%	Pectinex hydrolysed pectic polysaccharides and glucans resulting in an increased protein extraction.	Rommi et al. (2014)
Canola cake (defatted)	Pectinex (10 mg protein/g d.w.) 40 °C for 4 h. Proteins were then recovered by a two-step alkaline extraction into 0.2 M sodium phosphate buffer, pH 12.	35.5% d.w.	n.a.	67–78%	Pectinex hydrolysed pectic polysaccharides and glucans resulting in an increased protein extraction.	Rommi et al. (2015b)

Corn	Hydrolysis using a protease of *Bacillus liccheniformis*. The effect of temperature (55 °C and 60 °C) and time (1, 5, 15, and 24 h) on the protein recovery was evaluated.	6.13%	n.a.	71.5–86.8%	Optimal recovery was obtained at 55 °C and 24 h.	Campobiango et al. (2007)
Hemp seed meal	Hydrolysis with carbohydrases (4.5% w/w cellulase, 0.75% w/w hemicellulase, 0.75% w/w xylanase) and 0.5% w/w phytase for 4 h. Proteins were recovered using a 10 kDa UF membrane and the retentate was freeze-dried.	37%	74%	n.a.	Hydrolysate had higher protein solubility than other hemp products in the pH 3–9 range with a minimum of 76% at pH 4.0.	Malomo and Aluko (2015)
Peanut	Viscozyme® L (0.25–2.0%) at 35–70 °C, solid-liquid ratio 1:2–1:7, for 20–160 min.	24.44% d.w.	n.a.	81.5%	Optimal condition was 52 °C, solid-liquid ratio of 1:4, enzyme concentration of 1.35%, and hydrolysis time of 90 m.	Liu et al. (2020)
Peanut flour (defatted)	Alcalase (0.5–10 g/100 g protein) at pH 7.5 and 60 °C until the desired degree of hydrolysis is obtained. Followed by isoelectric precipitation of the proteins at pH 4.5 and neutralization to pH 7.0.	48.9%	Up to 77%	13.2–44.0%	Proteolysis increased the nitrogen solubility in acidic and basic conditions, as well as foaming capacity and foaming stability.	Zhao et al. (2013a)
Rice bran (full fat)	Alcalase (0–2 g/100 g bran) at 40–60 °C for 1–3 h.	14.12%	n.a.	36.2–68.2%	Optimal protein recovery was obtained at 2 g/100 g, 3 h and 50 °C.	Hanmoungjai et al. (2001)

(continued)

Table 3.4 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Rice bran (defatted)	Xylanase (250 units/g rice bran) and phytase (350 units/g rice bran) for 2 h. Proteins were then recovered by isoelectric precipitation at pH 4.0.	18.16–19.05% d.w.	n.a.	58.75–78.90%	Rice bran was stabilized by dry heat, microwave heat, and parboiling. Optimal protein yield was observed for the control without stabilization treatment.	Khan et al. (2009)
Rice	Termamyl (0.5%), at 90 °C for 2 h or Amylase S (0.5%) at 70 °C for 2 h. The solubilized starch was removed by centrifugation and the pellet was again mixed with 3 L of deionized water and incubated with 0.1% cellulase at 50 °C for 30 m. Proteins were recovered by isoelectric precipitation and adjusted to pH 7.0 before freeze-drying.	7.9% d.w.	Termamyl: 85.8% d.m. Amylase S: 81.0% d.m.	Termamyl: 85.2% Amylase S: 86.8%	Alkali- and salt-extracted proteins had higher solubility and emulsifying properties than those of enzyme extracted proteins.	Paraman et al. (2006)
Rice bran (Heat-stabilized)	Amylase (0.5%, w/v) at pH 6.0 and 90 °C for 1 h. Proteins were then recovered by isoelectric precipitation at pH 4.0. Then the dispersion was adjusted to pH 9.0 and subjected to HTC system at different temperature (120 and 150 °C) for 60 s.	16.93%	72.1–74.1%	44.5–50.0%	Combination of amylase treatment with HTC significantly improved protein yield and purity, when compared to the enzymatic treatment alone.	Xia et al. (2012)
Soy	Hydrolysis using two endoproteases Protex 7L (0.5%, pH 7, 50 °C for 1 h) and Protex 6L (0.5% and 1.0%, pH 9, 50 °C for 1 h).	32.0%	n.a.	Protex 7L (0.5%): 73% Protex 6L (0.5% and 1.0%): 85–87%	Protex 6L gave higher degrees of protein hydrolysis than Protex 7L.	de Moura et al. (2008)

Soy flakes (defatted)	Multifect pectinase (5%, w/w) at pH 4.0 and 60 °C for 3 h.	60.6% d.w.	89.0%	38.5–48.2%	Improvement of protein yields by 50% and 17%, when compared to laboratory and pilot-plant-scale trials, was observed following the enzymatic treatment.	Jung et al. (2006)
Soy flour (defatted)	Viscozyme L (15–45 FBG units) at flour/water ratio of 1:20 (w/v) and 45–65 °C for 30 min.	47.8%	n.a.	56.27%	Carbohydrate hydrolysis did not result in higher protein extraction, which was affected mostly by the pretreatment temperature.	Rosset et al. (2014)

Abbreviations: *d.w.* dry weight basis, *HTC* hydrothermal cooking, *n.a.* not available, *UF* Ultrafiltration

Table 3.5 Overview of ultrasound-assisted extraction for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Chickpea	Chikpea flour in distilled water (1:10 w/v) was processed at power density of 2.5 and 4.5 W/ cm^3 for 5 min. Proteins were extracted at pH range 8–8.5.	23.66% d.w.	34.17–35.44%	About 35%	A reduction in protein recovery was observed compared to the unsonicated control.	Byanju et al. (2020)
Ganxet bean	Ganxet bean flour was mixed with alkaline solutions of different concentrations (1:10 w/v) and was processed at 40 KHz, and 250 W for 30 or 60 min. Proteins were isoelectrically precipitated at pH 5.5.	24.7%	n.a.	45.11–78.73%	Extraction using 0.4 M NaOH followed by sonication for 60 min resulted in the highest protein recovery.	Lafarga et al. (2018)
Kidney bean	Kidney bean flour in distilled water (1:10 w/v) was processed at power density of 2.5 and 4.5 W/ cm^3 for 5 min. Proteins were extracted at pH range 8–8.5.	23.84% d.w.	39.91–48.66%	About 50%	No significant impact of power density was observed on the protein recovery, but protein content was lower at 4.5 W/cm^3 (39.91%).	Byanju et al. (2020)

(continued)

Table 3.5 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Peanut (partially defatted flour)	Peanut flour in distilled water (1:10 w/v) was processed at 24 KHz with amplitudes of 20% or 100% (20 or 100 μm, respectively) and processing times of 15 or 40 min.	54.86%	83–90%	50–68%	Highest protein recovery was observed at 100% amplitude.	Ochoa-Rivas et al. (2017)
Rice bran (defatted)	Defatted rice bran in distilled water (0.5:10 w/w; 1.0:10 w/w; 2.0:10 w/w) at pH 10 was processed at 100 W for 10 min at room temperature.	15.67%	73.3–75.6%	11.1–12.0%	The results showed a 7% increase in the extraction yield and a 4% in the protein content, in a 4.5 times lower time for ultrasound-assisted extraction, compared with what was obtained in the alkaline extraction.	Bedin et al. (2020)

(continued)

Table 3.5 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Rice bran (defatted)	Defatted rice bran in distilled water (1:5) at pH 11 was processed at 20 KHz under power of 40, 60, 80 or 100 W for 40 min.	9.26% d.w.	76.09% d.w.	n.a.	Extraction time decreased with increasing ultrasonic power. Extraction yield was higher than for conventional extraction but the residual bran exhibited more damage. Total extraction yield was 4.45%.	Chittapalo and Noomhorm (2009)
Rice bran (defatted)	Defatted rice bran in distilled water (10%, 30%, or 50%) was processed at 24 KHz and 400 W with titanium probe (H22D, 22 mm) at amplitude of 20%, 60%, or 100%, for 10, 20 or 30 min.	10.6%	7.94–39.45%	Up to 39.85%	Optimum conditions were estimated by surface response methodology as solid/liquid ratio of 43%, extraction time of 30 min, and ultrasound power amplitude of 48.25%.	İşçimen and Hayta (2018)

(continued)

Table 3.5 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Rice bran (defatted)	Defatted rice bran in distilled water (0.5–1.5 g/10 mL) was processed at amplitude of 50–90%, for 10–30 min.	14.13%	72.06%	n.a.	When compared to unsonicated control, the yield was found to be 1.62-fold higher for sonicated sample, while the extraction time was reduced by 3.33-fold. Total extraction yield was 4.73%	Phongthai et al. (2017)
Rice bran (defatted)	Defatted rice bran in distilled water (1:15 w/v) was processed at 15% power for 5 min using a ultrasonic processor (JY92-IIN). Proteins were then extracted at pH 9.5 for 120 min.	14.47%	67%	57.89%	Oil absorption capacity, emulsion stability, and foaming capacity were improved by the sonication treatment.	Sun et al. (2017)

(continued)

Table 3.5 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Soy	Soy flakes or soy flour in distilled water (1:10 w/v) was processed at power density of 2.5 and 4.5 W/cm^3 for 5 min. Proteins were extracted at pH range 8–8.5.	Soy flakes: 54.95% d.w. Soy flour: 53.11% d.w.	Soy flakes 57.00–64.08% Soy flour 59.32–59.48%	Soy flakes About 30% Soy flour About 50%	Soy flakes, when exposed to the power density of 4.5 W/cm^3, showed a reduction in the protein recovery (30.60%) compared to the unsonicated control and a lower protein content (57.00%).	Byanju et al. (2020)
Soy (defatted)	Defatted soy flakes in tap water (16% w/w) were processed at ultrasonic amplitudes of 21 and 84 μm_{pp} (power density of 0.30 and 2.56 W/mL, respectively) for 30, 60 or 120 s.	n.a.	85–94% d.w.	57.27–72.91%	Optimal protein recovery was obtained at high power treatment for 120 s.	Karki et al. (2009)
Sunflower	Sunflower meal in deionized water (5%) was processed at power density of 80, 150, or 220 W/L, for 5, 15 or 25 min, and under temperature of 25, 35 or 45 °C, at pH 8. Proteins were recuperated by isoelectric precipitation.	n.a.	Up 93.49%	28.00–54.26%	The optimal extraction points were observed at power density (220 W/L), temperature (45 °C), and extraction time (15 min).	Dabbour et al. (2018)

(continued)

Table 3.5 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Wheat (defatted)	Defatted wheat germ flour (1%) was processed at power of 250–450 W, for 10–30 min using a 20 kHz VCX 500 ultrasonic generator with a 12.7 mm probe. The pulse mode was set at 1 s:2 s, 2 s:2 s and 3 s:2 s.	31.48%	n.a.	37–57%	Optimum extraction conditions were determined by response surface methodology as power of 363W, for 24 min, and pulse mode 2.4 s on and 2 s off.	Zhu et al. (2009)

Abbreviations: *d.w.* dry matter, *n.a.* not available

principle of ultrasound-assisted extraction is the mechanical rupture of the cell wall by acoustic cavitation, which improves the release of proteins in the extraction solvent (Görgüç et al. 2019, 2020b; Li et al. 2017; Preece et al. 2017b, 2017c; Yagoub et al. 2017; Zhang et al. 2019b). It has been demonstrated that ultrasound-assisted extraction can improve protein extraction yield, reduce solvent consumption, and shorten processing times (Bedin et al. 2020; Li et al. 2017). Ultrasound power density, extraction time, solid/liquid ratio, and solvent characteristics are the main parameters that will influence the protein extraction yield.

2.4.3 Microwave-Assisted Extraction

Another emerging technique to improve protein extractability is microwave-assisted extraction. An overview of the microwave-assisted extraction process for the isolation of plant proteins from various sources is presented in Table 3.6. Similar to the ultrasound technique, microwave-assisted extraction breaks the cell wall and causes the release of proteins. However, in the case of microwave-assisted extraction, it is the microwave power which promotes uniform heating and generates intense pressure on the cell wall, which is responsible for the breaking of the cell wall (Bedin et al. 2020; Choi et al. 2006; Gorguc et al. 2020a; Luthria et al. 2018). In addition to improved protein extraction yield, other benefits of microwave-assisted extraction

Table 3.6 Overview of microwave-assisted extraction for the isolation of plant proteins from various sources

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Peanut (partially defatted flour)	Peanut flour in distilled water (1:10 or 1:25 w/v) at pH 9 was processed at power of 145, 290, 435, 580, and 725 W for 2, 4, 6, 8, and 10 min.	54.86%	up to 96.9%	up to 55.53%	The sample treated by microwave (725 W, 8 min) yielded an extraction of about 55%, i.e., 77% more protein when compared with the control without microwave treatment.	Ochoa-Rivas et al. (2017)
Rice bran (defatted)	Defatted rice bran in distilled water (0.5:10 w/w) at pH 9–11 and temperature 30–55 °C was processed at power of 400–450 W for 60–120 s.	15.67%	75.67–79.98%	11.57–15.68%	Optimal conditions in terms of yield was found to be pH 11, 55 °C, and 120 s.	Bedin et al. (2019)
Rice bran (defatted)	Defatted rice bran in distilled water (0.5:10 w/w; 1.0:10 w/w; 2.0:10 w/w) was processed at pH 10, 40 °C, a standard frequency of 50–60 Hz, at a power of 350–400 W for 90 s.	15.67%	75.0–75.9%	11.0–12.2%	The microwave-treated sample resulted in a higher protein extraction yield and a higher protein content in the extract, in a relatively shorter process time, than for the sample processed by alkaline extraction.	Bedin et al. (2020)

(continued)

Table 3.6 (continued)

Protein source	Process conditions	Protein content of starting material	Protein content of concentrate/ isolate	Protein recovery (based on the protein content of the starting material)	Remarks	Reference
Rice bran (defatted)	Defatted rice bran in distilled water (0.5–1.5 g/10 mL) was processed at pH 10, at a power of 600–1000 W for 60–120 s. Proteins were recovered by isoelectric precipitation.	14.13%	up to 71.27%	up to 22.07%	Optimal condition was 0.89 g rice bran/10 mL of distilled water, 1000 W and 90 s.	Phongthai et al. (2016)
Rice bran (defatted)	Defatted rice bran in distilled water (1:15 w/v) was processed by microwave at 20% power for 2 min using a domestic microwave oven (P70D20P-TD). Proteins were recovered by isoelectric precipitation.	14.47%	67%	n.a.	Microwave use led to a reduction in the extraction yield, which might be due to the thermal denaturation of proteins. Protein recovery based on the supernatant was 43.74%.	Sun et al. (2017)

Abbreviations: *d.w.* dry weight basis, *n.a.* not available

include a smaller amount of solvent required for proteins extraction (Ozbek and Bilek 2018). The microwave power density, extraction time, solid/liquid ratio, and solvent characteristics are the main parameters that will influence the protein extraction yield.

2.4.4 Other Technologies for Cell Disruption and/or Protein Extraction

Enzyme-, ultrasound- and microwave-assisted extractions have been identified as the most convenient technologies to improve cell disruption and protein extraction (Pojić et al. 2018). However, the applicability of other techniques, such as electro-activation, subcritical water extraction (SWE), aqueous two-phase extraction

(ATPE), pulsed electrical field (PEF), and high hydrostatic pressure (HPP), should be studied in more detail to assess their potential for cell disruption and/or protein extraction.

Electro-activation technology is based on applied electrochemistry. The electro-activation reactor consists of an anode and a cathode separated by ion exchange membranes. At the anode/solution interface, H^+ ions are generated, whereas OH^- ions are produced at the cathode/solution interface. These processes generate acids and bases in situ using only salts, water, and electricity. Electro-activated solutions have been used to produce plant protein concentrates from canola meal (Gerhzova et al. 2015a, b, c), and soybean meal (Gerliani et al. 2020) without using chemicals such as HCl or NaOH.

SWE is a process using water at subcritical conditions (100 °C < T < 374.2 °C under high pressure to maintain its liquid state) to extract molecules (Wiboonsirikul et al. 2007; Sereewatthanawut et al. 2008; Ndlela et al. 2012; Pojić et al. 2018). Under those conditions, proteins can be hydrolyzed since water acts as an acid or base catalyst in chemical reactions. However, the proteins and amino acids extraction yield is higher than for the conventional method (Sereewatthanawut et al. 2008; Sunphorka et al. 2012).

ATPE is based on the formation of an aqueous two-phase system when two water-soluble polymers or a salt and a polymer are dissolved in water beyond a critical concentration at which two immiscible phases form. Optimal conditions are the ones which would result in the complete recovery of the proteins in one of the two phases, with a high level of enrichment (Gu and Glatz 2007). Multiple factors will influence the proteins partitioning between the two phases, but protein surface hydrophobicity and hydrophobic differences between phases are two key factors. Gu and Glatz (2007) demonstrated that ATPE can be used successfully for the recovery of either hydrophobic or hydrophilic proteins from corn extracts. Aguilor and Rito-Palomares (2008) demonstrated the potential of ATPE to process fractionated soybean.

PEF treatment involves the application of electric fields of high intensity (>0.1 kV/cm) and short duration to disrupt cells. In their work, Sarkis et al. (2015) demonstrated that the application of PEF treatment prior to the extraction of sesame proteins helps to improve the extraction step by reducing the amount of solvent required, as well as the time and temperature of extraction. Similarly, Yu et al. (2015) observed that PEF treatments improved protein extraction from rapeseed stems. This illustrates the potential of PEF as a process to improve the protein extraction step. However, additional studies with other oilseeds, pulses, and cereals are to be carried out to fully assess the potential of this process for application at the industrial scale.

HPP involves the application of high hydrostatic pressure (up to 600 MPa) to food products previously sealed in flexible and water-resistant packaging for a few seconds to a few minutes. Its main application in the food industry is to disrupt microbial cells. Literature on the use of HPP treatment to improve protein extraction is scarce. However, Preece et al. (2017a) demonstrated that a single pass of soy slurry at 100 MPa improved the extraction yields of soy protein up to 82%. However, it was also observed that multiple passes reduced the extraction yield due to cell wall swelling. Thus, additional work is required to fully assess the potential of HPP treatment to improve plant protein extraction.

3 Drying Processes

Protein extraction and separation are the key steps in the production of protein concentrates and isolates. However, to enable their use as ingredients in food product formulation and for their storage, the protein extracts must be dried into a powder (Ghribi et al. 2015). Most of the research work carried out at laboratory scale used freeze drying as the drying process. Freeze-drying consists in the drying of the protein extract by sublimation. It requires the protein extract to be frozen before being dried. The equipment necessary to perform freeze-drying consists of a drying chamber, a condenser, and a vacuum pump. However, this method is not economically viable for drying protein extracts at industrial scale. Hence, functional properties of freeze-dried plant protein concentrates and isolates reported in the scientific literature may not have practical industrial applicability (Hu et al. 2010). On the other hand, spray-drying and vacuum-drying are used at industrial scale for the production of plant protein concentrates and isolates. Spray-drying consists in rapidly drying a liquid or slurry containing the soluble proteins with a hot gas (usually air) in order to obtain a dry powder. The liquid or slurry is fed into the drying chamber using some type of atomizer or spray nozzle to disperse the liquid or slurry into a controlled drop-size spray. Dry powder forms as moisture quickly leaves the droplets. The dry powder is usually collected in a drum or cyclone. Vacuum-drying consists in the evaporation of water present in the liquid or slurry containing the soluble proteins under reduced pressure. The equipment necessary to perform vacuum-drying consists of a drying chamber, a condenser, and a vacuum pump. In their work on soybean protein isolate, Hu et al. (2010) observed that spray-dried isolate had higher solubility than its counterparts dried by freeze-drying and by vacuum-drying, except at pH 4.5. Their emulsifying and foaming properties were also superior to those of freeze-dried and vacuum-dried isolate. Similar results were reported by Zhao et al. (2013b) for rice protein isolate dried by spray-drying versus freeze-drying. These results suggest that drying methods can influence the functional properties of plant protein concentrates and isolates.

4 Conclusion

To take advantage of the increasing demand for plant protein ingredients and plant-based foods, ingredients and food manufacturers must find innovative ways to produce plant protein concentrates and isolates, and make these ingredients into tasty, viable alternatives for consumers. Ingredient processors with in-depth knowledge of the factors which influence plant protein functionality and able to manipulate their processes intelligently to optimize these functional characteristics will have the much-needed competitive advantage in the marketplace.

AE-IP is the most widely used process to produce plant protein concentrates and isolates at the industrial scale. AE-IP has high productivity and is easy to scale up. On the other hand, the use of harsh chemicals for both the extraction and isoelectric precipitation steps has a negative impact on the functional properties of the resulting ingredients. For that reason, processes using mild extraction/separation conditions, such as membrane technologies, SED, and MP can be of interest when ingredients with superior functional properties are suitable. Furthermore, protein extraction can be limited by the interactions between the proteins and other components present in the cells, such as lipids and polysaccharides. For that reason, there is a growing interest in processes that can improve protein extraction, such as enzyme-assisted extraction, ultrasound-assisted extraction and microwave-assisted extraction. However, these processes are still in their infancy for their applications to produce plant protein concentrates and isolates at industrial level, thus these should be further investigated and developed intensively. More research studies should also be carried out to assess the potential of alternative techniques, such as electro-activation, SWE, ATPE, PEF, and HPP for plant protein extraction.

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Chapter 4
Food Processing Industrial Byproducts as Raw Material for the Production of Plant Protein Foods

Mian Anjum Murtaza and Kashif Ameer

1 Introduction

The demand for high-quality protein has been increasing over the past decade. The global protein ingredient market has seen a boon of approximately USD 38 billion and is expected to grow at compounds annual growth rate of 9.1% from time span ranging from 2020 to 2027. The rise in vegan, vegetarian and flexitarian communities has led to increased usage of plant-based proteins in food product formulations (Ismail et al. 2020). Owing to increasing population and increased consumer awareness on food supply sustainability issues, the food industry is keen on formulating products using plant-based protein ingredients derived from sustainable food crops to replace or partially replace animal proteins with plant-based protein ingredients for optimal delivery of flavor, functionality and nutrition to the intended consumers (Ismail et al. 2020; Loveday 2020).

In recent years, the consumer demand for plant-based food products has escalated, prompting the ingredient manufacturers to differentiate themselves to capture a share of the crowded marketplace (Ameer et al. 2017a, b; Etemadian et al. 2021). The average sales of plant-based foods and beverages raised to $3.7 billion in 2018 in United States and demonstrated about 17% rise as compared to 2017. About one-third (30%) consumers are actively trying to reduce meat consumption without sacrificing taste and making informed choices (Ameer et al. 2017c). Food manufacturers are constantly trying to develop innovative and better-tasting meat alternatives. Cargil Inc. conducted a comprehensive consumer research in October 2018, which showed that 60% of consumers expressed the satisfaction of getting enough and adequate nutrient from plant foods apart from meat and meat products (Good

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Food Institute 2020). Whereas about 79% of consumers are clean-label seekers who check on ingredients list especially proteins source and content (Ameer et al. 2017c; Daniel 2018).

Ongoing food science innovations must corroborate with the exponential rise in the demand of novel plant-based proteins. As extraction is the first step to achieve successful completion of production modalities, it is imperative to ensure the production of high-quality proteins with improved functionality, understanding of structure/activity relationship, and development of cost-effective protein functionalization strategies to overcome texture and flavor challenges. This chapter aims at providing an overview of innovation needed to develop plant-protein based foods.

2 Plant-Based Industrial Food Waste as Sources of High-Quality Proteins

2.1 Press Cakes/Oil Meals

Oil processing industry produces large quantities of industrial by-products, such as press cakes/oil meals after processing of oil-bearing fruits or seeds. Usually, the average proteins contents of these by-products are in a range of 15–50% in oil meals, which are potent sources for recovering high-quality proteins (Pojić et al. 2018). Major oilseed cops include sunflower, cottonseeds, rapeseed, peanut and soybean which yield significant amount of proteins meals on annual basis (about 200 million tones in 2015) (FAO 2018). Other significant oil-bearing crops are coconut, palm, olive and their residues which may as protein sources. Other alternative crops, such as hempseed, hazelnut, pumpkin seed, grape seed, sesame seed and walnut have also been cultivated for obtaining specialty oils and their meals are also high in protein contents (Ochoa-Rivas et al. 2017; Pojić et al. 2018). The initial pretreatment method (storage conditions, dehulling) affects the quality of oil meal/press cake and their protein contents. Processing of oilseed by either hulling or dehulling affects the resulting oil meal protein contents. In general, the dehulled meals consist of lower fiber and higher protein contents as compared to unhulled meals which are required to be fractionated into fiber and protein-rich fractions prior to carry out protein recovery (Roselló-Soto et al. 2015; Ochoa-Rivas et al. 2017). Chemically, the heat treatment to remove solvent from defatted meals increases protein aggregation and tightens bonding between carbohydrates and fiber. This not only leads to enhanced protein extraction from oilcake meals but also improves technological properties like emulsification, foaming and solublity, and so on (Rodsamran and Sothornvit 2018).

2.2 Cereals Processing By-products

Usually, cereal-processing by-products are excellent sources for protein recovery. After extracting oil, rice bran ranks at top followed by oat bran, brewer's spent grain and wheat bran. Rice bran proteins and rice bran dietary fiber have garnered considerable attention of researchers owing to well-balanced amino acid profile, high nutritional value and health-beneficial effects like hypolipidemic, hypoallergenic, anticancer and hypocholesterolemic effects (Pojić et al. 2018). Drying processing of rice and rice bran oil extraction yields rice bran and defatted rice bran as unavoidable by-products as suitable sources of proteins. Rice bran comprises of proteins ranging from 12% to 20%, however dense agglomeration of proteins with starches in endosperm limits rice bran utilization and mechanical pretreatment demonstrated better results to break down this complex starch-protein agglomeration as compared to hydrothermal pretreatment (Xia et al. 2012; Apprich et al. 2014).

Wheat bran has protein contents in a range of 13–18%, however, these wheat bran proteins are found in in form of tightly enclosed polysaccharides matrix which hinder their digestibility and hence, about 15.5 million tons high-quality usable proteins are wasted (Xia et al. 2012; Pojić et al. 2018). Therefore, strong alkaline-based extraction conditions are needed for wheat bran protein extraction, which limit their exploitation. Conversely, wheat bran prion could be utilized in producing γ -aminobutyric acid (GABA), bioactive peptides and high-quality free amino acids (Connolly et al. 2014). Defatted wheat germ has well-balanced high proportion of essential amino acids like methionine, lysine, threonine and could also be employed for bioactive peptides isolation (Balandrán-Quintana et al. 2015; Amagliani et al. 2017). Brewing industry also produces large quantities of brewer's spent grain as by-product comprising of 15–26% proteins. However, the protein interactions with other biopolymeric components cause impenetrable layer during spent grains processing operations like mashing that lowers protein extractability and necessitate modern extraction techniques to volarize cereals proteins (Apprich et al. 2014; Pojić et al. 2018).

2.3 Legumes Processing By-products

After cereals, legumes are ranked as the second-most significant source of high-quality proteins. Commercial milling of legumes produces several by-products, such as husks, powder, broken, shriveled and unprocessed seeds comprising of proteins contents of 14%, 12%, 13%, respectively (Pojić et al. 2018). The main issues in utilization of proteins from legumes is that legumes are processed in small-scale facilities and have specified market niches that limit their supply chain. Furthermore, the soybean processing to produce soy milk and tofu also yields soy pulp and such by-product known as okara which comprises of significant residual proteins in a range of 25–33%. Massive production of okara causes serious disposal issues until revalorization of the legume by-products (Oomah et al. 2011; Lu et al, 2016; Li et al. 2012).

3 Recent Technological Developments

Après® is one of plant-based beverage manufacturers, which recently developed a beverage enriched with plant proteins to replenish human body nourishment. In formulating this beverage, a blend of various plant proteins originating from chia, pea, cacao and hemp were blended with and coconut water and organic virgin coconut oil (Food Business News 2020). This drink is sufficiently enriched with all essential amino acids for lean physique and promotion of satiety. The blended coconut water provides electrolyte hydration and smooth texture to plant proteins while virgin coconut oil is a good source of energy and medium chain fatty acids (DrinkApres 2020).

NuGo Nutrition® is also expanding its plant-proteins based product line. They developed gluten-free pea protein cookies, which provide 10–12 g fiber and 16 g protein in 100 g cookie serving. Another venture is the introduction of low-sugar plant protein bars and antioxidant-rich chocolate prepared with natural cocoa butter proteins (NuGo Nutrition 2020).

Campbell Soup Co.® manufactured plant protein milk which contained 10 g of pea proteins per 8-oz serving. This plant-based product provides 50% more calcium as compared to conventional dairy milk. This is beverage for vegans and as non-GMO product, it does not contain common allergens like dairy, nuts and soy, nor intolerances like lactose and gluten (Food Business News 2020).

Until now, the seafood market share of plant-based protein alternative is limited; however, from an aquaculture product development technology perspective, the market has promising potent to replace animal-based counterparts with plant-based proteins. According to Good Food Institute, the sale of plant-based seafood alternatives raised to $9.5 million in 2019 (Good Food Institute 2020). Still the aquaculture market has potential to expansion up to $141 million due to lower manufacturing cost of plant-based alternatives, supply chain simplification, reliable production line, and longer shelf life potential (Yu et al. 2015; Food Business News 2020). Plant-based seafood proteins deem to be cheaper as compared to animal-based proteins due to less complex supply chain layer and direct processing of proteins to innovative food products. Plant-based proteins foods product line is highly controlled and may lead to the generation of finished products with higher degree of predictability as compared to animal-based products, which deals with biologically complex animals.

3.1 Technological Hurdles to Overcome Scale-Up Issues of Plant Proteins Globally

In order to scale up product development of plant-based proteins foods to full potential, there are significant technical hurdles, which need to overcome by food manufacturers. The most challenging task is to improve the taste and texture profiles of plant-based finished products to mimic taste similar to animal products (Bilek 2018). One example in this regard includes exploitation of Konjac powder to prepare shrimp, scallop alternatives by The Plant Based Seafood Co. (2020). Still, the plant-based ingredients options are limited for manufacturers, such as soy, chia, pea, cacao, and hemp proteins (Food Navigator 2020).

Soybeans are one of the main sources of plant-derived proteins. Moreover, new alternative plant sources have been already found in published literature and supermarkets and mainly include lentil, chickpea, sesame, peanuts, main, rice, potato, quinoa, walnut, hazelnut, hemp and wheat. Researchers have been even focusing on exploitation of aquatic flora as candidate plant-protein source. Even though, the plant-sources are rich in protein content, these have main issues of low bioavailability and digestibility (Bilek 2018; Fathi et al. 2018). One another main issue is their allegenicity owing to allergen compounds. In case of soybean, almost 15 antigens that bind to Ig-E antibodies have been identified to date. Gluten allergy is associated with celiac disease and gluten-based nutritional products increases the symptoms of this disease in affected people. Furthermore, some of the published reports have indicated exacerbation of cerebral ataxia and schizophrenia severity. Moreover, growing concerns about GMO food products have led to explore plant-based alternatives (Kammerer et al. 2014; Bilek 2018).

4 Development of Plant-Based Protein Hydrolysates

In food or feed industries, several technological strategies are employed to produce plant-based protein hydrolysate to improve human and livestock health for recovery of essential bioactive compounds (Etemadian et al. 2021). In this regard, microbial fermentation, enzymatic hydrolysis and solvent extraction technologies have been exploited. The enzymatic hydrolysis by far is the most preferable approach because of it is free from microbial, chemical or toxic contaminants (Moure et al. 2006). Moreover, the enzymatic hydrolysis is also favorable as compared to chemical hydrolysis due to mild reactions kinetics, recovery of high product quality, method performance and robustness, and low generation of undesirable products (Coscueta et al. 2019).

The processing units of soybean flour, rice, wheat, tomato, pea, etc. can be successfully scaled-up to identify effective bioactive compounds from plant matrices using high-efficiency innovative technologies to make economically affordable plant proteins by-products along with main product lines. Proteins of vegetables origin provide valuable bioactive compounds, which could be employed in preparing various food formulations (Ashaolu et al. 2017; Etemadian et al. 2021). Apart from nutritive value-addition, these vegetal proteins may be utilized as promising amino acid and energy sources for regulation of physicochemical properties of foods. It is evident from several published reports that plant hydrolyzed proteins exhibit better yield, physiological and functional properties in comparison with crude protein (Coscueta et al. 2019). This could be ascribed to the enhanced release of biologically active peptides of crude protein after subjected to enzymatic hydrolysis, which accelerates inhibition of lipid peroxidation (Ashaolu et al. 2017; Etemadian et al. 2021), and in a report by Fukudome and Yoshikawa (1992), it was implied that wheat gluten hydrolysis and exorphins play role in growth and survival and have opioid activity.

Generally, the safety of food products comprising of plant-derived hydrolysate as well as their multifunctional properties in regulation of chronic lifestyle disorders including oxidative stress, diabetes, cardiovascular, etc. have garnered attention of researchers and food manufacturers. One notable example in this regard includes soy proteins (Udenigwe and Aluko 2012). Soy comprises high glycine levels and owing to high proportion of proteins, isoflavones, and fatty acids, these soy compounds are regarded as the functional dietary components and exert beneficial physiological effect on human health (Etemadian et al. 2021). Hence, ingestion of these plant-derived peptides allows absorption of bioactive compounds which provide potent antioxidant, antimicrobial, cholesterol lowering, immunization and anticancer effects through various mechanistic approaches like scavenging of free radicals, electron donation or chelating of metal ions (Singh et al. 2017; Etemadian et al. 2021). Raw consumption of soy proteins may lead to several adverse health implications like anaphylactic shock and immune-mediated responses, such as asthma and hay fever. Moreover, raw and processed soy and soy products also comprise of anti-nutritive compounds like phytic acid and lectin, which adversely influence food absorption efficiency (Meinlschmidt et al. 2016). However, owing to plant protein hydrolysis yielding hydrolysate and biologically active peptides from soybean, canola, rice, peas, and wheat have shown a significantly increase in antioxidant capacity. Moreover, with regard to development of function met products, Zhang et al. (2010) have established that addition of soy protein hydrolysates in form of bioactive peptides, generated by enzymatic action of microbial proteases, to ground beef led to significant ($p < 0.05$) reduction in lipid peroxidation. Furthermore, the plant-based peptides from pea and chickpea proteins caused gastrointestinal stimulation which led to inhibition of angiotensin I-converting enzyme (ACE-1) (Etemadian et al. 2021) (Fig. 1).

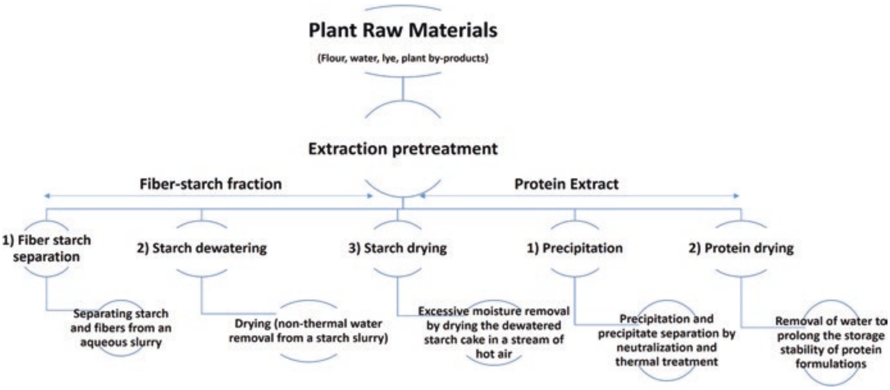

Fig. 1 Main manufacturing steps involved in production of plant-based protein ingredients and protein extracts from plants/plant by-products

4.1 Challenges for the Hydrolyzed Protein Production and Utilization

Despite the intense research by the researchers to exploit the bioactive peptides from plant hydrolysates until now, still the recognition of the best type of protein hydrlysates is challenging due to several inherent limitations. Pertaining to technological limitations, the main issues include the cost, lower degree of digestibility of thermally dried plant protein products and adverse effects on the structural conformations of the essential amino acids (specifically tryptophan) under acidic environment (Allaoui et al. 2019; Etemadian et al. 2021). The plant protein hydrolysate are inexpensive alternative natural replacer of animal-based counterparts, which stimulate the growth of intestinal absorption and improves digestibility of foods in animals and humans (Jahanbani et al. 2016). About utilization of plant-derived proteins, the hydrolysates does not comprise of all essential amino acids, and hence can be introduced in combination with aquatic protein hydrolysate as functional proteins. Still the research on optimization of hydrolyzed protein isolates and preparation of specific food formulations is lacking (Etemadian et al. 2021). Moreover, the recognition of molecular structures of peptide chains is essentially required. Moreover, plant-based proteins hydrolyastes can be essentially applied in formulations of foods intended for human consumption as emulsifiers, binders, and gel-producing substances as well as promising alternative to promote human health owing to excellent properties, e.g., anti-hypertensive property (Marcela 2017; Etemadian et al. 2021).

4.2 Plant Proteins as Replacer of Fish Meal in Aqua Feeds

In aquaculture, the feed constituents make up 60% of total operational cost, therefore, the plant proteins with economic affordability are great alternative to develop cheaper aqua feed. Fish meal is one of the key components in feed with good nutrient digestibility and deficient in anti-nutritional factors (FAO 2016). Fish meal compositional requirement of protein ingredient is 30–40% for omnivorous and 40% carnivorous. As non-traditional cost-effective alternative, researchers have recommended plant-based proteins (Daniel 2018). The main issues which have been highlighted by previous researches regarding plant-based proteins are as; presence of anti-nutritional constituents, deficiency of essential amino acids, poor nutrient digestibility and palatability and low bioavailability due to insoluble fibre and starches (Hardy 2010; Daniel 2017). Various processing modalities have been reported to prepare plant-based proteins aqua feed. These techniques include supplementation of dietary fish meal with deficient amino acids, aggregation of plant-proteins originated from different sources, hydrolysis by means of exogenous enzymes, adoption of meal provision strategy of one-day for plant-proteins based meal and next day fishmeal based feed, addition of certain additives and application of modern extraction techniques like microwave-assisted extraction, ultrasound-assisted extraction, and high-pressure processing (Bonaldo et al. 2015; Johnson et al. 2015; Daniel 2018).

4.2.1 Structure/Function Relationship

Structural configuration and characteristics mainly serve as the influential factors (involving amino acid sequence and composition) to determine functional properties. Other factors in this regard include physicochemical properties, such as cumulative presence of reactive groups like hydroxyl or sulfhydryl groups, net charge, hydrophobicity of surfaces as well as molecular configuration and sizes (Loveday 2020). These properties might be correlated to each other in such a way that hydrophobicity and net charge are usually affected by the amino acid composition, whereas molecular configuration could be affected by the sequencing which consequently exert significant effect on surface properties. Various functional properties of plant-based proteins like foaming, gelling, emulsifying capabilities as well as thermal stability and solubility are influenced by the surface properties of portions (Kumar et al. 2021). For example, soy proteins exhibit high molecular weight and high hydrophobicity and this may help to form polymers under specified conditions and hence could be subjected texturization to impart textural properties in the products similar to that of meat products. Any modification in protein structural configuration during purification or processing may cause significant change in plant-based proteins functionalities (Ismail et al. 2020).

4.2.2 Functionalization Strategies for Innovating Plant-Based Proteins Formulations

Various functionalization strategies are employed to improve functionality of protein powders. These functionalization strategies include high-pressure homogenization, lecithin coating, and agglomeration. These strategies usually exert significant influence on surface properties, shape configuration and particle sizes. Among these strategies, agglomeration leads to enhancement of particle sizes owing to formation of bridges through various binders, such as hydrocolloids, gums or starches (Ismail et al. 2020). This agglomeration process causes increased dispersibility and hence water diffusion may occur easily with the agglomerate. On the other hand, the lecithin coating improves wettability and prevents powder caking. Protein functionality is also modified through application of high-pressure homogenization accompanied by spray drying under controlled conditions (Ortega-Rivas et al. 2006). Viscosity and water-holding capacities tend to increase when proteins are subjected to high-pressure processing and these properties are desirable for plant-proteins applications as eco-friendly alternative of conventional meat products. Processing-induced manipulation of protein powder functionalization can also be carried out for targeted improvement of protein functionality (Ismail et al. 2020). Proteins exhibit sensitivity to various processing parameters, such as enzymatic activity, pH, temperature, and shear stress and these pose serious technological challenges in formulation of plant proteins-based products. Methods aimed at improvement of protein structure and functionality involve several facets, such as solubility improvement, flexibility enhancement, alternation of hydrophobic/hydrophilic equilibrium and promotion of protein cross-linking. The most commonly employed protein modification strategy is enzymatic hydrolysis (Ismail et al. 2020; Meinlschmidt et al. 2016).

Enzymatic hydrolysis has been utilized as one of the most researched technique for protein functionality improvement and provision of physiological benefits. Protein hydrolysates are produced through enzymatic hydrolysis and two prominent factors including choice of enzyme and degree of hydrolysis (DH) play pivotal role in determining functional properties of manufactured protein hydrolysate owing to exerting effect on peptide profile and protein structure (Meinlschmidt et al. 2016). DH at lower rates is of particular significance for functionality-enhanced protein ingredients because it provides enhanced control regarding the release of bitter peptides and structural loss, which is commonly attributable to the extensive hydrolysis with higher DH. Enzymatic hydrolysis at high DH might lead to production of protein products with higher proportion of free amino acids and short-chain peptides with minimal functionality enhancement (Ismail et al. 2020). Soy protein hydrolysis at limited scale with DH ranging 2–15% has been reported to cause improved solubility, emulsification, and foaming ability. Each hydrolysis process needs optimization depending on protein source to expedite the desired functionality enhancement (Sun 2011; Meinlschmidt et al. 2016).

Another widely employed protein modification modality is termed as Maillard-induced glycation. Glycation refers to the phenomenon involving addition of sugar molecules to the proteins or lipids for functionality enhancement. Although, researchers have explored the potential of Maillard-induced glycation at limited scale and in controlled manner for protein functionality improvement however, this has not been utilized at commercial scale until now. A review was published by de Oliveira et al. (2016) who gave an account of 31 studies on improved protein functionality enhancement through glycation. Various technological properties may results in significant improvement due to Maillard-induced glycation. The prominent characteristics include improved thermal stability, high foaming, gelation and emulsification capabilities because of increases in proteins cross-linking, viscosity and hydrophilicity while glycation also lowered the protein's isoelectric point and hence prevented protein denaturation (Wang and Ismail 2012; Wang et al. 2013; de Oliveira et al. 2016). In this regard, the degree of functionality improvement and structural modification depend on several factors, such as Maillard-reaction, conditions, chain-length and polysaccharide characteristics, and protein conformation. Therefore, it may be implied that Maillard-induced glycation needs process optimization for achieving desired functionality of particular protein products while minimizing the changes of reaction propagation to the extent more advanced undesirable stages causing generation of off-flavors and browning. Moreover, industrial scale of Maillard-induced glycation is a dire need for producing protein products with high degree of feasibility (Ismail et al. 2020). Non-thermal protein modification techniques has also been reported in published literature, including use of ultraviolet radiation, oscillatory magnetic field, pulsed electric field, ozonation, and recently cold plasma discharge has gained wide traction among researchers (de Oliveira et al. 2016).

In cold plasma technology, proteins are exposed to plasma in terms of partially ionized gas. Generated discharge of cold plasma comprises of reactive oxygen and nitrogen species including commutative proportion of negative ad positive ions along with free radicals at ambient room temperature. The type of gas used during cold plasma discharge operation usually determine the composition of reactive oxygen and nitrogen species (Ikawa et al. 2010). Proteins might be subjected to various chemical reactions, such as bound cleavage, oxidation, and polymerization. On pilot scale, cold plasma has been employed for surface modification in intensive manner. Cold plasma preserves the quality of processed proteins products as well as microbial decontamination. Tolouie et al. (2018) also reported the impact of cold plasma on structural modification, functionality enhancement and control of allergenicity in proteins from various sources. Studies have indicated that type of cold plasma treatment govern the degree of changes in protein structures. However, reports are limited on linking functional changes to structural modification through cold plasma, and hence results cannot be implied in comprehensive manner. Gaining of basic knowledge regarding cold plasma exploitation could be helpful for targeted development of protein functionality for particularly desired applications (Ismail et al. 2020).

4.2.3 Taste and Flavor Challenges Posed by Plant-Based Proteins

Utilization of plant-based proteins such as legume proteins has posed challenged while application in food formulations. Main challenge includes production of off-flavors in consistent manner, which might be easily perceptible by the consumers. For example, off-flavor production by the soybean proteins is also attributed as "beany, grassy and painty". Usually, the production of such off-flavoring or off-odorous compounds can be ascribed to peroxidation initiated by lipoxygenase enzyme in case of unsaturated fatty acids. In most cases, off-flavor production largely depends on type of raw materials, processing conditions and postharvest storage of plant-based proteinous foodstuff (Ismail et al. 2020). In a report by Malcolmson et al. (2014), pea flavoring compounds were investigated in cooked, raw and stored peas and flavoring components mainly comprised of methoxypyrazines, ester derivatives, alcohols, ketones, aldehydes as well as unsaturated alcohols. Similarly, in another report by Azarnia et al. (2011), have also reported that storage exerted significant influence on flavoring compounds of peas. Moreover, to the best of our knowledge, no published report until now has reported on retention of volatile flavoring compounds in pea protein isolates or concentrates or novel plant proteins ingredients. Hence, there is a need to innovate such production processes that ensure achieving yields of neutral products with bland taste profiles. Masking of flavoring profiles and taste attributes have been proved less successful in obtaining value-added plant protein products. Bitter after taste masking is acceptable, however, masking of off-flavor aromatic compounds is a complex process as the formation of aroma is governed by complex processes and involved patterns from wide array of receptors as compared to taste patterns which involve underpinning mechanistically single receptor type (Ismail et al. 2020).

Contemporary Technological Advancements for Alternative Protein Products

Alternative protein production from plant-based proteins is aimed at intended consumers to experience products similar to meat products accompanied by mimicry of taste, flavor, appearance, composition and structure to that of animal-based proteins. Complete generation of animal meat structure in reproducible manner is quite challenging when we take into account plant-based protein ingredients (Tolouie et al. 2018). Therefore, researchers around the globe has studied plant-based protein alternatives exhibiting functional and nutritional characteristics similar to that of animal proteins. Moreover, food scientists and technologists are working intensively on processing/structuring techniques to produce 100% plant-based protein products with desirable sensory attributes and provision of eating and appearance sensation similar to meat products. Various techniques are reported to produce traditional plant-based protein products with simple processing operations like chemical-based protein coagulation, fermentation, heating, pressing, steaming, washing and cooling (Malav et al. 2015). In recent years, modern processing techniques have been developed, such as three-dimensional (3D) printing, shear cell technology and extrusion. Continuous efforts are in progress to improve these processes as well as exploration is also underway regarding innovation of applicable processing technologies for plant-protein product manufacturing (Ismail et al. 2020).

4.2.4 Alternative Plant-Based Proteins Meat Production through Extrusion

Extrusion is a thermochemical process in which mechanical shear, pressure and heat are employed in synergistic manner. Several protienous raw materials of plant origins are being utilized during extrusion, including peanut protein, pea protein isolate and concentrate, defatted soybean meal, soy protein isolates and concentrates (Kyriakopoulou et al. 2019). On the basis of addition of water during extrusion, two types of processes have been reported, such as (1) high-moisture extrusion in which addition of water is ranged approximately 50–80% and (2) low-moisture extrusion which involves addition of water ranged 20–40%. In most cases, texturized proteins obtained through low-moisture extrusion need typically rehydration prior any further use with other ingredients. Whereas, proteins extrudates formulated through high-moisture extrusion do not require any processing prior to their usage as food products. Extrudates protein products exhibit significant functional characteristics, water and oil absorption capacities (if formed through low-moisture extrusion), particle size, shape, bulk and tapped densities (Ismail et al. 2020). Other important factors include secondary cutting, initial feed rate, die selection and specific extrusion conditions. Proteins flakes with low density may result in quick rehydration as compared to mince accompanied by slight loss of firmness. Excessive product expansion may result in formation of mush after re-hydration, during eating or processing. On the other hand, protein products with lower expansion may experience slower rehydration and seems to be hard chuck to intended consumers (Zhang et al. 2019; Kyriakopoulou et al. 2019). Protein extrusion involves preconditioning as a vital step that allows penetration of moisture to protein particles in uniform manner before their incorporation into the extruder. Elevated temperature and pressure during extrusion cause protein denaturation and melting. With movement of denatured proteins through screw, exposure of binding sites allow proteins to exhibit cross-linking. Such cross-linking in new way causes texturization of proteins and facilitate convention of globular protein structures to configurations resembling fibrous meat structures. Apart from generation/mimicking of meat-like structures, protein components may undergo changes in their flavors and colors. Most of the off-flavoring volatile compounds will dispose-off along with moisture evaporation during pressure release phase at end of extruder. Hence, extrusion is a versatile production method to improve proteins nutritional quality, however process control is one of the main challenge still posed until now and design specifications of proteins extrudates have not been defined in detailed manner (Ismail et al. 2020; Zhang et al. 2019).

4.2.5 Shear Cell Technology for Thermo-Mechanical Structuring of Plant-Based Proteins

A group of researchers based at Wageningen University, Netherlands first introduced this shear cell technology. This technique was primarily employed for formulation of plant-based meat analogues through combination of heat and shear and plan prions were prepared with layered fibrous structures that resembled to those of texture and mouth-feel of animal-based meat steak. The particular device which was used during this process was termed as shear cell whereby it was possible to apply shear stress in intensive manner. There are two kinds of reported shear cells; (1) cylindrical shape-Couette cell (originally developed for purpose of scaling-up) and (2) conical cell (on the basis of cone-plate rheometer) (Ismail et al. 2020). Overall, the structural configuration of finished protein products was reliant on the processing parameters and type of ingredients used. During shearing, well-defined product deformation occurs with low input energy requirement for structuring, hence shear cell technology may lead to plant proteins product with least variation in product quality in comparison with extrusion (Krintiras et al. 2016; Manski et al. 2007). Shearing capacity of the shear cell could be increased by increasing length and size of the Couette cell. In this regard, various combination based on plant proteins, such as wheat gluten with soy protein isolate and soy protein concentrate, and pectin and soy protein isolate were evaluated through use of shear technology for formulating fibrous protein structures (Dekkers et al. 2016; Manski et al. 2007). However, still the shear cell technology for production of plant-based proteins as potential alternative of meat cannot be scaled-up to commercial scale.

4.2.6 Three-Dimensional (3D) Printing for Developing Plant-Based Cultured Meat Products

3D printing has evolved in recent years as one of the most innovative and versatile technological development of ongoing era. 3D printing has encompassed wide array of practical applications in manufacturing sector in terms of prototyping. In 3D printing, usually recreation of muscle-like matrix is carried out though combining micro-extruding filaments originated from plant-based paste (Ismail et al. 2020). In 3D printer matrix, placement of this plant-based paste is usually performed through using a modeling software named Auto Computer-Aid Design (AutoCAD) (Carrington 2021). In another instance, NOVAMEAT food technology company has already initiated the production of plant-based meat products through 3D printing application and it was also announced by the company that recreation of steak with combination of pea protein, rice protein, seaweed, rapeseed fat, and beetroot juice was carried out to produce meat-analogue products exhibiting firm and fibrous texture with meat-like appearance (Liu et al. 2017; Carrington 2021). Another venture capitalist named Redefine Meat based in Israel also claimed to produce the meat-like products using plant-based proteins—mimicking the appearance, taste and flavor of animal-based muscle meat (Askew 2021). Usually the substrates are employed in the 3D printing of plant-based protein products and variety and speed of substrate may offer a significant opportunity to innovate plant-based protienous products in developing functional foods (Ismail et al. 2020).

4.2.7 Delivery of Protein Bioactive Ingredients by Micro- and Nano Particles

Better targeted delivery of bioactive peptides might be accomplished through micro and nanoscale particles formation with provision of properties like easy surface modification, scale-up feasibilities, microencapsulation etc. (Joye et al. 2014; Cirkovic Velickovic and Stanic-Vucinic 2018). Nanoparticles are usually exhibit greater preference over micro-particles for targeted nutrient delivery as nanoparticles have high extent of penetration in sub-mucosal tissue layers and possess high-nutrient bioavailability (Grancieri et al. 2019). Plant-based nutrient delivery systems in terms of micro or nanoparticles have several advantages, such as biodegradability, improved *in vivo* safety status, high loading capacity for plant-based peptides owing to multiple binding sites, amphiphilic structures possible binding mechanisms involving hydrophobic and electrostatic interactions, covalent and hydrogen linkages (McClements et al. 2007; Sagalowicz and Leser 2010; Joye et al. 2014; Pojić et al. 2018). Delivery of protein bioactive ingredients are described for various plant-based proteins in following sections.

4.2.8 Zein from Corn Gluten Meal

It is usually derived from corn gluten meal which exhibit α-helical structural conformation. Self-assembling capacity of zein protein form it an ideal candidate in formation of mesostructures with wide range of solvents and this peculiarity have significance with respect to processed foods and pharmaceuticals (Wang and Padua 2012; Wan et al. 2015). For hydrophobic active molecules, zein-based protein delivery systems have shown promising potential as compared to other plant proteins. In a report by Wang et al., self-assembled zein structures were used for encapsulation of lime and citral flavors in food, cosmetic and pharmaceutical industrials (Luo and Wang 2014; De Vries et al. 2014). For improvement of lading capacity of zein, a novel method of fabrication of hollow zein nanoparticles was developed by Yang et al. (2014) by employing sodium carbonate as sacrificial template for metformin delivery. Encapsulation of non-polar bioactive ingredients can be easily accomplished by zein nanoparticles occurring in dissolved state with aqueous alcohol solution (Zhong and Jin 2009a). Until now, published literature offer several examples of exploitation of zein-based micro and nanoparticles for various applications like stabilization, encapsulation, controlled release of targeted bioactive ingredients, such as food-grade antimicrobials, polyphenols, functional micronutrients, some food-coloring agents, polyphenols and bioactive lipids (Zhong et al. 2009b; Zhong and Jin 2009a; Chen et al. 2014).

Recently, some technological innovations pertaining to food processing have been developed for application of zein nanoparticles on industrial scale. Among these scalable approached include electrospraying, supercritical anti-solvent, and spray drying (Zhong et al. 2009a; Wan et al. 2015). In another research by Zhong and Jin (2009b), spray-dried zein microcapsules were prepared to render controlled release of antimicrobials including nisin, thymol, and lysozyme. Through supercritical anti-solvent process, the zein microparticles were also synthesized for controlled lysozyme release during extended period of 36 days. Similarly, controlled lutein release might also be achieved by means of using lutein-zein nanoparticles solution enhanced dispersion by supercritical fluid (SEDS) technique (Torres-Giner et al. 2010; Wan et al. 2015). Industrial scale-up of these techniques involve certain limitations. For example, the spray drying has not promising potential for encapsulation of temperature-sensitive bioactive compounds (Van Leeuwen et al. 2014).

4.2.9 Soy Proteins from Soy Oil Processing

Soy proteins are usually produced as a by-product from soy oil processing. Various food processing methods cause aggregates of proteins with diverse structures and functionalities. Along with zein, soy proteins-based micro-particles and nanoparticles are also employed in innovating nutraceutical delivery system (Guo et al. 2012). Several techniques including cold gelation, co-acervation and spray-drying have been reported to fabricate soy protein isolate (SPI)-based micro-particles (Wan et al. 2015). One such instance reported by Chen and Subirade (2009), cold gelation method was utilized to prepare SPI/zein complex to facilitate nutrient delivery of hydrophilic nutraceuticals (riboflavin). As compared to pure SPI or zein microspheres, the SPI-zien complex exhibited higher sustained riboflavin release for period of more than 4 h under both prandial and fasting states (Chen et al. 2010). Incorporation of SPI/zein microspheres into yogurt led to delayed release of riboflavin which would consequently enhance likelihood of gastric-sensitive nutrients for intestinal absorption (Tapal and Tiku 2012). Therefore, the exploitation of SPI/zein complex microspheres has promising potential to utilize as a nutrient-delivery vehicle to formulate novel functional foods, such as vitamins-enriched yogurt and potential carriers for hydrophobic and hydrophilic bioactive components, like vitamin B-12, cranberry polyphenols, resveratrol, curcumin, etc. with improved bioavailability, stability and water solubility (Teng et al. 2012; Roopchand et al. 2013; Wan et al. 2014). In another example, carboxymethyl chitosan and SPI nanoparticles were fabricated through Ca^{2+} induced co-gelation method for targeted delivery of hydrophobic vitamin D-3 (Liu et al. 2016; Teng et al. 2013).

4.2.10 Barley and Wheat Gliadins Proteins

Gliadin protein from wheat has also been employed for fabrication of nanoparticles to improve nutrient delivery systems and controlled release applications. In on such instance, gliadin nanoparticles were effective used to serve as carriers for trans-retinoic acid (Wan et al. 2015). The gliadin nanoparticles exhibited stability in phosphate buffer for up to period of 4 days, and cross-linking through glutaraldehyde cross-linking further led to enhanced stability of nanoparticles (Ezpeleta et al. 1996). However, gliadin nanoparticles fabricated by anti-solvent precipitation showed pH, temperature and salt-concentration stability over narrow ranges, and hence their commercial application are limited pertaining to food processing (Duclairoir et al. 2003). Moreover, researchers also fabricated barley protein-based micro-particles by pre-emulsification and micro-fluidizing without involving use of any organic solvents and cross-linking (Wang et al. 2011). Micro-particles showed improved oil loading and encapsulation efficiencies and could be employed to protect fish oil from oxidation. In another study, barley proteins showed protection for encapsulated β-carotene in harsh gastrointestinal environment and facilitated steady release of β-carotene (Wan et al. 2015).

5 Conclusion and Future Perspective

As the population is increasing, the global demand for protein ingredients and protienous products is on verge of rise across the globe. Comparatively, plant and animal-based proteins differ significantly with respect to their functionality and quality. The supply chains across the plant-based proteins must keep pace with technological innovations in food science and technology keeping in view novel protein sources. Evolving innovative technologies like extrusion, shear cell technology and 3D printing have widen the spectrum to produce plant-based protein products by manufactures to mimic the taste, flavor, appearance, texture as well as eating experience resembling to that of animal-based proteins. The most challenging task is to improve the taste and texture profiles of plant-based finished products to mimic taste similar to animal products. Still, the plant-based ingredients proteins are limited for manufacturers, such as soy, chia, pea, cacao and hemp proteins. Further research is needed to develop the eco-friendly protein extractions methods and improvement of existing delivery systems like hydrogels, films, fibers, nano- and micro-particles-based nutrient delivery modalities.

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Chapter 5
Enrichment and Fortification of Traditional Foods with Plant Protein Isolates

Mian Kamran Sharif, Makkia Saleem, Hafiz Rizwan Sharif, and Roma Saleem

1 Importance of Plant Proteins

The plants have always been part of the human diet to provide energy and nutrients for sustainable living. These are considered as the chief source of carbohydrates and proteins for human and animals due to their cost effectiveness, consumer acceptability, functional properties, sustainable production and being environment friendly. Plant protein refers to the protein from terrestrial plant origin. In most cases, plant protein resides in the seeds and grains of cereals, pulses and oilseed crops. These are usually consumed after milling, dehulling and oil extraction. The plant proteins are considered poor man's meat especially in developing economies. The health-conscious consumers are shifting their diets towards plant-based sources due to potentials health benefits, quality of life and longevity. The right combinations of plant proteins can supply sufficient essential amino acids to meet the human health requirements. In addition to their role as a macronutrient, plant proteins play an integral role in the structural formation of foods through processes such as emulsification, foaming, gelation and dough formation. Concerning land use, if the same amount of plant proteins is used directly for human consumption, less than 10% of the land will be required to grow food crops than that of required for feed crops to produce the same amount of animal proteins (de Boer and Aiking 2011). Furthermore, the production of animal proteins requires about 100 times more water than producing an equal amount of plant proteins (Pimentel and Pimentel 2003).

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The developing countries are greatly challenged by food and nutrition insecurity. With escalating population, this has emerged as a mega challenge for the agri-food industry. The shift towards a more sustainable diet necessitates less reliance on foods of animal origin and thus presents a huge potential for the agri-food industry to explore alternative sources of proteins (Aiking 2011). The development of new meat analog products has accelerated in recent years, with some of the most promising alternatives based on proteins from plant sources, such as soy, peas etc. Plant protein-based meat and dairy substitutes can deliver equivalent quality at lower costs while fulfilling the world's priority of reducing greenhouse gas emissions and limiting the destruction of forest land (Linnemann and Dijkstra 2002; Dijkstra et al. 2003). Moreover, the growing trend of being flexitarian increases the utilization of plant-based proteins to fulfill protein requirements. Flexitarian term refers to those vegetarians who occasionally consume animal foods. These consumers purposely configure their diets to eat less meat and more plants. This trend has increased the demand for non-meat protein sources and meat analogs that contained any vegetable proteins. This trend has engendered several business prospects, as recently numerous new firms have been emerged and start taking benefit of this movement. These companies are collectively developing various vegetarian options including beverages, meat analog, snacks, desserts and salad dressings.

Although plant proteins are relatively cheap and more abundant than that of animal proteins, their direct consumption in conventional human diets is still limited. Currently, most of the plant proteins are used as animal feed to produce functional animal proteins in the form of milk, meat and eggs. The conversion of plant proteins (e.g., from grains as feedstock) into animal proteins is inherently inefficient. In some cases, less than 15% of the plant proteins from feed crops are turned into animal proteins for human consumption and about 85% are wasted (Aiking 2011). Consequently, meat production is responsible for a disproportionate share of food-related environmental pressure (Gilland 2002). There is an urgent need to increase the use of proteins from a wide range of plant sources directly for human consumption. Increasing utilization of plant protein is required to support the production of protein-rich foods that can replace animal proteins in the human diet to reduce the strain that intensive animal husbandry poses to the environment.

There are multiple reasons why plant proteins are still underutilized as human food. This might be due to low nutritional value (on a single source basis) compared to animal proteins, poor functional properties, the economic cost associated with isolation and recovery of protein fractions and presence of some antinutrients. The composite flour technology and the use of food multimixes has significantly improved the nutritional quality of plant-based diets. Cereal and legume blends are widely used for the preparation of complementary foods, bakery flour mixes and diverse range of food products with improved protein contents, antioxidants and dietary fiber. Likewise, considerable development has been made to improve the nutritional and functional properties of plant proteins through research and development. Soy protein serves an excellent example of how scientific research can increase the consumer's awareness about the nutritional value of plant proteins and technological innovations can add value and diversify the use of plant proteins into a wide variety of food products. Consequently, a range of new food products are now available in super stores and retail markets, which utilize other grains, legumes and vegetables as sources of proteins (Asgar et al. 2010). The levels of antinutrients present in legumes, pulses and cereals can now easily be lowered using germination, roasting and numerous other technologies. The innovations in the isolation and extraction techniques have made it possible to obtain concentrated forms of protein like protein concentrates and isolates for diverse food applications.

Protein-energy malnutrition remains to be a problem in developing countries. As the traditional protein sources are usually expensive and are not always available, there is an increasing demand for alternative sources of proteins, which are relatively cheap and widely available. These proteins especially in the form of protein concentrates and isolates are now widely utilized by the food industry to increase the nutritional value of food products with negligible increase in cost and loss to quality attributes. There are several reasons for utilizing protein isolates by the food industries. These are rich sources of high-quality protein with higher essential amino acid contents. Isolates from various plant sources such as legumes, cereals and others have different compositional, physiochemical and functional properties. They are considered satisfactory constituents in the food industry because of their functional characters such as acceptable flavour, colour, particle size, fine dispersibility, emulsion stability and emulsification. Finally, plant-based proteins are economical and environmentally sustainable compared to animal-based proteins. Hence, these isolates are utilized to fortify various foods, such as baked products, pasta items, meat analogs and milk alternatives, to improve their nutritional, compositional and functional characteristics (Garba and Kaur 2014).

2 Potential Sources of Plant Proteins and Isolates

The legumes, cereals and oilseeds are usually considered as preferred sources for the extraction of protein concentrates and isolates. The typical protein contents of major cereals, legumes, oilseeds and other vegetable sources are given in Table 5.1. Most of the developed countries are producing these ingredients as coproducts, while extracting edible oil from soy, canola, sunflower and starch especially from the cereals and tubers. The characteristics of protein isolates differ based on their parent sources and extraction techniques. The major sources of plant protein concentrates alongwith their production potential and food applications are discussed below:

Table 5.1 Typical protein content of major vegetable sources

Cereals	Protein content (%)	Other constituents
Soy	35–40	20% oil; 30% non-starch polysaccharides
Lupin	35–40	10% oil; 35–40% non-starch Polysaccharides
Pea	20–30	60–65% starch; 5% non-starch polysaccharides
Chickpea	20–25	60% starch; w10% non-starch polysaccharides
Canola	17–26	40% oil; 12–30% non-starch Polysaccharides
Wheat (flour)	8–15	75% starch; 1–2% lipids 5% non-starch polysaccharides
Rice	7–9	90% starch
Maize (corn)	9–12	70–75% starch; 3–18% oil (from the germ)
Barley (dehulled)	8–15	60–64% starch; 23% lipids, 3–10% soluble dietary fibre (in which 4–6% beta glucan) and 11-14% insoluble dietary fibre
Sorghum	9–17	2% lipids; 70–75% starch

2.1 Legumes

Legumes are eatable seeds, which include pulses like beans, chickpeas, mung bean, lentils, peas etc. In 2017, the global production of pulses was around 55 million metric tons, up from 50.19 million metric tons in 2015. Beans are considered as the most commonly produced type of pulses that is apparent from their production in 2017 (22.55 million metric tons). The production of lentils was 13.34 million metric tons during the same year (Statista 2017). The numerous countries are cultivating legumes to cater the needs of local population whereas surplus commodities are exported to net users. Likewise, there are great variations among the countries with respect to utilization in daily life. Based on a comparison of 128 countries, Niger was ranked at the top in per capita pulse consumption (34.3 kg) followed by United Arab Emirates and Ethiopia. On the other hand, the lowest consumption was noticed in Uzbekistan (0.031 kg), Romania (0.051 kg) and Poland (0.053 kg), respectively (FAO-UN 2019).

2.1.1 Soy

Soy is unique among the legume species due to its edible oil and high quality protein (35–40%). Soy is the second largest source of edible oil in the world superseded by palm oil. Besides edible oil and protein, the other valuable compounds present in soy include isoflavones, oligosaccharides, phospholipids, polysaccharides, minerals and vitamins. However, the concentration of protein, oil and other compounds vary depending on the geographic location and varieties. Although, soy is grown globally, the primary producers are in the Western hemisphere. In 2020, the global production of soy was about 362.85 million metric tons (USDAFAS 2020). Nearly 80%

of the global soy production is from Brazil, United States and Argentina. Nearly 86% of the total global output is used for processing to produce oil and defatted soy meal, whereas 7% is directly used for feed and remaining 6% as food including tofu, soy milk etc. (Fig. 5.1). The schematic diagrams for the production of defatted soy flour, soy protein concentrate, and soy protein isolates are presented in Figs. 5.2, 5.3, 5.4, and 5.5, respectively. Defatted soy meal is mostly used as animal feed and only small amount is used for human consumption (Alibhai et al. 2006; FCRN 2020). Most of the oil from soy is used by human consumption. In East Asia and Western countries, soy is used to produce protein rich products having well-balanced amino acid composition that potentially replace dairy and meat proteins. The mature soy is mainly comprised of storage proteins β-conglycinin and glycinin, which account for about 65–89% of the total seed proteins. These proteins are also known as globulin proteins and are the major constituents in soy protein isolates and concentrates. Additionally, lectins, trypsin inhibitors, and lipoxygenases are also present in seeds. Numerous purification processes are adopted for their inactivation or removal; however, these may affect the nutritional and hedonic attributes of soy protein ingredients. Although soy protein is by far the most utilized plant protein, still its utilization is not as high as anticipated. Currently, about 5–6% of the total soy production is utilized for human. However, it is expected that the utilization of soy and its coproducts will increase in future due to evidence-based research related to their potential health benefits against chronic diseases and alleviation of protein-energy malnutrition, especially in developing countries (Friedman and Brandon 2001; USB 2020).

The modern soy processing industry produces soy flour, soy concentrates, and soy protein isolates as soy protein ingredients, which are classified based on their protein content. Among these ingredients, soy flour has the least protein content whereas soy protein isolates are considered as the richest source of plant protein. These ingredients are usually produced in dry powder or granular form and

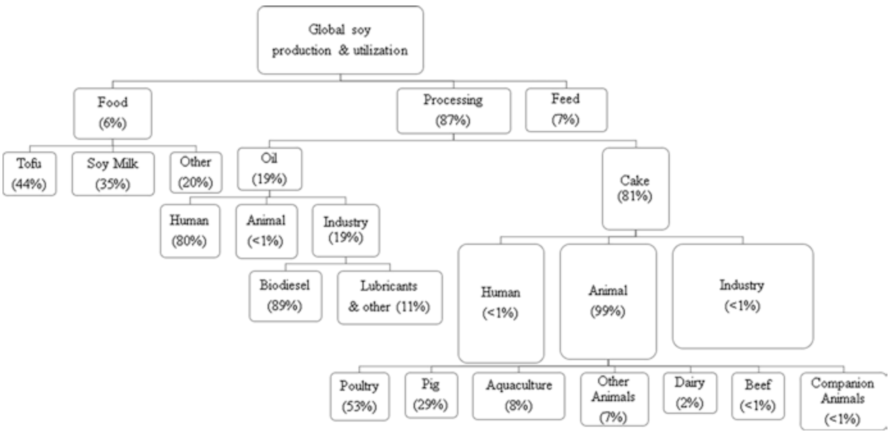

Fig. 5.1 Global production and utilization of soy

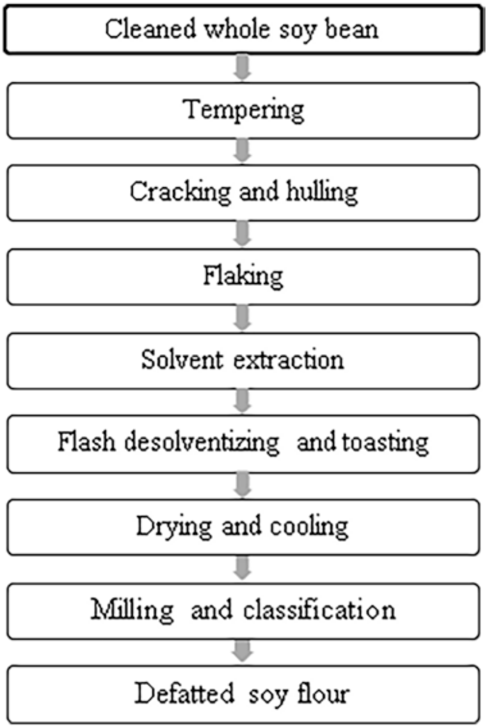

Fig. 5.2 Preparation of defatted soy flour from whole soybean

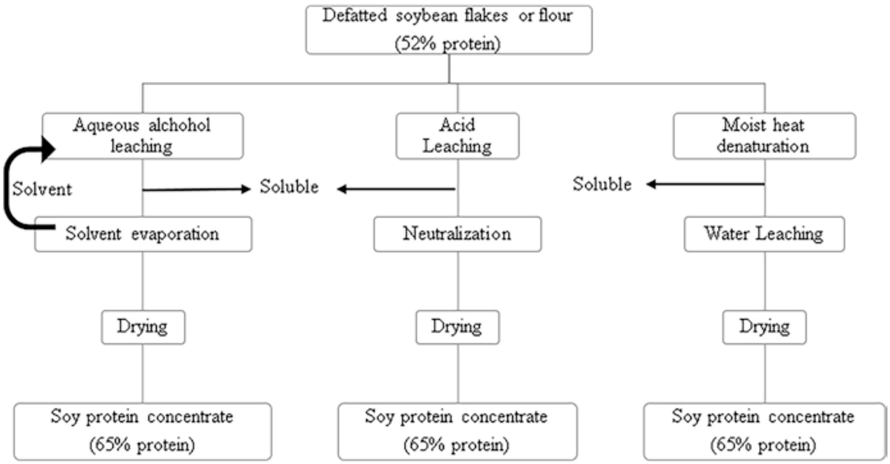

Fig. 5.3 Preparation of soy protein concentrate from defatted soybean flakes or flours through different methods

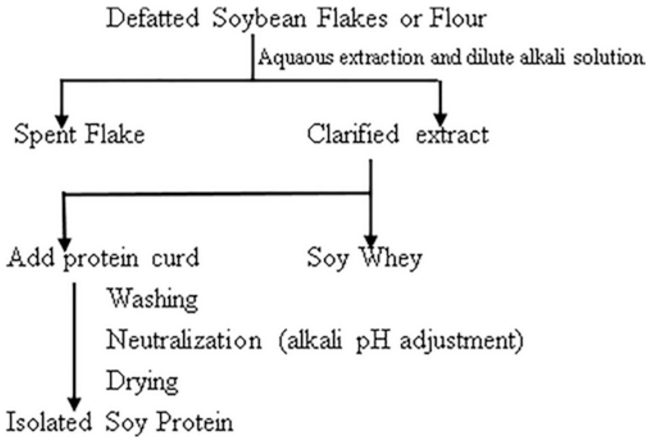

Fig. 5.4 Preparation of soy protein isolates from defatted soybean flakes or flour

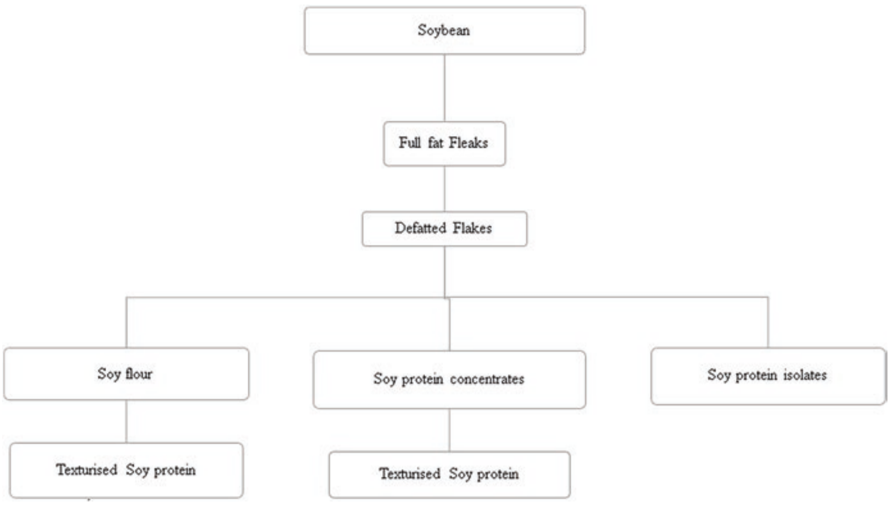

Fig. 5.5 Preparation of soy flour, soy protein concentrates and soy protein isolates from soybean

product's features like dispersion and wettability, water holding capacity and gelation, emulsification, fat binding, or low viscosity at high solids are demarcated by the potential food applications. After removal of the most of the soluble cell wall materials, soy protein concentrates with more than 65% protein content are manufactured from defatted soy flakes. Soy protein isolates are the most refined form of soy protein (>90% protein content) and are usually produced through alkali extraction and isoelectric precipitation techniques. Textured soy protein (TSP) is produced using extrusion technology and their texture has resemblance with meat chunks.

These are used as a protein source in vegetarian meat alternatives by partially or totally replacing the animal proteins in various food products. Soy flour, soy protein concentrate, soy protein isolate and their texturized products, are mainly used as ingredients in formulated foods for their functional properties. Soy protein products are used to extend or replace animal proteins. Soy protein is also used as a protein source in infant formulae. Soy milk is used for replacement of cow milk by vegans and persons with intolerance to milk protein.

2.1.2 Lupins

Lupins are non-starch leguminous seeds with comparable protein content to soy (Evans et al. 1993). These are grown for human and animal consumption and have been used in cosmetics and medicines in ancient Greece and Egypt before 2000 BC. Lupines are unique among the peas, beans, and lentils due to their high protein content (30–40%) and complex carbohydrates. Globally more than 85% production is from Western Australia, which has now become the largest grower and exports most of it as animal feed. Australian sweet lupin (*Lupinus angustifolius*) is more suitable for direct human consumption due to low alkaloid contents. Currently, less than 4% of global production is consumed as human food. It has been estimated that about 0.5 million tonnes of food containing lupin ingredients is consumed annually in the European Union. Ruminants are the major consumers followed by pigs and poultry. There is also an increasing utilization in aquaculture in recent years (Lupins 2020).

The major cultivated species of lupin are yellow lupin (*L. luteus*), blue narrow leaf lupin (*L. angustifolius*), and white broad leaf lupin (*L. albus*). The taste of primitive lupin cultivars was bitter due to the presence of high alkaloids contents. The acceptable limits of alkaloids for human and animal consumption are 0.02%. The presence of quinolizidinic alkaloids prevents the direct utilization of yellow lupins as food, however, these may be removed by simply soaking in water. In contrary, the white lupins, are generally consumed directly in the Mediterranean countries. The processing of 1000 kg lupin batch, delivers 600 kg carbohydrate, 24 kg hulls and 150 kg protein concentrate. When lupin protein is further extracted, the resulting protein isolate is devoid of alkaloids and consequently can be utilized as a functional ingredient in human food. Alkali extraction produces lupin protein isolates. Proteins, fats, and sugars are present in the alkali solution. The extraction is followed by isoelectric precipitation of two classical storage globulins (conglutins α and β) and one albumin (conglutin δ). The supernatant contains another main lupin protein i.e., conglutin γ. Further purification of conglutin γ is done to yield conglutin δ, a 2S sulfur-rich lupin protein (Duranti et al. 2008). Afterwards, the protein and carbohydrate fractions can be spray-dried. Similarly, lupin protein isolates (LPIs) with improved water solubility are manufactured for diverse food applications. Lupin flour and protein isolates have been successfully tested as ingredients in various food products such as in muffins (egg and milk proteins were totally substituted), biscuits (high protein contents), dairy, bakery and meat products (Pollard et al. 2002; Drakos et al. 2007; Duranti et al. 2008).

2.1.3 Pea

Peas have also been exploited extensively as an important source of commercial proteins. Besides their usage as an ingredient in an array of food products, these are widely used in numerous cuisines. These are easy to process due to their week and thin hull. Several types of peas are grown globally for end-use markets. The fresh garden and snap peas are picked fresh (72–80% moisture) for further processing in canning and frozen food industries, whereas Austrian pea and field pea are mostly harvested in dry form (10–15% moisture) for global feed (Elzebroek and Wind 2008) and food applications. Peas are rich in protein, carbohydrates, insoluble dietary fibre whereas their fat levels are extremely low. The protein content of peas is more than 25% with wide variation among the species and varieties. Peas are commercially grown in Canada, Russia, the United States, France, and Australia within variable growing and soil zones. Since 1970s, the cultivation of dry peas in the Western Canada has increased many folds as a result of Canadian government initiatives. Similar growth patterns of pea production have been observed in USA since 2000s. The US farmers increased dry pea production in the Western North Dakota and Eastern Montana after pea's inclusion US food Aid Programs (Tulbek et al. 2017).

Besides regular use in various cookeries, peas are commercially used for the production of pea flour, pea protein concentrate and pea isolates for an array of food applications (Pavek 2012). Whole peas are processed by cooking, canning, frying, or milling processes depending upon their color, size, shape, uniformity, soaking quality, and canning properties. Split peas are obtained after dehulling of whole peas and are mainly utilized in soup, and global food aid supply. Pea flour is made from dehulled whole or split peas into numerous diverse granulations based on end-product utilization such as baked goods, extruded snacks, pasta, and extruded and canned pet food. The main constituents of pea flour include protein (22–28%), starch (40–53%), and dietary fiber (6–20%) based on cultivar, processing technology, and particle size distribution. Pea flour is frequently used as a source of protein in cereal-based composite flours (Huisman and Van der Poel 1994). The functional attributes of pea flour such as water- & oil-binding, emulsification, gelation, and texturizing, make it an ideal ingredient for combination with cereals, pulses, meat, and gluten-free formulations. Pea flour is widely used in extrusion processing as it can withstand longer cooking times, shear, and thermal stability. The retrogradation of pea amylose generates a firm gel. Pea protein concentrates and isolates with 48% and 90% protein contents can be produced through dry- and wet-milling technologies, respectively (Boye et al. 2010). Pea seeds can be processed into protein and starch isolates through different technologies such as isoelectric precipitation and extraction, water/salt-based extraction and enzymatically assisted isoelectric precipitation and extraction (Tulbek 2014).

2.1.4 Chickpea

It is one of the most noteworthy pulse crop consumed in the Indian subcontinent. It provides same amount of protein that is available from peas. Chickpea protein and starch are valuable sources of daily diet due to their versatile functionalities (Ma et al. 2011). In 2017, the global production of chickpeas was around 14.78 million metric tons whereas India was the chief producer with nine million metric tons (60% of the total supply) share in the global supply. The second leading county is Australia, which contributes around two million metric tons in the global supply. Nevertheless, India is the leading producer of chickpea but at the same time it is also the principal importer due to high domestic demand. Australia with 40% share is at the top among the chickpea exporters (FAO-UN 2019). Chickpea protein isolates with good emulsification properties are manufactured from chickpea flour using procedures similar to those used for pea proteins (Boye et al. 2010; Karaca et al. 2011). Similar to other pulse flours, chickpea flour is widely used as an extender in emulsified meat products due to its quality protein, superior technological functionality and minimal effects on flavor (Sanjeewa et al. 2010).

2.2 Cereal Grains

2.2.1 Wheat

Wheat is one of the most important cereal crop consumed by more than one billion people to obtain substantial amounts of calories and protein compared to any other foodstuff (USDA-FAS 2012). The chemical composition of wheat has revealed presence of about 8–15% protein depending on variety. According to Food and Agriculture Organizations (FAO), the annual production of wheat is around 761.5 million metric tonnes. It is the third most cultivated cereal after corn and rice. Wheat is cultivated on 224 million hectares worldwide. The major wheat producing countries include the EU (153 million tonnes), China (133 million tonnes), India (102 million tonnes), Russia (59 million tonnes), the USA (55 million tonnes), and Canada (29 million tonnes). Most of the wheat production is converted into flour for food consumption, whereas a handsome amount is used for animal feed. Additionally, approximately eight million tons of European wheat is processed into wheat starch and protein with the production of approximately 560,000 tonnes wheat gluten (FAO 2019).

Wheat proteins are distinguished from their structure (monomeric vs polymeric) and solubility or insolubility in water and alcohol. The monomeric proteins are albumins, globulins and gliadins whereas the polymeric proteins are mainly in the form of glutenins. Gliadins and glutenins are the major (75–80%) wheat proteins. When gluten is extracted from wheat flour, albumins and globulins are mainly removed in the washing water (MacRitchie and Lafiandra 1997). Wheat gluten is a natural protein derived from wheat or wheat flour. Once dried, it has a creamy color, neutral taste, and is free flowing. After rehydration, dried gluten is able to recover its unique viscoelastic structure. There are 3 types of gluten obtained from processing of wheat i.e. vital wheat gluten, devitalized wheat gluten and solubilized wheat proteins. The vital wheat gluten is also known as wheat gluten. It exhibits high viscoelasticity after hydration and its protein content is >80%. The devitalized wheat gluten is also known as devital wheat gluten. It has protein content >80% but reduced viscoelasticity due to denaturation. The solubilized wheat proteins are also famous as soluble wheat proteins. These have >60% protein content; however, viscoelasticity is too low due to partial hydrolysis of wheat gluten.

Wheat gluten is a vital ingredient in leavened baked products as it forms a unified, viscoelastic proteinaceous network necessary to produce leavened products (Wrigley 1996). Commercial gluten is manufactured through simple physical separation of wheat flour since 1850s. Today, modified gluten with enhanced functionality is commercially produced through chemical or enzymatic treatments of normal gluten. This process further elevates the protein content of the normal gluten. The key usage of gluten in developed countries is in baked products, breakfast cereals, pasta, and noodles (Day et al. 2006; Day 2011). For this purpose, wheat flour is fortified with gluten by flour millers and bakers to achieve the desirable protein content. Gluten is also used as a fat and water binding constituent in restructured poultry, meat, and fish products. It is frequently used as a meat replacement in vegetarian foods, and in the preparation of analogues of expensive foods such as seafood and crab meat, mostly in Japan. Texturized wheat proteins, manufactured through extrusion processing, are widely used as meat replacement. These exhibit the same look and texture of meat products (Day 2011).

2.2.2 Rice

Rice (*Oryza sativa L.*) is the second largest grown cereal crop worldwide. It is the most universally eaten dietary staple for nearly half of the global population (>3.5 billion people) mostly from Asia, Africa, some parts of Latin America, and the Caribbean. Rice plays vital role in the food and nutrition security of developing countries. The major portion of production (~95%) is used for human consumption (World Atlas 2020). In 2016/2017, the worldwide per capita consumption of milled rice was approximately 53.7 kg. In 2018/2019, approximately 486.62 million metric tons of rice was utilized worldwide. The daily per capita consumption of rice is the highest among the Asian nations (Statista 2021). About 50% of the global rice is consumed in China and India (FAO 2000). The Chinese utilized around 143 million metric tons rice in 2019/2020 followed by India (100 million metric tons). India was the top exporter of rice in the year 2018/19. India exported about 12.5 million metric tons of rice in 2018/19 followed by Thailand (7.56 million metric tons). Although China is the top producer of rice globally, its consumption of rice cannot be gratified by national production. In 2018, the estimated global paddy rice production was around 782 million metric tons whereas milled rice yield was 495.9 million metric tons.

Among the cereals, rice has the lowest protein content (7–9% by weight); however, its regular consumption in South and Southeast Asia, make it one of the major sources of protein (Shih 2003). It not only supplies substantial amounts of dietary protein to about 520 million people living in Asian region but also provides up to 50% of the daily caloric supply (Muthayya et al. 2014). It is considered one of the most abundant vegetable protein source in Japan (Kubota et al. 2013). Rice production in Asia has deep sociopolitical and sociocultural roots and accounts for about 90% of global production. Paddy is the end product that is obtained from harvesting and threshing of the mature rice plant. On average, paddy rice produces 25% husk, 10% bran and germ, and 65% white rice (Chen et al. 1998). Rice endosperm has only a small concentration of protein and very low levels of minerals, vitamins, or oil. Rice bran has more protein content (>13%) than that of milled white rice (Satter et al. 2014). However, it can provide substantial amounts of vitamins, mineral and dietary fiber when consumed in the form of brown rice.

Rice is not an ideal commodity for the production of protein as compared to soy or other pulses (Shih 2003). It is considered as an expensive starting material for the production of protein rich products. However, co-products of rice milling industry such as rice bran, broken rice kernels, and residue of rice starch extraction, are used as inexpensive sources to obtain rice protein. The rice protein concentrates, isolates, milk etc. are now available in the geographical regions like North America where rice consumption is low. The addition of rice proteins in rice based infant formula can improve their protein quality. Historically, the most economical and sustainable use of rice bran is in animal feed formulations (Hoogenkamp 2015). Rice bran oil is a high value product obtained from rice bran through solvent extraction. Afterwards, defatted rice bran is an ideal ingredient for the preparation of rice bran protein concentrates and isolates. However, rice bran proteins are less soluble in water. Numerous methods are now available to obtain protein products from stabilized rice bran. Physical processes like colloidal milling, homogenization, and highspeed blending are frequently used to release and concentrate rice bran proteins without forming chemical artifacts. These methods provide shear forces to disrupt cell wall leading to cell lysis rupture of intercellular membrane structure. In case of broken rice enzymes like α-amylase, gluco-amylase, and pullulanase assists in the disintegration and removal of starch thus producing protein concentrates (25% protein) and isolates (90% protein).

2.2.3 Maize

Maize or corn (*Zea mays*) is one of the most significant food and industrial crop especially in developed countries. Its protein content ranges from 9% to 12%. In 2018–19, the global production of corn was over 1.09 billion metric tons. The United States of America is the global leader in corn production (345.89 million metric tons) followed by China and Brazil. Likewise, the share of US in the international corn export is one third of the total corn exports. In 2019–20, the US export was over 47.5 million metric tons. Japan and Mexico with 9.6% and 8.5% of the total corn imports are the largest importers of corn from USA (Workman 2020). Furthermore, the United States of America (12.3 billion bushels of corn) and China (10.98 billion bushels of corn) are the chief consumer of corn worldwide (Statista 2020a).

By 2027, the corn utilization is expected to surge by 16% due to fast expanding livestock sector in developing countries. Likewise, human consumption of corn is expected to boost especially Sub Saharan Africa due to escalating populations which utilize white maize as dietary staple (OECD-FAO 2018–2027). In developed countries like USA, major portion of the total yield (about 50%) is used directly as animal feed whereas around 25% is used for ethanol production. Currently, only a small amount is consumed by human in the form of corn chips, tortillas, corn syrups. Corn is commercially processed by using two methods i.e., dry and wet milling. In case of dry milling, corn flour is one of the major products which is used in diverse products such as muffins, doughnuts, pancakes, breading, batters, baby foods, meat products, and some fermented products. On the other hand, starch and oil are obtained after wet milling of corn. Corn gluten meal (with a protein content of 60%) is also obtained as protein byproduct. Afterwards, this corn gluten meal is used for the preparation of zein protein (Shukla and Cheryan 2001). Zein has numerous industrial applications; however, it is rarely used directly for human consumption owing to water solubility issues. Hence, the prime applications of zein are as a polymer material for film, coatings and plastics (Lawton 2002).

2.3 Other Oilseeds: Canola and Sunflower

2.3.1 Canola/Rapeseed

Rapeseed is one of the primitive oilseed crop used for the production of edible oil. However, it is not liked by the consumers due to the presence of undesirable components. Subsequently, canola was developed using plant-breeding techniques in order to get rid from these antinutrients. Canola after soy is considered the second most important oil seed crop in the world (USDA-FAS 2012). In 2019–20, the worldwide production of rapeseed oil reached approximately 27.3 million metric tons. Canada with 19 million metric tons yield was the top producer followed by China (13.1 million metric tons). In 2018, the top exporters of rapeseed were Canada ($4.45 billion), Ukraine ($1.01 billion), Australia ($945 million), France ($743 million), and Romania ($569 million) whereas the top importers were Germany ($2.36 billion), China ($2.18 billion), Japan ($1.08 billion), Belgium-Luxembourg ($885 million), and Mexico ($569 million), respectively (OEC 2018; Statista 2020b).

After oil extraction, the meal (a byproduct after oil extraction) is solely used as a protein source in livestock and aquaculture feedstuffs. Canola meal is rich in protein; however, it has comparatively less protein content (17–26%) than that of soy. Globally, the cultivation of canola is on the rise, hence, the quantity of canola meal is also increasing. The high contents of glucosinolates, phenolics and phytates left in canola meal limit its utilization for human consumption (Tan et al. 2011). Canola protein is ranked above several plant proteins in quality indices and is considered rich source of sulphur-containing amino acids and lysine than that of pulses, and cereals, respectively. Based on human efficacy studies, canola protein has been ranked as a high quality protein and declared equivalent with milk and egg protein (Wanasundara et al. 2016). The amounts of essential amino acids in rapeseed protein isolate and protein concentrate are almost same as recommended by the WHO for daily intake in humans (WHO/FAO/UNU 2007). Histidine, leucine, isoleucine, valine, lysine, threonine, phenylalanine and tyrosine were present in sufficient amounts whereas cysteine and methionine were below the recommended amounts (Haar et al. 2014). Oil-free canola meal contains about 36–40% protein on a dry weight basis; however, separation of canola protein from non-protein components like fibre, polymeric phenolics, phytates and sinapine is challenging (Wanasundara et al. 2016). However, these can be easily separated using sophisticated extraction and fractionation techniques such as ultrafiltration and membrane separation. Consequently, canola protein isolates with >80% protein contents have been manufactured (Xu and Diosady 2002; Logie and Milanova 2010). These protein isolates have wide range of food applications in beverages, dressings and sauces, meat substitutes, baked goods and protein snack bars.

2.3.2 Sunflower Seeds

After soy and rapeseed, sunflower is the third major source of edible oil in the world. After lipids, the second most abundant constituent is protein. The dehulled sunflower seeds contain about 20–40% crude protein. After oil extraction, defatted sunflower meal has around 30% protein, which can reach to as high as 53–66% with solvent extraction of edible oil. With increase in global cultivation, this crop is abundantly available for the preparation of protein products like concentrates and isolates. Sunflower protein meets the requirements of all amino acid except lysine. Sunflower protein contains about 20% branched-chain amino acids which are vital for muscle repair. According to the estimates of the United States Department of Agriculture (USDA), the global production of sunflower in 2020–21 was about 56.69 million metric tons (WAP 2020/2021). In 2018, Netherlands ($321 million), Turkey ($298 million), Russia ($291 million), Ukraine ($269 million), and Germany ($253 million was the leading importers of sunflower seeds whereas Romania ($733 million), China ($557 million), Bulgaria ($459 million), France ($444 million), and United States ($333 million) were the major exporting countries (OEC 2018).

Sunflower is mainly cultivated for the production of cooking oil whereas meal (a by-product of oil extraction) is primarily used for animal feed. The meal is even more rich in protein; hence, could be used for human consumption after getting protein rich products. Though the high protein content makes sunflower meal an attractive source of proteins, the suitability for food applications depends chiefly on the oil extraction method. The sunflower meal proteins are denatured to a large extent during the preconditioning, expelling and desolventising processes, rendering not suitable for human consumption (Gonzalez Perez and Vereijken 2007). However, with the advancements in oil extraction and processing techniques, sunflower protein powders with above 50% protein contents are now available for human consumption. Inexpensive, large-scale processing methods to isolate sunflower proteins, however, are currently lacking.

The technological and functional properties of sunflower proteins are comparable with those of leguminous proteins (Gonzalez-Perez et al. 2005). These proteins have low levels of antinutrients and free from toxic compounds; hence, considered as a valuable alternative food ingredients (González-Pérez and Vereijken 2007; Rajasekaran and Kalaivani 2013). However, the usage of sunflower meal protein isolate (SMPI) is limited due to the existence of antinutritional components like polyphenolic substances like chlorogenic and caffeic acids. Consequently, the nutritional worth of SMPI is lower due to their interaction with amino acids like lysine and methionine. After the successful removal of antinutrients, sunflower meal as well as SMPI can be used for diverse food applications replacing the more costly protein sources such as soy proteins (Salgado 2011; Shchekoldina and Aider 2014). The sunflower proteins are mostly incorporated into livestock feed, whereas only a small amount is used in protein powders for human consumption mainly due to the existence of branched-chain amino acids. Limited research is available on sunflower protein functionality for food applications. The sunflower protein concentrate has been used in protein rich food bars, crispbreads, instant soups, smoothies, cookies, and numerous other bakery products (González-Pérez and Vereijken 2007).

2.4 Proteins from Tubers and Nuts

Potato is considered as the king of vegetables due to its usage in global cuisines in a variety of ways. The protein content of potatoes is very low (1–1.5%); hence, these are not considered as a valuable source of protein (Camire et al. 2009). However, potato is an excellent candidate for the production of starch at industrial scale. This process produces potato fruit juice, which contains most of the tuber soluble protein. After extraction and refining, these proteins have great potential for utilization in foods. However, protein recovery process is challenging and expensive. Furthermore, this process negatively affects the functional quality attributes solubility, foaming and emulsifying properties (van Koningsveld et al. 2001; Vikelouda and Kiosseoglou 2004; Bartova and Barta 2009). The potato protein ingredients are now widely used for the preparation of meat free analogs, gluten free bakery products, dairy free ice cream, toppings and desserts. Nuts are another rich source of plant protein. These are rich in oil and protein and primarily are used for edible oil production. The co-products like nut meals, skins and hulls have high levels of protein, fibre and polyphenolics. However, the usage of these coproducts as food ingredients is very limited, primarily due to extreme allergenicity from nut proteins. However, nut protein containing products like almond milk, and intact nuts are very popular among the consumers.

3 Preparation of Plant Protein Concentrates and Isolates

Globally, soy, peas, lupins, chickpea, wheat, rice, maize, barley, sorghum, canola, and sunflower are widely used for the production of protein ingredients in the form of defatted flour, concentrates and isolates. Protein rich plant materials cannot be used in some products beyond a certain level. Hence the concentrated form of protein ingredients like protein concentrates as well as isolates have been developed using different processing techniques such as air classification, water extraction, salt extraction, alkaline extraction, acid extraction, and ultrafiltration. The major industrially produced protein ingredients from plant sources are given in Table 5.2. The protein content and quality of these ingredients can vary depending upon the extraction and refining techniques, composition of raw materials, and processing conditions. The commercially used methods for the manufacturing of protein ingredients are discussed below:

3.1 Air Classification

This is a physical technique, which is in use since the 1980s. The procedure used today for the preparation of commercial protein fractions dates back to 80's. Pulse crops are fractionated into protein- and starch-rich parts. For the purpose, whole as well as dehulled seeds can be used. Seeds are grounded into a fine powder and air is used as a separating agent working on the basic principle of sorting out lighter protein particles from the heavier starch particles. Protein fractions from different legumes and pulses such as lentils, mung beans, peas etc. are separated using air classifiers with varying degrees of success. From the dehulled pulses, the cotyledon is separated and then subjected to milling preferably using pin mills and converted

Table 5.2 Major industrially produced protein ingredients from plant sources

Plant source	Protein products	Protein content
Soy	Soy protein concentrates (SPC) Soy protein isolates (SPI) Texturized soy proteins	65–70% >90% 60%
Peas	Pea protein concentrate Pea protein isolate	85–90%
Wheat	Vital wheat gluten (VWG) Isolated wheat protein (IWP) Texturized wheat proteins Enzyme hydrolyzed protein	75–80% 90% >90%
Rice	Rice protein concentrate Rice protein isolate	w80% 90%
Maize/corn	Zein	88–96%
Canola	Canola protein isolate Hydrolyzed protein	90% 83%

into very fine particles. The adherent protein is derived from the membranes and stroma of the chloroplasts in which the starch granules developed. The starch fraction consists of starch granule entrenched in a protein matrix, hence further purification is carried out by repeated pin-milling and air classification. Grain reduction to fine particles ensures maximum separation of proteins. Afterwards, lighter fine flour fraction, rich in protein, are separated from heavier coarse fraction, rich in starch, through an air classifier. The effectiveness of the air classifier for protein separation is calculated by protein separation efficiency (PSE) as the proportion of the total flour protein recovered in the fine fraction. The efficiency of air classification varies based on type of classifier used and composition of the material to be separated. Materials having more moisture and fat contents tend to decrease PSE (Tiwari and Singh 2012) The mung beans and lentils have been found most suitable pulses for air classification, while lima beans and cowpeas were the least ones. The fractionation process is greatly affected by the pulse characteristics such as rigidity of the cell wall, adhesiveness between the cell contents and the cell wall, and stiffness of the proteinaceous material (Tyler et al. 1981).

During milling, the particle size is very much important as it ensures effective separation during the air classification. The seed is broken to a point that separates proteins and starch. To decreases the particle size, protocols like multiple passes, variations in grinding speed and types of air classifiers are followed for improved yield. With decrease in moisture content, there was improvements in the yield where protein content in protein faction was decreased (Tyler and Panchuk 1982). Another disadvantage of air classification is presence of high levels of oligosaccharides and antinutrients like phytic acid, hemagglutinin and trypsin inhibitors all get separates in protein fractions. In most of the pulse flours, the protein fraction ranged from 20% to 30% whereas starch contents are around 40%; the dehulling process before air classification did not increases the protein separation efficiency. Furthermore, the presence of high fat contents in some pulses like chickpea (~7%) also end up in protein fraction resulting in further low yield (Sosulski and Youngs 1979). The end protein yield is an important determining factor in most of the plant proteins. In nutshell, air classification method can effectively be used to obtain protein-rich factions from several legumes; however, the major disadvantaged are low protein contents and high levels of antinutrients than that of original raw flours.

3.2 Water Extraction

This is another method of protein extraction from plant seeds and grains. Generally, pulses have high number of water-soluble proteins, which can be separated in the form of supernatant by blending pulses with water at the ratio of 1:10 (grain:water) at low-temperature (around 4–5 °C). The amount of protein obtained may vary depending on the type of grain, number of water-soluble proteins in them and on extraction conditions. Repeated extraction (3–4 times) is usually required to increase the protein recovery. Protein content in the first extraction is greater than

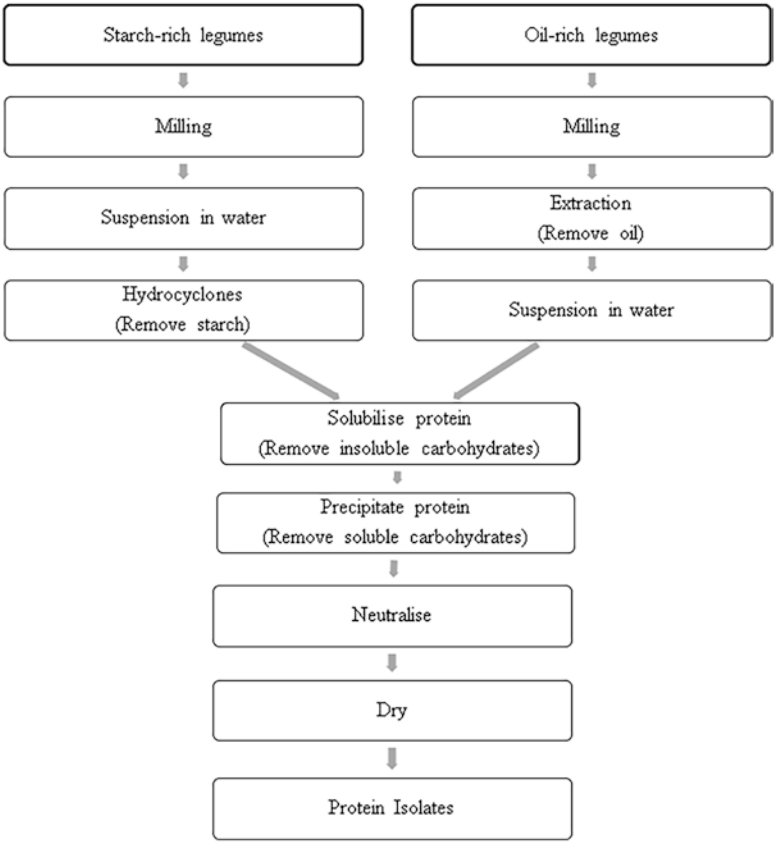

Fig. 5.6 Preparation of protein isolates from legumes through wet and dry fraction process

that of second one and onwards extractions. The schematic diagram of the preparation of protein isolates from legumes through wet and dry fraction process is given in Fig. 5.6.

3.3 Salt Extraction

This method is based on the simple phenomena of salting in and salting out. Salting in, at low concentration of salt, stabilizes the numerous charged group existing on the proteins and thus fascinates protein into the solution enhance its solubility. With increased salt concentration, a point comes where maximum protein solubility reaches and water to solubilize salt is not available and thus protein is precipitated. This phenomenon of protein precipitation in the presence of excess salt is known as salting-out. Protein extraction by salt micellization is affected by salt concentration, and the type of salts used for precipitation and purification. The common and inexpensive salt in use is ammonium sulfate, it has high solubility. A salt solution of desired ionic strength is used for protein extraction followed by its dilution for protein precipitation. Afterwards, these proteins are then either centrifuged or filtrated or dried. In a study, protein isolates with 87.8% protein content were prepared from defatted chickpea flour suspension (10% w/v) using 0.5 M sodium chloride at 7.0 pH (Paredes-López et al. 1991).

3.4 Alkaline Extraction

In this method, the pulse flour is submerged in water (1:5 to 1: 15 flour and water), the slurry pH is made alkaline (pH 8–11) by adding sodium hydroxide and stirred for different periods (1–3 h). The temperature is often raised to about 50 °C to recover maximum protein within minimum time. The soluble part from insoluble part (fiber) is separated by filtration or centrifugation. The pH of the extracted slurry is then adjusted to isoelectric point (pH 4–5) causing the protein precipitation, which is then retrieved by centrifugation or filtration. At a laboratory scale, starch and protein separation occur by hydrocyclones in the wet milling process. Dehulled chickpea is milled by pin milling into fine flour followed by defatting by adding isopropyl alcohol. The pH of the diluted slurry (1.5%, w/w) is adjusted to 9, with continuous stirring for 1 h and overnight stay. This slurry is then subjected to a hydrocyclones to yield overflow and underflow. Hydrocyclone separate the starch and protein-based on the difference in their densities. The low-density protein-rich part forms the overflow in hydrocyclones to the high-density starch-rich fraction hence resulting in separation. The overflow of the first-pass process yielded the highest protein separation efficiency. Other than laboratory scale, this process can be adopted for large scale commercial production using Flottweg process by forming large number of hydrocyclone to separate starch from the protein (Emami et al. 2010)

3.5 Acid Extraction

The process works in a very similar manner as that of alkaline extraction except it takes place under acidic environments. The schematic diagrams of the preparation of plant protein isolates through dry and wet fractionation are given in Figs. 5.7 and 5.8. The pulse proteins are soluble under both acidic (i.e., pH <4) and alkaline conditions (i.e., pH <9). Protein solubilization takes place at low pH, trailed by protein precipitation at isoelectric point. The precipitated protein is then retrieved by any of the several methods like centrifugation, filtration, hydrocyclones, or drying. The acid extraction method is less commonly used than alkali extraction (Tiwari and Singh 2012).

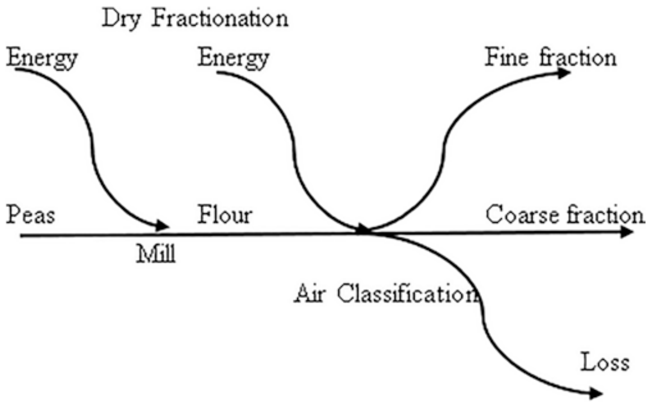

Fig. 5.7 Preparation of plant protein isolates through dry fractionation

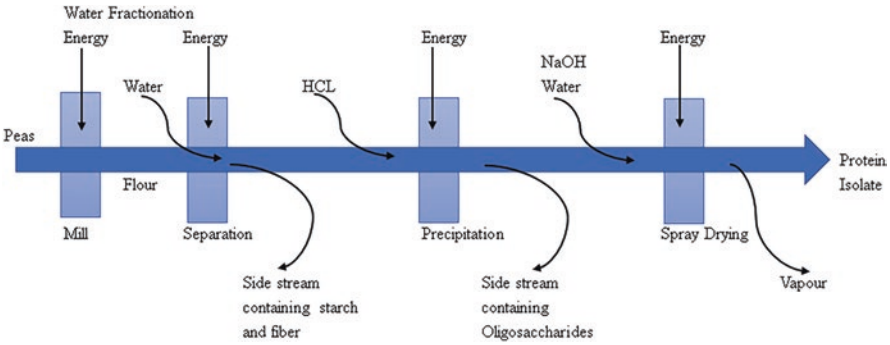

Fig. 5.8 Preparation of plant protein isolates through wet fractionation

3.6 Ultrafiltration

Ultrafiltration is another physical technique that refers to a pressure-driven separation of the protein-based on molecular size. The ultrafiltration membrane is employed having a pore size of less than 0.01 µm. The water molecules at 20–150 psi pressure are passed through the membrane while the colloidal solids and salts are retained. Afterwards, protein present in supernatant is precipitated either by acid or alkaline extraction followed by ultrafiltration to concentrate the proteins. Apart from protein extraction, ultrafiltration can also be used for purification of proteins by removing undesirable components such as anti-nutritional factors (oligosaccharides and phytic acid). This technique also makes it possible to produce purified proteins with superior functional properties. The protein isolates obtained through isoelectric precipitation and ultrafiltration possess are comparatively more stable as compared to isolates obtained through ultrafiltration, which exhibit lower stability during the storage. These emulsions can be destabilized by the addition of salts that alter the structure of proteins, while xanthan gum increases the stability of emulsions by enhancing protein absorption (Makri et al. 2004).

4 Enrichment and Fortification of Traditional Foods

Plant-based protein ingredients like concentrates and isolates from soy, pea, lupin, lentils etc. are now widely used in an array of traditional food products like baked products, breakfast cereals, pasta, extruded snacks, snack bars and chips, meal analogs, plant-based drinks, infant formulas and baby foods for the enrichment and fortification of protein, dietary fiber and bioactive components. Soy and pea-based protein rich materials are added in several food products owing nutritional and functional properties (Sandberg 2011). Protein content of these ingredients may vary from 48% to 90% based on extraction technologies like dry-milling or wet-milling. Lupin possesses a variety of applications in the food industry owing to higher fiber and protein content. Moreover, it exhibits better taste than beans, pea and lentils. Moreover, it has better amino acid profile; hence, frequently used in variety of sweet and savory foods. In Europe, being gluten-free vegetarian food, is incorporated in a variety of foods. Hulls of lupin are removed and milled in fiber-rich flour, which has been found suitable for the enrichment of bread and other bakery items. Owing to better functional properties, lupin protein isolates (LPIs) can be used as animal protein replacers (Fraunhofer 2011).

Prolupin, a leading lupin food manufacturer, produces a variety of vegan products using lupin protein isolates (Prolupin 2020). The brand is named as "Made with Luve", having a range of products such as ice cream, desserts, drinks, dressings, mayonnaise, pasta and yogurt alternatives. Likewise, another German company, produced LPI through a gentle process, which are suitable to replace milk or chicken proteins in the food products for the emulsification purpose (Lopino 2020). In majority of the South Asian countries, lentils alongwith beans and peas are essential part of numerous cuisines (Thomas-Patel 2014). In India, rice-lentil batters are generally consumed in dinner, lunch, or breakfast. These nutrient-rich combinations provide adequate amounts of protein (Decker 2018). Dehulled split lentil and lentil flour are extensively used in gluten-free diets to fulfill the protein and minerals requirement. Moreover, split lentils can be used both as a main or side dish and in salads. Likewise, lentil flour has a wide applications in stews, soups, stews, and purees. Furthermore, it can be mixed with cereals for the preparation of cakes and bread and used as a food for infants and a meat extender (Williams and Singh 1988). The other potential protein applications of lentil include nutrition and sports bars, TVP, meat extenders, infant complementary foods, protein supplements, as well as aquaculture feed and pet food. Whole grain nature, protein quality and levels make lentil an ideal ingredient for incorporation in traditional as well as modern recipes especially in Germany, France and Canada. The utilization of plant protein concentrates and isolates in the following food products have been reviewed:

4.1 Bakery products
4.2 Meat products
4.3 Pasta
4.4 Extruded snacks
4.5 Breakfast cereals
4.6 Beverages
4.7 Fat replacers
4.8 Infant formulas and baby foods
4.9 Flavour enhancers
4.10 Miscellaneous applications

4.1 Bakery Products

The baked products are among the most popular snacks and breakfast items in the world. Among the bakery products, bread market has grown steadily since 2007 and reached to 129,000 tonnes in 2016. The countries that accounted for 41% of total global bread and baked product consumption included USA, China, Russia, UK, Germany, Egypt and Italy with 14.7, 9.3, 8.7, 6.2, 5.2, 4.6 and 3.9 million tonnes consumption, respectively (FMCG 2018). Keeping in view the current situation and increasing trend, bread and baked products are considered ideal for fortification of nutrients, especially those deficient in respective communities. The additional benefit can be better utilization of under-utilized crops. The cereals and their products are usually low in protein content and being vital part of regular diets across the globe are excellent target for protein and micronutrient fortification. The protein contents of bread, pasta, or yogurt fall in the range of 5–13% and are classified as low protein foods. According to the EU, foods claimed as a "source of protein" or "high-protein" must contain 12% or 20% of the energy content from proteins, respectively (EU Commission 2006). The addition of plant protein in such foods can be challenging to achieve the desired technical, sensory, and nutritional quality.

Soy protein isolates are generally used as ingredients in bakery, cereals and pasta items for its nutritional, functional and economical characteristics. The defatted soy flour and SPI are widely used as partial replacement of milk powder in baked products. Various milk replacer mixtures with protein content ranging from 20% to 40% are now available. The nature of the desired blend depends upon the nutritional and functional requirements of the product. Primarily, defatted soy meal is used in nonfat dry milk blends, whereas isolates and concentrates are used for complete or partial replacement of milk-based blends (Stauffer 2006). Ordinary white bread has 8–9% protein content however, in specialty bread the protein content is increased from 13% to 14% by incorporating soy flour, concentrate or isolates along with gluten and lipid emulsifier. The addition of high levels of soy flour without emulsifier or surfactants may alter the bread attributes like poor crumb and loaf volume. The addition of 12% of the soy flour in these formulations, may boost protein content by 50% and protein efficiency ratio from 0.7 to 1.95 (McWard 1995).

Gluten and soy proteins are similar in amino acid composition however, soy proteins have less concentration of sulfur-containing amino acids but are rich in lysine that is deficient in gluten proteins. This unique composition of soy proteins, make it a potential ingredient for use in different food items to produce products with high protein quality. Blending of 3% soy flour in the dough resulted in the production of breads with equal or slightly superior quality compared to those having 3% nonfat dry milk (Singh et al. 2008). Moreover, the addition of 1–3% defatted soy flour helps to improve absorption, crumb body, crust color, elasticity, freshness and toasting properties of bread. However, the dark color of the crust can be maintained with the help of high lysine content soy flours having low lipoxidase activity. Similarly, heavily toasted grits of soy can be used in multigrain and wholegrain bread to augment color and a nutty flavor (Singh et al. 2008). However, high levels of soy in the bread dough may harm the formation of gluten gels (Apichartsrangkoon 2002). Therefore, the addition of soy proteins in bread dough should not be done to such extents to avoid disturbance in attributes of the end-product (Bainy et al. 2010). The breads of acceptable loaf volume and hardness can be prepared with 3% protein isolates made from pea, chickpea and lentils. The acceptability of breads decreased with higher supplementation levels (6–9%) due to poor texture and more green color (Aider et al. 2012).

Doughnuts are deep-fried white flour-based food product that absorbs much fat while frying. Whereas doughnuts having soy protein absorb less fat probably due to the thermal protein denaturation that hinders fat penetration. This ends up in the better quality doughnut with the more economical and healthy formulation. The incorporation of 3–3.5% soy flour in formulations results in better shape, crust color, texture and high water absorption capacity. Different types of cakes have been formulated using soy isolate-whey blends replacing 50%, 75%, or 100% nonfat dry milk without deteriorating cake characteristics. Similarly, full-fat and defatted soy grits and flours, soy concentrate and isolates alongwith emulsifier were incorporated at various concentrations (2–15%) in wheat flour to prepare bread, cake, waffle, pancake and other baking mixes (Golbitz 1995). The resultant doughs were less sticky, pliable, smoother and uniform in texture. Moreover, the finished products were obtained with better crust color, grain size, longer freshness, symmetry and texture. In a study, lecithinated soy protein was added at 3–5% in wheat flour for the preparation of pound and sponge cakes. This addition of lecithinated soy flour improved the emulsification process and thus reduced the utilization of shortening and eggs (Singh et al. 2008).

The addition of pea ingredient in food products can improve the protein content, functional properties as well as replace allergens such as soy or egg. The type or amount of the pea ingredient in food products depends on its application in that particular food. The precooked or unflavored pea flours are frequently used in the preparation of products such as bread, donuts, cookies, muffins, tortillas and cakes. The purpose of this addition is to increases the protein content of staple foods since pea flour is rich in lysine amino acid. Likewise, the use of precooked pea flour did not impart typical pea flavor or taste in food that has higher inclusions rates. The maximum levels of recooked pea flours (50%) may be used in doughnuts followed by bread (30%), hamburger buns (30%) tortillas (20%), and whole wheat bread (15%). Pea flour and pea starch concentrates contain lipoxygenase enzymes, which can be used in premixes as a soy flour replacement. However, such flours have high protein dispersibility index (PDI) and should not be exposed to heat treatment (Tulbek et al. 2017).

In the recent era, consumer's attention towards plant-based and nutritious foods has increased drastically. Incorporation of pea protein ingredients in bread can bring some techno-functional difficulties like gluten-aggregation, pasting and other bread characteristics. However, the impact on dough properties as well as bread characteristics can differ. In the study, the substitution of 15% pea protein in wheat flour resulted in doughs with weakened gluten-network and breads with slightly inferior quality (Hoehnela et al. 2019). Pan cakes were fortified with different levels of pea proteins (0–40%). There was gradual decrease in the batter air volume leading to an increase in the cake density. There were significant improvements in the rheological properties and batter stability mainly due to the larger particle size of the pea proteins resulting in higher water-binding capacity, and network of interconnected "bridged" particles in the continuous phase thus acting as a filler. However, solubility was not affected by the large particle size of pea proteins (Bustillos et al. 2020).

Lupin finds its application in the baking industry as a bread improver and egg protein replacer. The native lupin constitutes about 1–5% of the bread weight. While in cookies, waffles, specialty breads and cakes, toasted lupin flour-based bread improver is used to impart yellow color and provide texture, structure, and other functional properties such as water-binding and emulsification, similar to imparted by eggs. The water binding ability of lupin helps in keeping the bread quality (Kohajdova et al. 2011). As an egg protein replacer, lupin is unable to replace 100% egg protein due to low quality than that of egg proteins, hence some other ingredients are also required to achieve 100% egg protein replacement in bakery products. In a study, 50% egg replacement was achieved by using a combination of lupin protein concentrate (20%), potato starch (7%) and whey protein concentrate (3%) along with water (Noort 2017).

Physical properties such as emulsification, fat uptake, functionalities of adhesion, barrier formation during frying, freeze-thaw tolerance, crispiness, etc. are important for the preparation of batters. Depending upon the types of batter, 5–10% lupin protein is incorporated as dry ingredient batter premixes. The addition of lupin helps in the emulsification (stabilizes the batter), viscosity (results in the increased addition of water while reducing the costs), and exceptional adhesive properties (forms a stable film around the product surface). This results in improved eating properties due to less uptake of fat during frying, freeze-thaw stability and better crispiness and expansion of layer (Noort 2017).

Gluten is vital ingredient in baked products, breakfast cereals, meats, cheese, snacks, and texturized meat analogs (Day et al. 2006). It is considered as prerequisite in bread manufacturing as it improves the mixing and handling properties of the dough due to better water absorption and dough strength. The film-forming property of gluten helps in improved volume, gas retention, and uniform texture in bread. The improved water absorption helps in retention of bread softness alongwith yield improvements. The proportion of gluten in bakery flours varies depending on the type of product, texture required and shelf life (Maningat et al. 1994). For example, pretzel with optimum breakage can be manufactured using 1% gluten whereas elevated levels of gluten (>2%) makes them too hard to eat. Similarly, about 2% gluten is used in hamburger and hot dog buns formulations for desirable crust characteristics. Gluten is also incorporated in the wheat flour to make both thin and thick crust pizzas from the same flour. Gluten strengthens the crust and chewiness by reducing the moisture transfer from the sauce to the crust. Gluten addition in traditional foods as a protein enhancer may pose a risk for the people which are on gluten-free diets. Baked products in which gluten can be used are breakfast cereals, meats, cheese, snack, and texturized meat analogs, (Day et al. 2006). Food labeling laws assist in identifying such products (Jones and Russell 2004).

Globally rice is mostly consumed in the form of intact or broken kernels. Moreover, rice-based baked products, breakfast cereals, crackers, noodles, pasta, rice flakes and snacks are also popular among the masses. Rice is considered as one of the least allergen food, hence is an excellent ingredient for the manufacturing of a wide range of gluten-free products (Bhattachrya 2011). Rice proteins have unique nutritional properties such as a well-balanced amino acid profile and hypo-allergenicity compared with other cereals and legume proteins; therefore, rice protein concentrates, and isolates can serve as valuable ingredients in many food applications such as baby foods, sports nutrition etc. Parboiling is almost universally applied to improve the technological, functional and edible qualities of rice. During this process, protein properties could be altered both in the rice endosperm and bran (Reza et al. 2005). Moreover, extruded rice protein is used for crunchy texture in cereals bars (Hoogenkamp 2015). Commercially, rice proteins are extracted from rice bran and use for the preparation of rice bran protein concentrates and rice bran protein isolates. The addition of 1–5% of alkali-extracted RBPC the protein content up to 12.10% alongwith corresponding increase in fiber content. The sensory panelist declared bread containing 1% RBPC similar to made from 100% wheat flour with respect to texture, taste, flavor, color and overall acceptability (Jiamyangyuen et al. 2005). Similarly, leavened bread was manufactured substituting wheat flour with protein concentrate at 5%, 10% and 15%, respectively. Consequently, protein levels of supplemented breads were raised to 12.3, 16.5 and 21.1 as compared to control (9%). Bread with 5% rice bran protein concentrate was comparable with the control in terms of all the sensory attributes considered. The sensory quality of bread was affected negatively when the level of substitution was beyond 5% respectively (Sadawarte et al. 2007). Apart from bread, RBPC prepared by the wet alkaline extraction method was also incorporated in biscuits by gradually replacing wheat flour with 5%, 10% and 15% RBPC levels. The biscuits' protein concentration was raised from 7.3% to 15.4% (with 15% RBPC). The biscuits with 5% RBPC were declared similar in texture, taste, flavor, color and overall acceptability as the control (Yadav et al. 2011). Rice bran, a by-product of rice milling industry, has better protein quantity and quality as compared to white milled rice. It can successfully be incorporated in biscuits (5–15%), flat bread (10–12%) and *Parotha* (oily flatbread) without any adverse sensory effects (Saeed et al. 2009).

Other than oat flour, oat protein concentrates (OPC) and oat protein isolates (OPI) has been in use as a part of wheat formulations (D'Appolonia and Youngs 1978; Ma 1983). However, the higher levels of OPI (>5%) in wheat flour significantly decreased the volume and increased the hardness and chewiness of bread (Pastuszka et al. 2012). Canola protein products have been found appropriate for a range of food products, including bakery products, beverages, meat binders, cheese-like products (Wanasundara et al. 2016). Likewise, protein concentrates and isolates from oilseed meal has been used in the preparation of baked products. The fat absorption and water holding capacity of sunflower meal protein isolate (SMPI) incorporated in wheat flour was assessed. This intervention resulted in improved dough consistency, plasticity and elasticity and also augmented the biological and nutritional value of the bread. At a 10% supplementation level, the overall content of amino acids increased by an average value of 20%. The consumption of about 150 g of white bread supplemented with 10% SMPI is sufficient to fulfill 23.2% protein and 38.9% essential amino acid daily requirements of a child. The use of SMPI can further be extended to other products such as naan, spaghetti and macaroni (Shchekoldina and Aider 2014).

4.2 Meat and Meat Analogs

Meat and meat analogs, being important component of a daily diet, are largely consumed throughout the world. These are major source of biological proteins and are usually fortified with different plant-derived protein ingredients for binding and extending purposes. This practice not only reduces the price but also improves the nutritional value of the products. Cottonseed proteins, peanut proteins, rice bran proteins, soy protein isolates, wheat gluten and whey protein have been extensively studied for the development of films (Rhim et al. 1998). Soy flour performs similar functionally and is nutritionally more economically compared to meat. Utilization of soy protein isolates can replace or minimize the use of dairy proteins, egg, fish, meat and poultry in an array of food products with higher protein content and lower cost. The film-forming ability, water holding capacity and fat absorption characteristics of SPI are important in meat products. These results in hard, firm gels, compared to soy concentrates and flour that forms fragile and soft and gels (Feiner 2006), however, it also depends on soy protein formulation and preparatory conditions. Some SPI form gels upon heating under pressure while others are unable to form a gel. Meat batters with soy protein showed structural changes upon thermal treatment. Stronger gel network results with the addition of soy protein upon thermal treatment of meat batters that is associated with increased formation of β-sheet structures. Moreover, this procedure develops stronger hydrogen bonds between water and protein (Herrero et al. 2008). Neutralized soy protein isolates are highly recommended where water and fat binders are required in meat-based products including loaves, patties and sausages.

Textured soy protein (TSP), another type of isolated protein, is used globally in meat products and meat analogs. After hydration, TSP exhibits meat-like texture and appearance alongwith similar high-quality protein. Moreover, TVP has the ability to absorb synthetic as well natural flavors. TVP based formulations are effective in reducing the product cost by replacing expensive lean meats, improve yield and overall nutrition (Riaz 2006). It is also incorporated in comminuted meat products such as meatballs, sauces and patties (Berk 1992). TVP can replace 30–40% beef in the patties and chicken in the nuggets. Furthermore, these products can hold more water that allows more moisture retention during secondary processing i.e., freezing, thawing, cooking, and reheating. These products are more likely to serve in circumstances where food is served fresh and is kept ready again and again in extreme conditions like heat lamps and steam tables. Utilization of above 10% soy concentrates and isolates has resolved odd flavor, mouthfeel, texture, dryness associated with soy flour used in meat items (Kinsella 1976).

For several years, soy protein products remained the most widely used ingredient for the enrichment and fortification of food products for functional attributes as well as nutritional quality. Afterwards, functional as well as nutritional properties of pea protein isolates and concentrates such as water & oil binding capacity, foam expansion and stability, whip ability, gelation, emulsion ability and stability have been extensively studies for food applications. Pea protein utilization in meat products like meat patties, hams and sausages have been reported. The utilization of pea flour and protein ingredients in meat-based products as a binder and filler results in firm texture and improved functional properties of the end products due to better amylose content, starch retrogradation and gel formation, which binds water and fats (Dzudie et al. 2002; Modi et al. 2003; Serdaroglu et al. 2005).

The major drawback of pea protein isolates is development of weak gels as compared to soy protein isolates. However, this issue can be rectified through enzymatic treatment, transglutaminases can improve the gel strength (Shand et al. 2008; Sun and Arntfield 2011a, b). Similarly, pea protein emulsification property can be improved by using acid proteases (Periago et al. 1998). After enzymatic modifications, functional properties of pea proteins can be comparable to soy protein isolates and egg-white proteins. Likewise, taste and flavour issues associated with pea proteins and pea flours in meat products can be handled by using de-flavored pea protein isolates and thermal stable pulse ingredients. However, such pea protein isolates do not contain lipoxygenase enzyme that has a detrimental effect on meat quality as seen in certain meat products. On the other hand, the addition of pulse flours in meat-based products, can overcome weight reduction and water retention issues alongwith improving the fat binding. Certain veggie meat analogs such as matzah balls and pakoras can also be fortified by pea protein isolates (PPI). PPI, lentil flour and rice protein have been used to develop fortified beef patties for the elderly individuals. The addition of these protein sources reduced the chewiness, cohesiveness, gumminess and hardness of patties making them more acceptable by the older adults along with fulfilling the protein requirements (Baugreet et al. 2016).

The impact of lupin protein isolates (LPI) on the emulsion stabilization property has been evaluated in the meat system using in the concentration of 2% (w/w). This was attributed to the globulin aggregates bridging effect that strengthens the droplet-droplet interaction in the emulsion droplet gel network. In another study, conducted on salad dressing, the addition of LPI stabilized the emulsion due to the presence of globulins (Papalamprou et al. 2006; Drakos et al. 2007). Low-fat bologna has been prepared from konjac blends and soy protein isolate by replacing meat protein. The replacement of meat protein with 2% SPI did not alter the most characteristics of bologna (Chin et al. 1999).

In an attempt to decrease the animal meat consumption and to increases the vegetable intake by humans, several meat alternatives from vegetable proteins have been developed. Soy in the form of tofu and tempeh has been acting as a meat alternative for quite a long time as for vegetarians. Afterwards, another meat alternative, "Seitan", has been prepared from wheat and is used in different recipes like vegetable soup or can be deep-fried. Next to seitan, wheat protein can be texturized in an extruder by giving high temperature, high shear, and low moisture environment. This texturized wheat proteins can be used as meat extenders or in the recipe with egg white or soy protein isolates (Areas 1992; Akdogan 1999). The wheat gluten is also used to produce texturized proteins by using twin-screw extrusion processes for use in cereals, snacks and fried foods.

The rice bran (10%) with high amount of protein, fiber and fat was found suitable in the preparation of pork meatballs. The texture analysis showed decrease in hardness, gumminess and chewiness by the addition of the bran (Huang et al. 2005). Canola protein isolates (CPI) have been found suitable for use in comminuted meat products due to the gelling properties. Similarly, their usage in cereals can complement the protein quality and improve the nutritional quality of baked products (Arntfield 2011). Similarly, the utilization of rapeseed concentrate (RC) and rapeseed protein isolate (RPI) has been investigated in sausages, cakes and mayonnaise. The products containing RPI were rated slightly higher in quality attributes compared to containing the protein concentrate (Haar et al. 2014).

4.3 Pasta

Among the pasta products, spaghetti and macaroni especially for public sector feeding programs, military and emergency rations are usually fortified with soy proteins. These products are often enriched with defatted soy flour, whole flour and soy protein isolates (SPI) upto 15% level and also fortified with vitamins and minerals premixes (Tsen et al. 1975). The addition of soy protein in pasta dough enhances absorption and firmness essential to survive during the long cooking procedures. SPI usually produces lighter colored food items. Likewise, sweet potato flour containing 15–45 g/100 defatted soy flour (DSF) and soy protein concentrate (SPC) produces pasta with five times more protein compared to traditional potato-based pasta alongwith better appearance, cohesiveness, firmness and springiness with (Limroongreungrat and Huang 2007).

Besides soy protein ingredients, pea flour and pea proteins are also used as a base ingredient in durum wheat-based pasta, noodles and Chinese vermicelli noodles. The supplementation of 5–20% yellow and green pea flour in durum wheat pasta improves the nutritional value without affecting much of the quality characteristics (Tulbek 2007). The textural properties of pasta are dependent on the type and inclusion rate of pea ingredients. Changes in the pasta color were significantly affected by the type of pea flour. The addition of 10% green pea flour lowers the yellowness and brightness of the resultant pasta significantly while the addition of yellow peas flour at the same concentration did not change the color. Likewise, cooking the pea flour fortified pasta improved the texture by increasing firmness, these changes are attributed to additional protein contributed by animal proteins. The same properties were also seen in pea fortified spaghetti (Bahnassey and Khan 1986; Zhao et al. 2005).

Chinese vermicelli noodles traditionally were made from mung bean starch, but due to decline in the mung bean production, pea starch is now widely used for their production (Tulbek 2014). Pea has high starch level (98%) with some unique pasting properties necessary for the quality of vermicelli. The addition of pea flour and other ingredients not only raises the nutritional profile of vermicelli, spaghetti and noodles but also improves their end-use quality. The negative attributes associated with higher levels of protein flour (20% and more) can be minimized by using unflavored or precooked protein flours (Tulbek et al. 2008).

4.4 Extruded Snacks

Apart from products like cakes, bread and pasta, pea ingredients can also be added in ready to eat extruded snacks and breakfast cereals especially developed through extrusion technology. In this process, starchy and proteinaceous ingredients are cooked at elevated moisture content, temperature, pressure, and mechanical shear. The extrusion process works by gelatinizing starch and denaturing protein. Corn, rice and potato are excellent ingredients for extrusion processing due to high starch and low protein and fiber levels. In the extrusion cooking, pea flour-based products exhibited similar expansion ratio and bulk density as from corn or rice flour. Among the single- or twin screw extruders, coarse pea flour is suitable for both types of systems whereas fine pea flour is better ingredient for the twin-screw extruders. The extruded products are also influenced by the quality of raw materials. In a study, pea flour varying in protein and carbohydrate levels was studied for its extrusion characteristics. The pea flour with low starch contents exhibited less expansion (Hood-Niefer and Tyler 2010; Vadukapuram et al. 2014). Likewise, the solubility of pea proteins increases in extrusion processing resulting in loss of certain amino acids. Lysine, an important amino acid in pea protein, is heat and mechanical shear sensitive. In several studies' lysine losses in extruded products have been reported. These losses are due to interaction of free amino acids and reducing sugars (Maillard reactions). The potential of blending pea flours with cereals needs to be further investigated. Pea protein has the potential to increase protein and nutritional value of extruded snacks and breakfast cereals, however, efforts should be made to reduce the interactions among the free amino acids and reducing sugars by devising some suitable strategies. The addition of pea protein (0–42%) and pea fiber (0–24%) in rice starch increased the specific mechanical energy inputs, bulk densities and cell densities of extrudates compared to control sample. However, there was gradual declines in expansion with higher levels of protein and fiber (Beck et al. 2018).

4.5 Breakfast Cereals

The global market of breakfast cereals is growing in the current era due to modern and busy lifestyle. The most of the breakfast cereals are poor in protein and fiber contents. The demand of nutrient-dense breakfast cereals and snacks has increased the utilization of soy proteins in granola bars, hot cereal mixes and breakfast products (Zind 1998). In a study, SPI have been used in combination with wheat bran to prepare extruded corn-based cereals with various moisture contents and sugar concentrations. The product containing 25% moisture and 10% sugar were declared as the best due to better consumer acceptability. In this formulation, soy flavour was masked by added sugars (Faller et al. 2000). The most recently new cheerios oats and honey cereal having lentil proteins have been launched. One serving of this product has been claimed to provide about 11 g proteins when taken with milk (Samaranayaka 2017).

4.6 Beverages

Soy milk is a by-product of tofu manufacturing industry. It is one of the most popular plant-based dairy milk alternative, widely consumed in the world. It is a stable emulsion of oil, water and protein. It is prepared by soaking soybean in water followed by grinding, boiling and filtration to obtain white colored liquid loaded with plant proteins. Physicochemical changes in soymilk are directly related to protein composition and processing techniques. New soy varieties with lower antinutritional compounds (trypsin inhibitor) have been developed for human consumption. Genotypic changes in protein composition and heat treatment directly affect the particle emulsion and stability of soymilk (Malaki et al. 2009).The ratio between glycinin to β-conglycinin also greatly affects the quality of soy protein and soymilk.

4.7 Fat Replacers

Fat in foods contributes to softness, structural stability, lubricity and mouthfeel. These attributes can also be gained by the inclusion of protein ingredients in the formulations. Rice bran protein (RBP) because of its superior emulsifying and foaming properties has been found to effectively replace fat in the production of low-fat franks (Bloukas and Paneras 1996). RBP is considered promising fat replacers in a wide range of food commodities, especially in bakery products. Lupin as a cheese replacer has been investigated in several studies. For this purpose, lupin paste was prepared from the seeds by soaking in water for seven days followed by boiling for about 2 h. Afterwards, the seeds were peeled, minced and blended to a very fine paste and kept in the freezer until the use. In another study, different levels of mature (3 months old) Egyptian Ras cheese (25–100%) were replaced with lupin based analog. The cheese analogs prepared by all four levels of cheese replacer were acceptable, however, cheese blend with 25% lupin paste was the most acceptable blend. Additionally, in all the mixed cheeses, the properties such as fat separation, penetration and meltability, decreased. Subsequently, the effectives of product against blood sugars were established through animal feeding trials (Awad et al. 2014). Lentil proteins-based edible films with comparable optical, mechanical and barrier properties to other edible protein films have been developed (Bamdad et al. 2006). Likewise, microcapsule prepared using lentil protein isolates was found efficient for the entrapment and gastrointestinal (GI) delivery of flaxseed oil (Karaca et al. 2013). Furthermore, microcapsules prepared by lentil and chickpea along-with maltodextrin coating exhibited a protective effect against lipid oxidation at room temperature during 25 days of storage.

4.8 Infant Formulas and Baby Foods

The unique nutritional and hypoallergenic properties of rice proteins make them appropriate constituent for infant food (Helm and Burks 1996). Rice bran protein (RBP) has been utilized as milk replacer in infant formulas (Landers and Hamaker 1994). These can also replace cereals containing allergens especially used for the production of baby foods. Thus, these products can be made available to the children without any fear and restrictions (Helm and Burks 1996).

4.9 Flavor Enhancers

Rice bran proteins (RBPs), being low cost and abundantly available ingredient, have been used frequently in product development. Protein hydrolysates have long been used as flavour promoters, especially in combinations with glutamic acid and its salts as monosodium glutamate (MSG). However, MSG has been banned in several countries because of health concerns. The bran proteins have also shown prospects in producing flavour or flavour enhancers. Glutamines and asparagines are present in large amounts in RBPs, if deaminated these may serve as excellent flavour enhancer in a variety of food systems. However, the peptides are also known to induce bitterness and careful production of proteolysates plays a significant role in flavor development (Lemieux and Simard 1992). Partial hydrolysis of RBPs using 0.5 N HCl produced free amino acids and peptides that were aroma-producing compounds and may act as potent flavour precursors. They were formed through the Strecker degradation. These peptides or proteins present in the hydrolysate may also react with reducing sugars to form Maillard compounds that are components of pigments like melanoidins (Jarunrattanasri et al. 2007). The enzymatic hydrolysis has certain disadvantages as well, including the development of bitter taste due to the formation of polypeptides having hydrophobic ends (Alder-Nissen 1979). The debittering of protein hydrolysates at low (10–20%) or high (50% and above) degree of hydrolysate (DH) has been achieved by the use of flavorzyme, which shows the ability to cleave such hydrophobic ends of the peptides and the resulting end products do not produce a bitter taste (Pommer 1995).

4.10 Miscellaneous Applications

Plant proteins, besides regular food applications, also perform a variety of functions in food systems. Rice bran proteins (RBPs), like other proteins, form stable color conjugates and act as carriers for homogeneous distribution of colourants in the food systems. Moreover, the tryptic digestibility of colour-bound proteins is least affected, showing that bioactive sites of neither the enzyme nor the substrate, are involved in binding (Badaruddin et al. 2007; Abdullah et al. 2008). Parboiled and stabilized rice bran is a granulated powder with cream colour and no taste and odour. These attributes pave the way for its utilization as a bulking and thickening agent in various formulations. An emulsifier of excellent surface activity and potential commercial applications in food processing has been developed from rice bran (Yun and Hong 2007). The edible protein coatings and films for preservative and cosmetic purposes are drawing significant attraction from both processors and consumers. Protein enrichment, although not intentional in film coating, is another point in value addition. RBPs have been found suitable for the production of edible films of high quality using glycerol as the plasticizer. The functional properties of these films were comparable to soy protein-based films (Adebiyi et al. 2008). Rice protein products have been used as ingredients in gels, puddings, ice-creams (Chrastil 1992), snack foods, edible films (Adebiyi et al. 2008) and breakfast cereal (Bakar and Hin 1985). Protein concentrates from rice bran have been incorporated into beverages, pasta, confections (Saunders 1990), gravies, meat products, sauces, soups and savoury applications (Giese 1994). Similar to hydrolysates from other protein sources, rice bran protein hydrolysates may also be used as nutritional supplement and functional ingredients in foods such as coffee whiteners, confectionery, drinks and juices (Fabian and Ju 2011).

5 Conclusion

The commercial production and availability of plant protein ingredients has opened new horizons for their utilization in conventional food products for their fortification and enrichment. This strategy can be helpful in alleviating nutrient discrepancies among the vulnerables especially in developing and underdeveloped countries. Moreover, these value added ingredients can further be used for the production of designer foods. The more consumption of plant-based protein can also mitigate the prevalence and severity of non-communicable diseases in health conscious consumer from the developed nations.

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Chapter 6
Plant-Based Protein Meat Analogues

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1 Introduction

Meat analogues, also referred to as meat substitutes, mock meat, faux meat or imitation meat, are designed food products that mimic the appearance, flavour, mouthfeel, fibrous texture, and chemical characteristics of traditional meat but made from non-animal protein sources (Joshi and Kumar 2015; Boukid 2021). Based on the origin, current meat analogues can be divided into three major groups: culture-based, fungi-based, and plant-based. Culture-based meat analogues are developed from the tissue culture of animal's stem cells (Sun et al. 2021). Mushroom is a typical source of fungi that is used to produce fungi-based meat analogues. Plant-based meat analogues (PBMA) are commonly made from proteins of oilseeds, legumes, and cereals. The term "meat analogues" used in this chapter only is refered to plant-based meat analogues.

The idea of developing plant-based meat replacers has been in existence throughout human history (Bohrer 2019). The earliest documented product, tofu, made from soybean and produced by the Chinese, can be traced back to the tenth century (Shurtleff and Aoyagi 2014). Other plant-based products, including seitan, yuba, tempeh, and so on, were introduced to the dining tables in many parts of Asia (Shurtleff and Aoyagi 2014). However, except the protein content, other quality parameters such as appearance, texture, and mouthfeel of these products are not like the animal meats. Therefore, they were not well-accepted by Western consumers. This trend started to change beginning mid 1900s when modern technology (such as

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extrusion) was introduced to the food industry, especially after texturized vegetable protein (TVP) was developed in 1960s for the production of vegetarian bacon, burger, and so on (Bohrer 2019; Riaz 2011). Today, many traditional plant-based meat replacements have successfully evolved into modern meat analogues that captivate the interest of researchers/technologists from the industry and academia, as well as gaining acceptance from the consumer. The increasing popularity of meat analogues can be attributed to the rising concerns on: (1) food security; (2) environmental impact of food production; (3) consumer dietary preference and religion constraints; and (4) perceived health benefits of plant-based food products.

With the increasing pressure from the growing population, it has been predicted that meat and dairy productions need to be doubled by 2050 (Kyriakopoulou et al. 2019). However, meat production is less sustainable and efficient in term of land, electricity, and water uses, than plant-based food production. For example, on per kg basis, the production of chicken consumes around 50 MJ electricity and 16.3 kg water, while soy meal-based product needs 37.04 MJ electricity and 2.7 kg water, and wheat 5.51 MJ electricity and 0.6 kg water (Smetana et al. 2015). Moreover, livestock is responsible for 9%, 39% and 65% of annual carbon dioxide, methane, and nitrous dioxide emissions, respectively, which contribute significantly to global greenhouse gas effects (Kumar et al. 2017). Therefore, it is important to take all possible measures to reduce the environmental footprints of meat production and explore other substitutes.

Although meats are favored by many consumers, the consumption of plant-based foods is rising in recent years due to various reasons, including personal preference, animal welfare, religious constraint, and so on. Meat analogues are ideal substitutes for meat-based products with advantages of low saturated fat and cholesterol-free, which may be beneficial in reducing the risks of heart diseases and cancers (Boukid 2021; Joshi and Kumar 2015). Driven by the consumer need and robust production technologies of meat analogues, this product category has become one of the major foci of research and development in the food industry and academia.

2 Processing Technologies

2.1 Principle of Plant Protein Texturization

To mimic the fibrous structure of meat (muscle texture), globular plant proteins must be transformed from their native form to linear form through the texturization process (Fig. 6.1). The process generally begins with protein hydration, followed by shear and heat processing, during which parameters such as pressure, pH, moisture content, and so on are controlled. The original disulphide bonds, and non-covalent bonds (e.g., hydrogen bonds, ionic bonds, and hydrophobic bonds) of the proteins are disrupted, thereby denaturing the protein molecules and causing them to unfold substantially (Vatansever et al. 2020; Samard et al. 2019). With further orientation

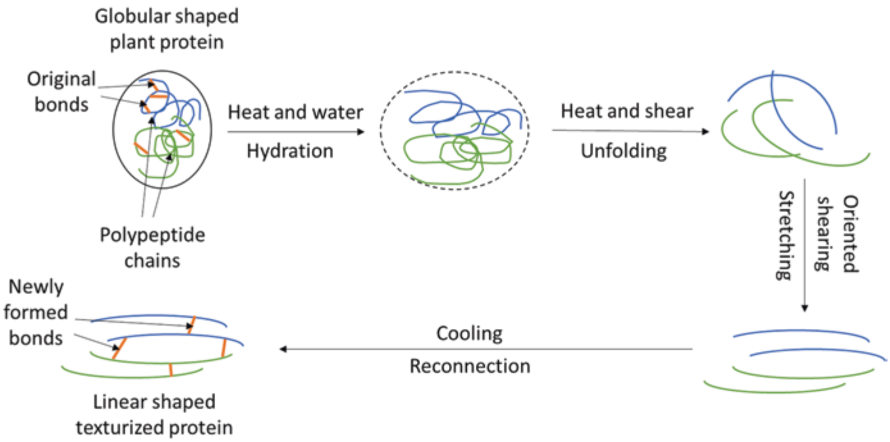

Fig. 6.1 Conceptual representation of plant protein texturization

through shearing, the polypeptide chains stretch in the direction of force field. Upon cooling, new polypeptide-polypeptide covalent and non-covalent bonds re-established, resulting in fibrous layered structures (Joshi and Kumar 2015; Samard et al. 2019).

Current technologies for creating the fibrous structure of meat analogues can be based on either top-down (e.g., extrusion, shear cell and freeze structuring) or bottom-up (e.g., spinning) strategies (Fig. 6.2; Dekkers et al. 2018). The top-down strategy simulates the fibrous structure on large length scale (100 μm–1 cm), which does not mimic the hierarchical structure of meat. On other hand, the bottom-up strategy creates the structural elements with 1 μm–1 mm length scale and then assembles them to a full-size product, by mimicking the hierarchical structure of meat. The top-down strategy is relatively simpler and less costly than the bottom-up strategy, while the latter stimulates meat more closely.

2.2 Extrusion

Extruder was originally invented in the third century as a tool to lift water. After a long time of development, extrusion is now a well-established technology for processing of liquid/semi-liquid products widely employed in food, plastic, and metal fabrication industries (Maskan and Altan 2012). Extrusion is a continuous thermal process. In the food industry, it is often used to produce breakfast cereals, pastas, puffed snacks, meat analogues, and so on. Extruders may be single- or twin-screw in configuration, with the co-rotating intermeshing twin-screw extruder being the most optimal for producing extruded food products. Co-rotating extruder has many processing advantages, like flexibility, large operation windows, mixing efficiency, and ability of handling a wide range of materials (e.g., viscous materials) (Fellows

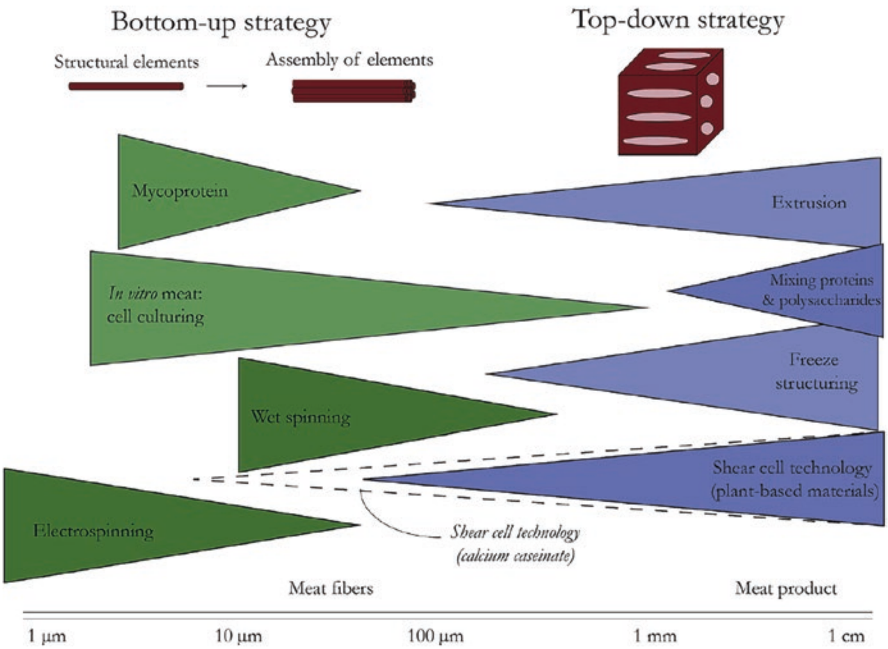

Fig. 6.2 Approaches used for the creation of fibrous structure in meat analogues (Dekkers et al. 2018)

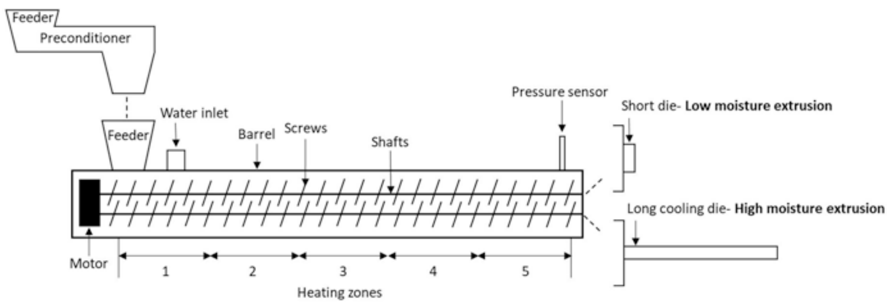

Fig. 6.3 Diagram of a co-rotating intermeshing twin-screw extruder used for low- and high-moisture extrusion

2009; Maskan and Altan 2012). A typical twin-screw extruder is composed of 5 essential components, i.e., motor, feeder, barrel, screws, and die (Fig. 6.3). Motor is used to provide energy to rotate the screw shaft. Feeder stores raw ingredients before they are being fed via the feed throat into the barrel. Advanced extruder feeder may be equipped with a preconditioner section, where steam is injected to precondition the feed materials at specific temperature and moisture content (Maskan and Altan 2012; Vatansever et al. 2020). Extruder barrels usually have 3–6 zones that can be heated to different temperatures. By optimizing the configuration (e.g., hexagonal and sinusoidal) of the screw elements and their positions on the shaft, the assembled screws can effectively mix, convey, compress or shear the materials at different zones of the barrel (Maskan and Altan 2012). Finally, the plasticized materials are being forced through the die to shape the extrudate. In general, there are two types of extruder dies, i.e., short and long cooling dies, typically used for low moisture and high moisture extruded products, respectively. Apart from these main components, modern extruders are also equipped with sophisticated controller systems, pressure sensor, water inlet, water pump, cutting system, and so on.

Extrusion is the most common technology for producing meat analogues, typically via low-moisture (<35%, using short die) and high-moisture (40–80%, using long cooling die) approaches (Boukid 2021). Low-moisture extrusion has been applied to produce meat analogues since 1960s, while high moisture extrusion began to appear around 1990s (Palanisamy et al. 2018; Cheftel et al. 1992). Despite the difference in the moisture level, the material processing within the barrel is similar for both approaches. Powdered raw ingredients are dropped into the barrel from the feeder, mixed with water, and conveyed towards the die by the rotating screws. With the application of moisture, heat, compression, shear, and accumulated pressure, materials are hydrated, blended, and transformed into a homogeneous "melt" (Guy 2001). At the molecular level, the protein is hydrated and denatured substantially, loosing their globular structures. The polypeptide chains are stretched and preferentially aligned in the direction of shear as the molten extrudate passing through the die located at the end of the barrel (Vatansever et al. 2020; Samard et al. 2019).

In low-moisture extrusion, due to the short die and large pressure difference between the barrel and atmosphere, as the extrudate materials leave the die at high temperature, moisture in the materials expands and evaporates rapidly. When the extrudate reaches its expansibility limit, it ruptures and water vapor escapes (Guy 2001). The resulting cellular structures created after cooling, along with the new intermolecular interaction and disulfide bonds established between the polypeptide chains, form sponge-like structures. Low-moisture extruded meat analogues need rehydration before cooking, and often time lacking the fibrous texture seen in cooked meats (Kyriakopoulou et al. 2019). However, due to the low moisture content, they tend to have a long shelf life (Boukid 2021; Palanisamy et al. 2018; Samard et al. 2019). By contrast, in high-moisture extrusion, the unidirectional shear and cooling of the extrudate in a long cooling die promotes polypeptide chain alignments. Moreover, the slow cooling rate applied and high moisture level in the extrudate prevent severe expansion of the extrudate, which is essential in stabilizing the fibrous structure formed. As a result, the high-moisture extruded meat analogues have a meat-like fibrous texture and do not require rehydration before cooking (Kyriakopoulou et al. 2019; Boukid 2021).

Overall, extrusion cooking is a cost-effective, energy-efficient, productive, and versatile technology for meat analogue production. Moreover, by substantially denaturing the protein, extrusion can improve its digestibility, as well as destroying anti-nutritional factors (e.g., trypsin inhibitors) and alleviating unpleasant odor/bitterness attributes often associate with plant-based protein ingredients (Boukid 2021; Samard et al. 2019).

To obtain desirable meat analogues, extrusion processing parameters such as moisture level, barrel temperature, and screw speed, must be carefully controlled. Typical ranges of moisture level for low- and high- moisture extrusions are 25–30% and 50–80% on wet basis, respectively (Tehrani et al. 2017; Boukid 2021). Increasing moisture level usually decreases the extent of shear and the springiness of the final products (Grahl et al. 2018). Maximum barrel temperature usually ranges from 130 to 180 °C (Kyriakopoulou et al. 2019; Cheftel et al. 1992). Extrusion at elevated temperatures may benefit the protein digestibility (Nosworthy et al. 2017; Frias et al. 2011). The practical range of screw speed is 200–1000 rpm depending on the screw diameter and extruder length (Tehrani et al. 2017; Grahl et al. 2018). Screw speed has a positive relationship with shear force and negative relationship with residence time (i.e., the time taken for the materials to convey through the extruder) (Maskan and Altan 2012). Cheftel et al. (1992) reported that the extrusion of meat analogues requires a residence time of around 150 s to ensure optimal protein denaturation and formation of desired textures. Besides extrusion process parameters, raw ingredients formulation (e.g., protein purity, starch content, plasticizer) will also affect the material and sensory properties of the final products.

Studies on extruded meat analogues are extensive. Chemical, physical, and sensory properties of meat analogues produced from various protein sources and ingredient formula, can be found in the studies of Chiang et al. (2019), Sreeitthiyawet et al. (2019) and Caporgno et al. (2020) and others. Tehrani et al. (2017) and Rehrah et al. (2009) studied the effects of extrusion parameters (barrel temperature, screw speed, moisture content) on meat analogues properties. Effects of different liquid additives on extruded meat analogues were studied by Wi et al. (2020) (Fig. 6.4) and Palanisamy et al. (2018).

2.3 Shear Cell Technology

Recently, inspired by the design of cone-plate rheometers and the concept of flow-induced structuring, researchers have developed a simple method based on a combination of elevated temperature (typically 90–140 °C) and intensive shear flow to create structured proteins. This new method is known as shear cell technology (Boukid 2021; Dekkers et al. 2018; Krintiras et al. 2015; Kyriakopoulou et al. 2019). The batch process usually has a residence time of more than 20 min (Berghout 2021).

The shear cell can be based on cone-on-cone (also called shear cell) or cylinder-in-cylinder (also called Couette cell) configurations (Kyriakopoulou et al. 2019) (Figs. 6.5 and 6.6). In cone-on-cone devices, the materials are placed between the outer rotating cone and inner stationary cone. The assembled cones are placed in an oil bath, which is heated during the structuring process and cooled when the process is complete. Cylinder-in-cylinder devices are based on a similar principle, where the inner cylinder rotates while outer cylinder is stationary. A temperature-controlled

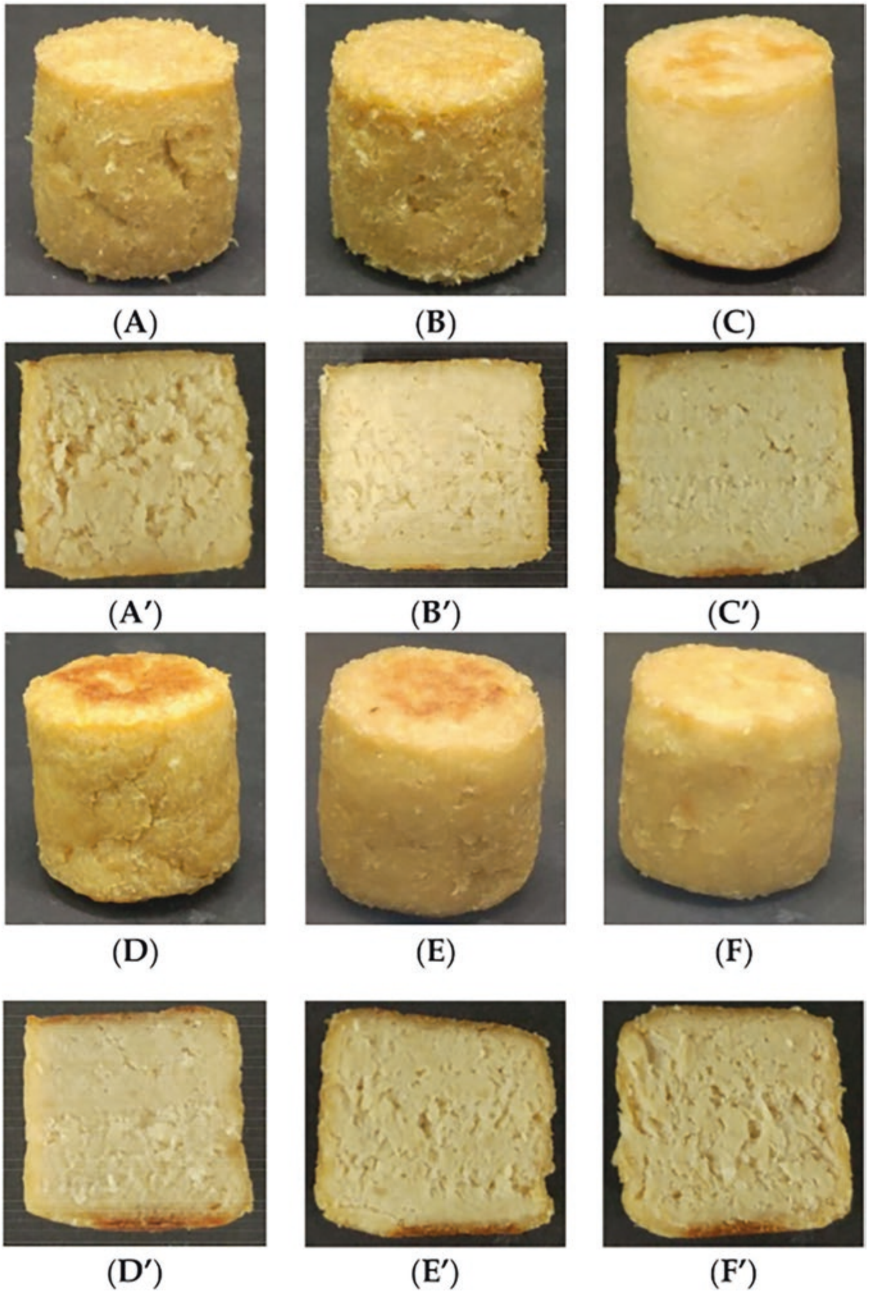

Fig. 6.4 Exterior (**a**–**f**) and interior (**a′**–**f′**) images of TVP (made from soy protein isolate (SPI), wheat gluten and wheat starch)-based meat analogues produced by extrusion with different additives: water (**a** and **a′**), water and SPI (**b** and **b′**), canola oil (**c** and **c′**), canola oil and lectin (**d** and **d′**), O/W emulsion (**e** and **e′**), and water, canola oil, SPI and lecithin (**f** and **f′**) (Wi et al. 2020)

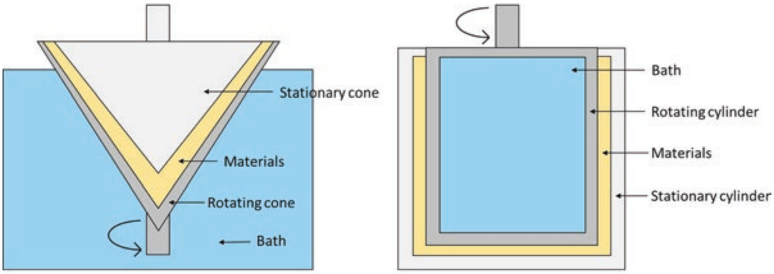

Fig. 6.5 Schematic diagrams of cone-on-cone (left) and cylinder-in-cylinder (right) shear devices

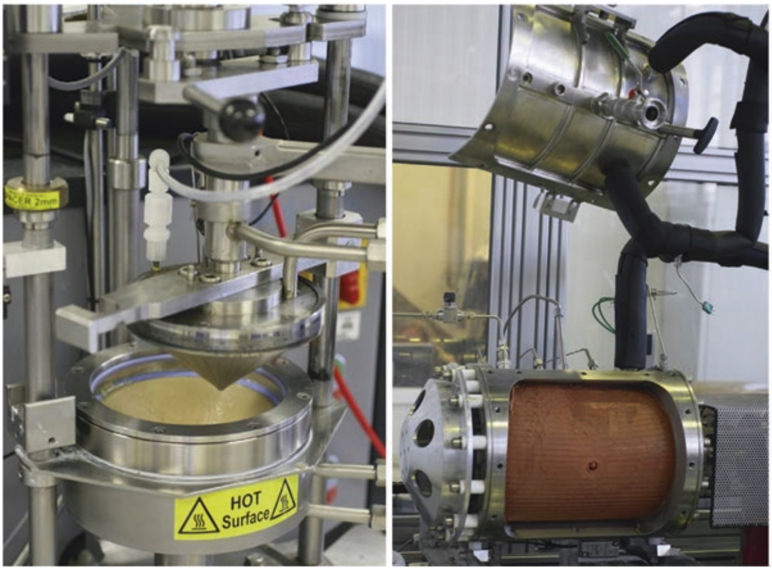

Fig. 6.6 Shear cell (left) and Couette cell (right) devices from the Laboratory of Food Process Engineering at Wageningen University (Kyriakopoulou et al. 2019)

bath (or referred to as solution chamber) is placed within the inner rotating cylinder (Boukid 2021).

The shear cell technology is relatively new; it has been successfully applied on the pilot scale level to make meat analogue products from soy protein concentrate, SPI-wheat gluten blend (Fig. 6.7) and SPI-pectin blend (Dekkers et al. 2018). Krintiras et al. (2016) reported a 30 mm thick textured soy-based meat replacer prepared using an up-scaled Couette cell, which was considerably thicker than the typical extruded products of 5–10 mm thick (He et al. 2020). Reportedly, the shear cell technology is more energy-efficient than extrusion (Kyriakopoulou et al. 2019).

Fig. 6.7 SPI-wheat gluten blend-based meat analogue produced by a Couette cell device from the Laboratory of Food Process Engineering at Wageningen University (Kyriakopoulou et al. 2019)

2.4 Freeze Structuring

Freeze structuring (or freeze alignment) is a technology that uses the isotropic structure of well-mixed frozen solutions to create new structures (Dekkers et al. 2018). Freeze structuring of proteins was reported around four decades ago (Hashizume et al. 1971; Consolacion and Jelen 1986) but it is still not extensively applied in meat analogue production. On the other hand, freeze structuring technology is commonly used in other industries to produce porous metallic and ceramic materials (Dekkers et al. 2018).

The production of meat analogues using freeze structuring starts with the preparation of a protein emulsion (or slurry) and heat treatment to unfold the globular native proteins. After cooling, the protein emulsion is molded and frozen for a specific duration (e.g., at −20 °C for 48 h) to allow for the formation of new linear-structured proteins, due to the formation of ice crystal needles in the protein emulsion. Then, the frozen protein emulsion is freeze-dried to remove the ice crystal needles under vacuum without melting them, resulting in the formation of parallelly oriented sheet-like protein product with porous and fibrous microstructures (Dekkers et al. 2018; Yuliarti et al. 2021; Consolacion and Jelen 1986). Studies have found that the solubility of protein is key to obtaining the fibrous structure in the final product. Moreover, the size of ice crystal needles formed can be controlled by freezing temperature and rate (Dekkers et al. 2018; Lugay and Kim 1978).

Studies on using freeze structuring to produce meat analogues are limited. One of the earlier studies by Consolacion and Jelen (1986) investigated the effects of protein extraction process and freezing rate on protein interactions in the freeze structuring. Kolakowski et al. (1997) later studied the effect of trypsin treatment on freeze structuring process using animal proteins. Yuliarti et al. (2021) recently studied various blends of wheat protein and pea protein for producing chicken nugget analogues using freeze structuring (Figs. 6.8 and 6.9).

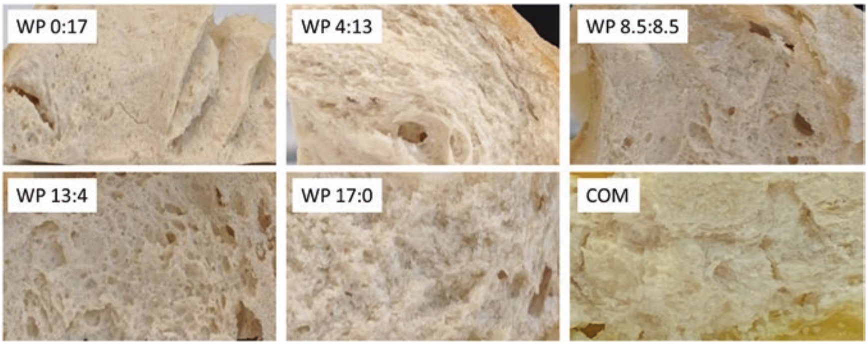

Fig. 6.8 Images of the cross-sectional area of wheat protein (W) and pea protein (P) based meat analogues with 0:17, 4:13, 8.5:8.5, 13:4, and 17:0 ratios (W:P, wet basis) in the formulation produced by freeze structuring. An image of commercial chicken nuggets (COM) is included for comparison (Yuliarti et al. 2021)

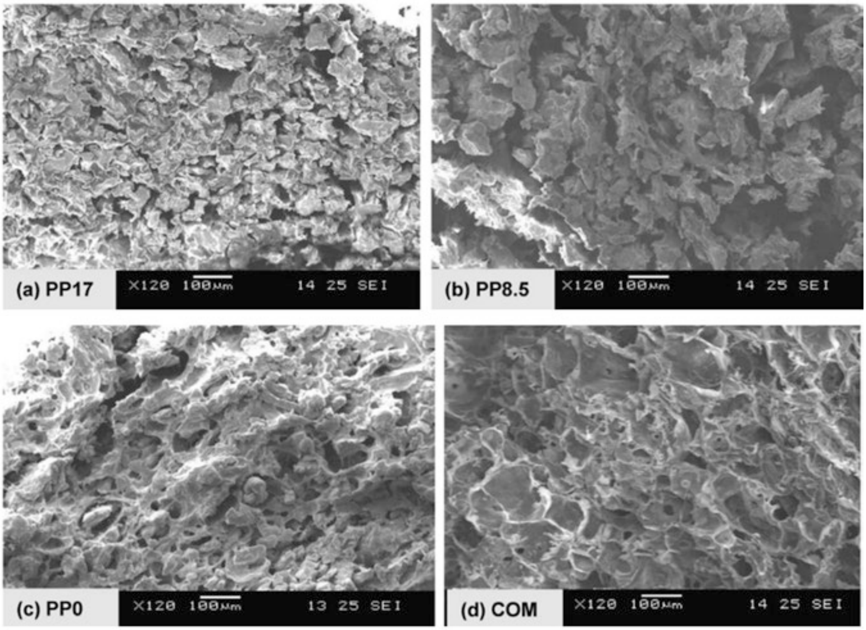

Fig. 6.9 Scanning electron micrographs of the cross-sectional area of wheat protein (W) and pea protein (P) based meat analogues with (**a**) 0:17, (**b**) 8.5:8.5, (**c**) 17:0 ratios (W:P, wet basis) in the formulation produced by freeze structuring. A micrograph of commercial chicken nuggets (COM) is included for comparison (Yuliarti et al. 2021)

3 Protein Sources

In general, the plant proteins used to produce meat analogues are derived from legumes (e.g., soy, pea, bean, lentil and lupine) and cereals (e.g., wheat, rice, barley and rye). Legumes are known for high protein contents, but their proteins generally are low in methionine. Cereals contain relatively lower proteins compared with legumes and are usually low in lysine. However, most of the cereal protein structures are viscous and elastic, which have the advantages on creating the fibrous texture of meat analogues (Bohrer 2019). Based on the report of Mintel (an UK market research company) in 2020, 63.3% of meat analogue products on the market included soy protein in their formulas. Wheat protein was the second popular ingredient and used by 46.8% of products. Pea protein (40.2%), rice protein (7.2%) and vegetable proteins (4.7%) followed up (Boukid 2021). Except cereals and legumes, oilseeds like rapeseed, novel materials like jackfruit by-product (i.e., blend of rind, rag, and seed cotyledon) and algae have also been studied by researchers to develop high quality and diverse meat analogue products (He et al. 2014; Hamid et al. 2020; Grahl et al. 2018).

3.1 Soy Protein

Soy protein is the most widely used plant protein in meat analogues production (Bohrer 2019). After eliminating the antinutritional factors, the protein digestibility corrected amino acid score (PDCAAS) of processed soy protein such as soy protein isolate or concentrate could reach 1.0, which is close to the score of animal derived products (meat and egg) (Bohrer 2019). Cheftel et al. (1992) reported that soy protein concentrate-based meat analogue is easier to produce than SPI-based formula under similar processing conditions. Soy protein is also often mixed with wheat protein to produce more satisfying meat analogues (Fig. 6.10) (Chiang et al. 2019).

Beta-conglycinin and glycinin are two key functional proteins in soybeans that are suitable for producing meat analogues (Renkema et al. 2001; Joshi and Kumar 2015). However, glycinin is a major food allergen, which excludes many consumers from consuming soy protein-based meat analogues (Sun et al. 2008). Soy protein-based products have beneficial effects on lowering cholesterol level and the risk of cardiovascular diseases (Sun et al. 2021).

3.2 Pea Protein

Pea protein is an emerging alternative to soy protein, especially in the production of high-moisture extruded meat analogue mainly due to the low-cost and clean label (not genetically modified) (Schreuders et al. 2019; Yuliarti et al. 2021). Two major

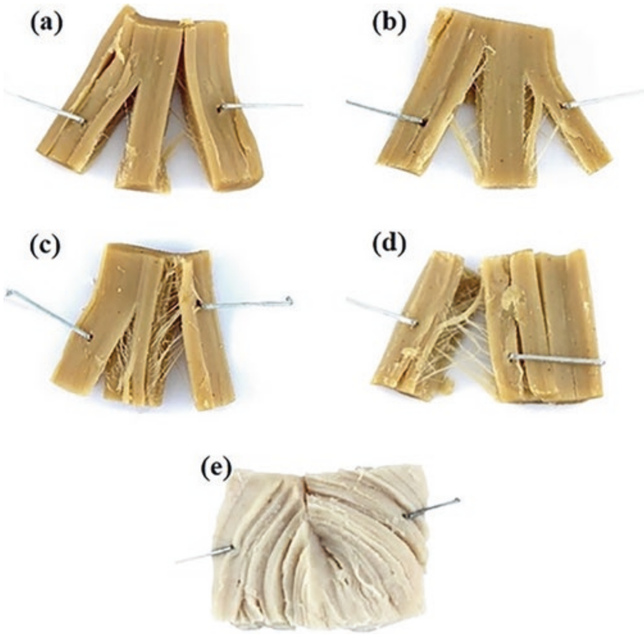

Fig. 6.10 Images of high-moisture extruded soy protein concentrate-based meat analogues with (**a**) 0%, (**b**) 10%, (**c**) 20%, (**d**) 30% wheat gluten in the formula, and boiled chicken breast (**e**) (Chiang et al. 2019)

proteins present in pea that are suitable for producing meat analogues are glycinin and vicilin, which have great emulsion capacity and foaming stability (Joshi and Kumar 2015; Sun et al. 2021). However, the overall gelling capacity of pea protein is lower than soy protein, resulting in softer and less elastic final products (Sun et al. 2021; Schreuders et al. 2019). It has been reported that the addition of salts with chaotropic ions (e.g., sodium thiocyanate and sodium chloride) and pH adjustment could enhance the gelling properties of pea protein (Sun and Arntfield 2011, 2012). The product quality could also be improved by optimizing the particle size of pea protein powder and processing temperature (Fig. 6.11) (Osen et al. 2014).

3.3 Wheat Protein

Gluten is the major storage protein and the main functioning protein in terms of producing meat analogues in wheat, accounting for 60–85% of wheat protein (Kumar et al. 2017; Zilic 2013). It is also the by-product when isolating starch from wheat flour. Except wheat, gluten is also present in barley, rye and oat (Joshi and Kumar 2015). Gluten is composed of gliadin and glutenin. Gliadin is a single-chained polypeptide, having low to medium molecular weight and being

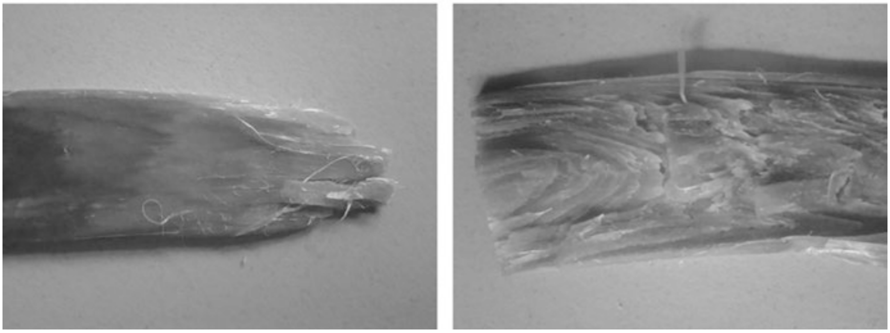

Fig. 6.11 Images of pea protein-based meat analogues showed predominant lengthwise fibrous structures when extruded at 160 °C (left) and parabolic fibrous structure when extruded at 130 °C (right) (Osen et al. 2014)

responsible for viscous behaviour. Glutenin consists of multiple chains of polypeptides, having high molecular weight and being responsible for elastic behavior. Both gliadin and glutenin are connected by intermolecular disulphide bonds (Singh and Singh 2013; Wieser 2007).

Wheat gluten has high protein digestibility (85–95%) comparable to soy protein (98%); however, its PDCAAS value (25%) is lower than soy protein (100%) (Berrazaga et al. 2019). Although gluten is also identified as a major food allergen like soy, the outstanding solubility, viscosity, swelling, binding and water holding properties make it the second most widely-used ingredient in the production of meat analogues (Sun et al. 2021; Boukid 2021).

The natural viscoelastic properties of gluten form thin protein film upon elongation, which can be then transformed into a fibrous structure (Kyriakopoulou et al. 2019). Moreover, wheat gluten can serve as a binding agent to hold the fiber structure in meat analogues and may reduce the cooking losses (Boukid 2021). Besides being mixed with soy protein and pea protein (Chiang et al. 2019; Schreuders et al. 2019), wheat gluten is also often blended with novel materials to develop new meat analogues products. Kumar et al. (2012) studied the effects of the blending ratio of wheat gluten and mushroom on sensory properties of developed nugget analogues and found that the optimal wheat gluten ratio in the formula was 18%. Hamid et al. (2020) reported that 58% jackfruit by-product and 20% wheat gluten included recipe had great potential to develop novel heathy meat analogues.

3.4 Algae

Algae is described by Bleakley and Hayes (2017) as "oxygen-producing, photosynthetic, unicellular or multicellular organisms excluding embryophyte terrestrial plants and lichens", including macroalgae (i.e., seaweed) and microalgae (Cavalier-Smith 2007). Although algae is not biologically defined as a plant, some algae species (e.g., green seaweed) have plant-like form (i.e., green and leafy). Recently, algae protein is being used as a novel and promising ingredient source for the development of animal-free meat analogues (Nadeeshani et al. 2021).

The annual global production of seaweed is 7.5×10^6 tonnes dry matter. South Korea, China and Japan are the top 3 consumers of seaweed (Bleakley and Hayes 2017). Seaweed is a popular food in Asia that can be used in soup and salad. The production of microalgae is smaller than seaweed, which is only 5000 tonnes dry matter per year. It is usually sold in powder form as functional foods due to high vitamin and mineral contents (Bleakley and Hayes 2017). Algae is also known for its high protein content (50–60%), which is greater than soy (35–40%). Moreover, different from traditional terrestrial high-protein plants, algae does not require freshwater and land to grow, and having higher growth rate, productivity, and protein yield (Grahl et al. 2018; Caporgno et al. 2020; Bleakley and Hayes 2017). In short, algae are more sustainable than terrestrial plants. Under the threats of increasing population and animal protein shortage, using algae protein to produce meat analogues is an attractive alternative. In fact, seaweed protein-based meat alternative products have already been commercialize in German market, trade named Remis Algen (Fig. 6.16). Progress on developing microalgae-based meat analogues is still on research stage. Studies of Grahl et al. (2018) and Caporgno et al. (2020) showed that when microalgae protein ratio was less than 30%, microalgae protein and soy protein blends had a potential to produce promising meat analogues under low moisture (<35%), high temperature, and high screw speed extrusion condition (Fig. 6.12).

4 Product Forms

Commercial meat products are usually sold in three forms: ground, comminuted and whole muscled (Dekkers et al. 2018). Depending on the form of meat products they are mimicking, meat analogues can be classified as coarse ground, loose filled and emulsified forms, aiming to satisfy specific consumer's needs (Table 6.1) (Joshi and Kumar 2015; Kumar et al. 2017; Borders 2007). Typical coarse ground-formed meat analogues include burgers, meatballs, nuggets, sausages etc., which is a relatively well-developed form of meat analogues and already has various mature products (Kumar et al. 2017). Loose filled-formed meat analogues include chili mix, taco filling, sloppy joe (an American cuisine), bulgogi (a Korean cuisine), etc. Emulsified-formed meat analogues include deli "meat", frankfurter, spreads, etc. (Kumar et al. 2017; You et al. 2020).

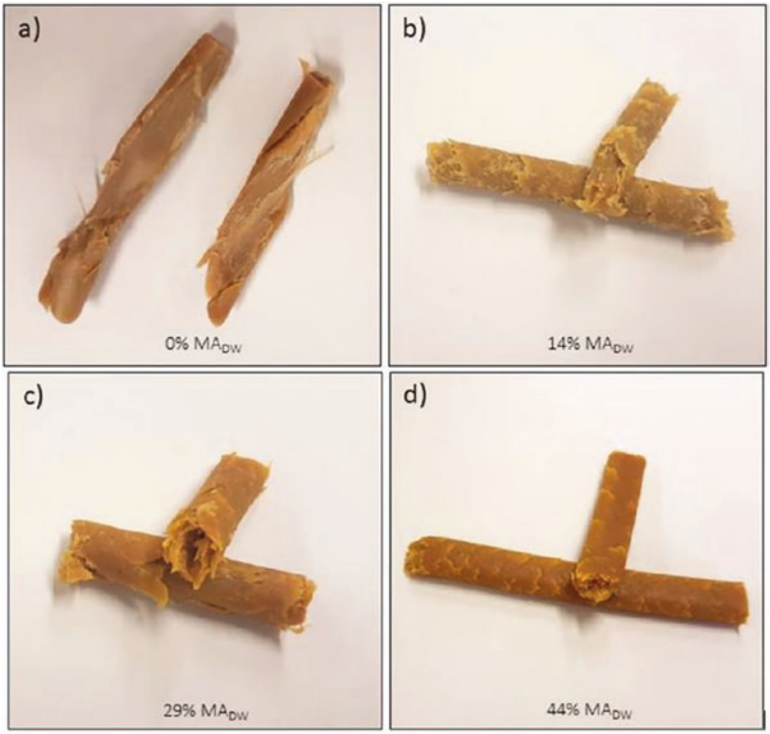

Fig. 6.12 Images of low-moisture extruded microalgae protein and soy protein blended meat analogues with (**a**) 0%, (**b**) 14%, (**c**) 29%, (**d**) 44% microalgae (MA) ratio by dry weight (DW) (Caporgno et al. 2020)

5 Quality Parameters

5.1 Microstructure

Microstructure is an important parameter used to evaluate the fibrous structure of meat analogues. Generally, scanning electron microscopy (SEM) is used to characterize the microstructure of meat analogues (Fig. 6.13) (Okano 2018). The sample preparation is an important step for SEM. The moisture content of meat analogue samples needs to be reduced significantly (usually by drying at 30–60 °C for 24 h) before imaging. However, drying might not be required for the low-moisture extruded samples. Dried samples are then cut into small pieces (0.5–1.0 cm cube) using a sharp blade, glued to aluminium stubs using silver conductive adhesive and finally sputtered coated with gold (Caporgno et al. 2020; Chiang et al. 2019; Krintiras et al. 2015). After loading the sample onto the microscope, the focused electron beam irradiates samples, and incident electron interacts with atoms on the surface of samples to generate various signals with respect to the texture of the samples. Finally, this information is transformed into image form, showing the shape including the structure of the test samples.

Table 6.1 Examples of commercial meat analogue products and their forms

Product	Product form	Commercial name	Company	Country
Burger	Coarse ground	Beyond burger®	Beyond Meat	America
		Impossible™ burger	Impossible Foods	America[a]
		Quinoa burger	Risenta	Sweden[a]
		Juicy burger	LikeMeat	Germany[a]
Meatball	Coarse ground	Gardein meatless meat balls	Gardein	Canada
		Vege mixed balls	Vegefood	South Korea[a]
Nugget	Coarse ground	Quorn™ chik'n nuggets	Quorn Foods	United Kingdom[a]
Taco filling	Loose filled	Loma Linda taco filling	Loma Linda	America
Sloppy joe	Loose filled	Loma Linda sloppy joe	Loma Linda	America
Bulgogi	Loose filled	Vegan-bulgogi	Vegefood	South Korea[a]
Deli "meat"	Emulsified	Smart Deli® turkey	Lightlife Foods	America
Frankfurter	Emulsified	Classic smoked plant-based frankfurter	Field Roast	America

[a]You et al. (2020)

SEM can reveal great microstructural details of samples at high magnification. It can detect changes due to modifying material formulation and, processing conditions. Chiang et al. (2019) investigated the microstructures of high-moisture extruded soy protein and wheat gluten blended meat analogues. They found that products started to show layered fibrous structure when wheat gluten ratio reached 20% in the formula (Fig. 13). For freeze structuring process, Yuliarti et al. (2021) reported that the pea protein and wheat protein based meat analogues had smooth and porous microstructure when wheat protein reached 17% in the formula. These results suggest that wheat protein may have better ability to form fibrous structure than soy protein and pea protein. Palanisamy et al. (2018) and Wi et al. (2020) investigated the effects of different additives, such as carrageenan, canola oil, lectin and so on., on the microstructure of soy protein-based meat analogues, indicating that proper additives and addition levels can modify products' fibrous structure substantially (Figs. 6.14 and 6.15).

5.2 *Textural Properties*

Textural properties of meat analogues are usually determined using cutting or compression testing system such as a texture analyzer. Meat analogue samples are cut into uniform pieces and placed on the centre of the device platform. For cutting tests, the blade cuts the sample in both parallel and perpendicular directions

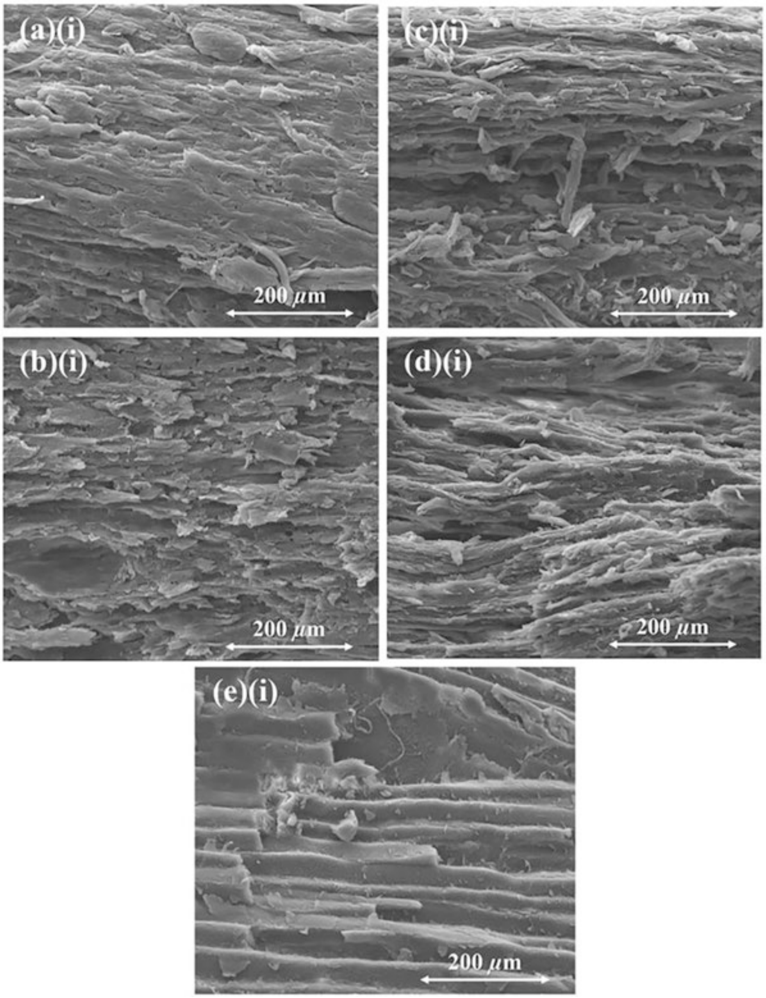

Fig. 6.13 SEM images of high-moisture extruded soy protein concentrate-based meat analogues with (**a**) 0%, (**b**) 10%, (**c**) 20%, (**d**) 30% wheat gluten in the formula, and boiled chicken breast (**e**) (Chiang et al. 2019)

to the formed fibers in the samples. Data such as real-time cutting/compression force, deformation distance, total compression distance, reversible deformation distance and so on are recorded and analyzed with the associated software (Caporgno et al. 2020; Chiang et al. 2019; Palanisamy et al. 2018). Besides material formulations and processing conditions, texture results can be affected by the moisture content and uniformity of the sample, pre-set cutting/compression depth, cutting/compression speed, cutter/probe type and so on. Common parameters of

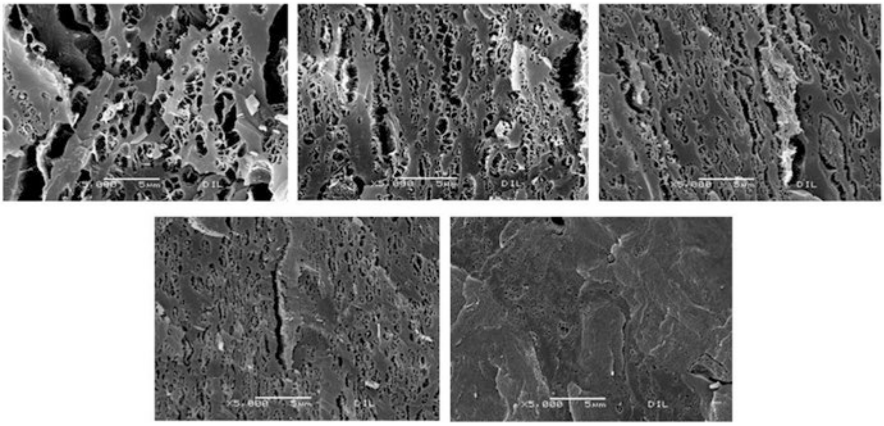

Fig. 6.14 SEM images of soy protein-based meat analogues with 0%, 0.75%, 1.5%, 2.25%, and 3% carrageenan (binding agent) addition levels (from left to right). Scale bars indicate 5 μm (Palanisamy et al. 2018)

meat analogues derived from cutting and compression tests, and their definitions are presented in Table 6.2.

Soy protein is a functional material to imitate the texture of meat products, especially in terms of obtaining optimal hardness (Egbert and Borders 2006; Yadav et al. 2015; Kitcharoenthawornchai and Harnsilawat 2015). For blended materials, Yadav et al. (2015) reported that equally blended wheat flour, corn flour, texturized soy grit, and mushroom paste (each material accounted for 25%) can produce chicken analogue rolls with satisfying texture. Meat analogue texture is also affected by processing temperature and moisture (Osen et al. 2014; Caporgno et al. 2020). In general, increasing extrusion moisture decreases meat analogues' hardness (Caporgno et al. 2020). Additives (e.g., carrageenan) can affect the texture of meat analogues by affecting formed protein network (Palanisamy et al. 2018).

5.3 Sensory Evaluation

Sensory evaluation is a tool to understand consumers' acceptance and preference towards products, which is widely used during food product development (Kemp et al. 2018). Descriptive analysis is adopted by far the most common. Different from results that generated from machines, descriptive analysis uses trained panelists as instruments to judge samples from the aspects of appearance, flavour, texture, mouthfeel, odour and overall acceptability, thereby obtaining more detailed, objective and reliable data than consumer studies (Grahl et al. 2018; Chiang et al. 2019;

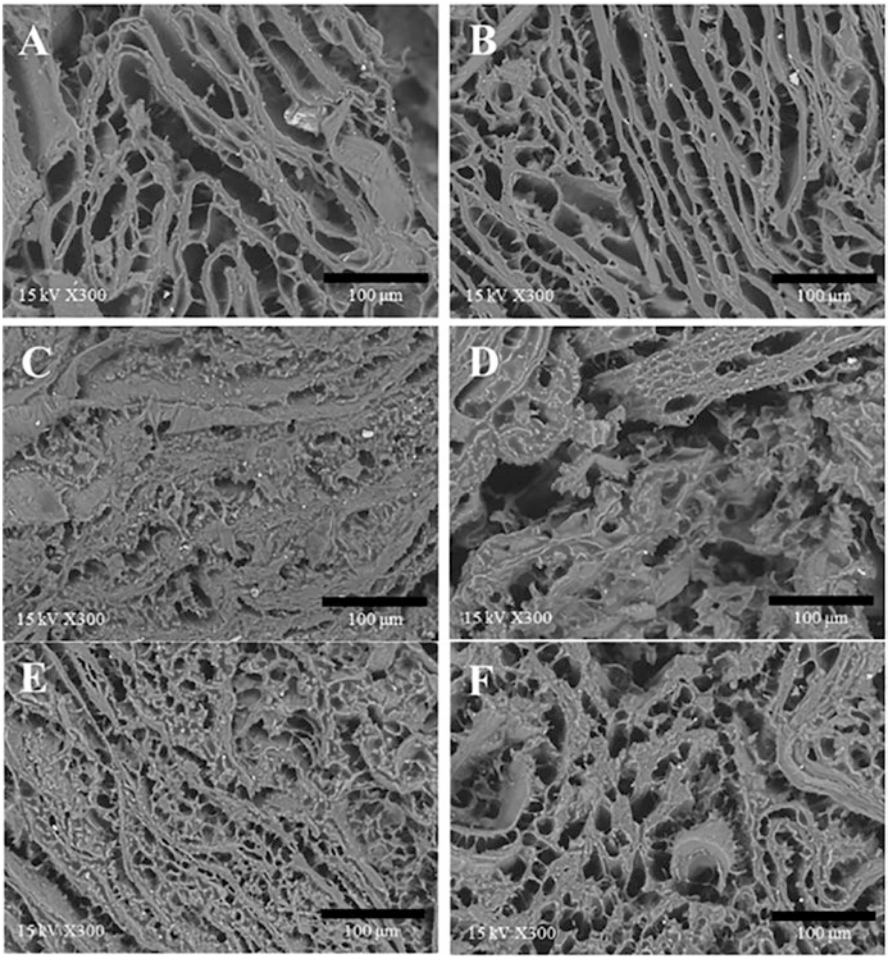

Fig. 6.15 SEM images of TVP (made from SPI, wheat gluten and wheat starch)-based meat analogues produced by extrusion with different additives: (**a**) water, (**b**) water and SPI, (**c**) canola oil, (**d**) canola oil and lectin, (**e**) O/W emulsion, and (**f**) water, canola oil, SPI and lecithin (Wi et al. 2020)

Kemp et al. 2018). In terms of evaluating meat analogue products, special attributes such as hardness, chewiness, fibrousness, odour, color, flavour, aftertaste and so on need to be well-defined in advance (Table 6.3) for panelist training. During the evaluation, panelists are introduced to individual booths, where lighting, room temperature, and humidity are controlled to minimize variability and bias. Samples coded with random digital numbers are served to panelists for scoring, along with water for panelists to clean mouth between samples (Grahl et al. 2018; Chiang et al. 2019; Kemp et al. 2018). Sensory evaluation can examine food products with a broad range of characteristics that machine-based experiment cannot achieve.

Table 6.2 Typical quantitative attributes (instrumental parameters) used to evaluate meat analogues and their descriptions

Attribute	Unit	Description
Hardness	N	Maximum force required to compress the sample[a]
Springiness	mm	Ability of the sample to recover its original form after removing deforming force[b]
Cohesiveness	No unit	Ratio of total work required for first compression to second compression[b]
Elasticity	No unit	Ratio of reversible deformation distance to total deformation distance[c]
Gumminess	N	Force required to disintegrate the sample for swallowing (hardness * cohesiveness)[b]
Chewiness	N * mm	Work required to swallow the sample (springiness * gumminess)[b]

[a] Attributes and descriptions modified from Chiang et al. (2019)
[b] Attributes and descriptions modified from Yadav et al. (2015)
[c] Attributes and descriptions used by Palanisamy et al. (2018)

Table 6.3 Typical qualitative attributes used to evaluate meat analogues and their assessment methods

Attribute	Assessment method
Hardness	Bite the sample through completely between the molar teeth[a]
Chewiness	Chew the sample for at least 24 chews[a]
Elasticity	Press the sample with fingers[b]
Firmness	Section off a piece of the sample and evaluate the force needed[b]
Fibrousness	Tear the sample into half and observe it visually[a]
Juiciness	Chew the sample for 5 times and evaluate released moisture[b]
Crumbliness	Chew the sample for 5 times and evaluate the amount of small pieces that the sample break up to[b]
Odour	Hold the sample 2 cm under the nose, smell the bottom part 3 times every 15 s[b]
Color	Observe the sample visually under the standard light of the booth[b]
Flavour	Chew the sample for 5 times and evaluate the flavour intensity[b]
After-taste	Swallow the sample, wait for 5 s and then evaluate the aftertaste intensity[b]

[a]Information gathered from Chiang et al. (2019)
[b]Information gathered from Grahl et al. (2018)

However, sensory evaluation can be time-consuming and expensive as it involves trainings and labours, which can limit its application in many studies (Kemp et al. 2018).

Among the extrusion parameters (i.e., temperature, moisture, and screw speed), Grahl et al. (2018) found that moisture had the greatest impact on sensory properties of meat analogues, which was positively correlated with juiciness, mouthfeel, and appearance. In terms of additives, Palanisamy et al. (2018) reported that the addition of carrageenan had a positive and negative quadratic relationship with the firmness and elasticity of soy protein-based meat analogues, respectively. The addition of emulsion can improve meat analogues' juiciness, tenderness and overall acceptability in general (Wi et al. 2020). The flavour and odour of meat analogues are tightly associated with protein sources. Rehrah et al. (2009) found that consumers' acceptability to peanut-based and soy-based meat analogues was high and comparable, in terms of flavour and after-taste. However, elevated microalgae content increased the intensity of the earthy flavour and musty odour of meat analogues, resulting in reduced consumer acceptability (Grahl et al. 2018).

6 Nutritional Values

Typical meat analogues contain 50–80% moisture, 20–50% texturized and non-texturized proteins, 2–30% polysaccharides (e.g., starch and fiber), 0–15% lipids (e.g., coconut and canola oils), 3–10% flavouring agents (e.g., sugar, spices and herbs), 1–5% binding agents (e.g., carrageenan) and 0–0.5% coloring agents (e.g., lycopene, beet juice extract) (Egbert and Borders 2006; Boukid 2021). In addition to mimicking the appearance and texture of meat products, meat analogues provide similar nutritional value (Table 6.4). Processed plant proteins have enhanced protein digestibility, but they may still have imbalanced amino acid profiles, lacking one or more essential amino acids. For example, pea protein and wheat protein is low of methionine and lysine, respectively (Boukid 2021; Bohrer 2019). Therefore, it is recommended to blend whey protein or egg white in the formula to maximize the nutritional value of meat analogues (Sun et al. 2021).

Besides protein, meat analogues can be fortified with other nutrients. Jackfruit by-product based meat analogues developed by Hamid et al. (2020) contained more than 9% of dietary fiber, which was almost double of commercial meat analogues. Caporgno et al. (2020) fortified microalgae meat analogues with vitamins B and E, and successfully retained >95% of additions in the final product. Sreeitthiyawet et al. (2019) reported that the blend of 22.5% Jerusalem artichoke (a low-calorie healthy herb) flour, 40% white kidney bean (a high-dietary fiber pulse) flour and 37.5% soy flour can be used to produce consumer-acceptable and nutrient-comprehensive meat analogues.

7 Commercial Products

Based on the investigation of Market and Market (an American market research company), the global meat analogue market accounted for 1.6 billion USD and was estimated to reach 3.5 billion USD in 2026 (Boukid 2021). Mintel's market report showed that Europe, North America, Asia Pacific are the top 3 markets holding 51.5%, 26.8% and 11.8% global share, respectively. Markets of Latin America (6.3%), Middle East and Africa (3.6%) are smaller (Boukid 2021). Beyond Meat, the most famous and mature meat analogue brand, has grown explosively since founded in 2009. They produce plant-based meat crumbles, nuggets, sausages, patties and so on, with multiple flavours (Fig. 6.16). They partnered with global or

Table 6.4 A comparison of nutritional composition of commercial meat products and meat analogues (per 100 g serving)

Commercial products	Energy (kcal)	Protein (g)	Total lipid (g)	Carbohydrate (g)	Calcium (mg)	Iron (mg)	Potassium (mg)	Sodium (mg)	Zinc (mg)
Meat products									
Ground beef (70% lean meat and 30% fat, raw)	332	14.4	30	0	24	1.64	218	66	3.57
Chicken nuggets (raw)	298	13.36	13.92	17.88	52	1.06	229	600	0.87
Ground pork sausage (raw)	296	13.6	25.1	3.78	19	1.41	308	788	1.68
Meat analogues									
Beyond Meat, burger patties (raw)	230	17.7	15.93	4.42	88	3.72	248	310	n.d.[a]
Impossible Foods, burger patties (raw)	212	16.81	12.39	7.96	150	3.72	540	327	6.64
Quorn Foods, chicken nuggets (raw)	247	11.76	10.59	30.59	0	0.42	n.d.	482	n.d.
Simulate, chicken nuggets (raw)	235	15.29	11.76	18.82	35	2.35	282	471	n.d.
Lightlife, ground sausage (raw)	107	14.29	0	12.5	71	1.93	500	536	n.d.

[a]*n.d.* no data. Information in the table was obtained from FoodData Central (2021) of U. S. Department of Agriculture

national chain restaurants (e.g., KFC, A&W, Panda Express, Dell Taco, Veggie Grill, etc.) to market their products successfully in a short time. Other brands such as Gardein and Lightlife Foods are also gowning rapidly and ready to take a share of this booming market (Fig. 6.16). According to Mintel, more than 6485 meat analogue products were launched since 2015 (Boukid 2021).

Fig. 6.16 Beyond meat beyond sausage, Gardein breakfast patties and lightlife smart dogs sold on Hy-Vee (2021). Remis Algen Algae Currywurst sold in Germany (Vegetarischer Versand.de 2021)

8 Conclusion

Meat analogues mimic the appearance, texture, mouthfeel, and flavour of traditional meat products, while providing similar or greater nutritional values. Soy, pea and wheat proteins are the most widely used raw materials for producing meat analogues. Novel materials such as algae is also gaining researchers' attention for the development of diversified meat analogue products. Current technologies that are employed to produce meat analogues are extrusion (low-moisture or high-moisture), shear cell (cone-on-cone or cylinder-in-cylinder) and freeze structing. During this process, native globular plant proteins are hydrated, unfolded, aligned and reconnected to form linear fibrous meat-like structure. In general, the SEM is used to determine the microstructure of meat analogues for their meat-like texture. The sensory evaluation and instrumental textural properties such as hardness, springiness and chewiness are vital to assess their acceptability by consumers. At present, the commercial meat analogues are available in three major forms such as coarse ground, loose filled and emulsified. The meat analogue is a good starting point for consumers who are interested to include more plant proteins in their diet.

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Chapter 7
Fermented Plant Protein Products

Tariq Ismail, Anam Layla, and Saeed Akhtar

1 Introduction

Fermented foods of plant origin are important components of human diet and are thought to be a biocultural heritage that has emerged centuries ago by the interaction of societies and their environment (Nabhan 2010). Non-dairy foods or plant matrices are of immense health significance to human for being source of essential nutrients including vitamins, minerals and fibers, and non-nutrients bioactive compounds (Hugenholtz 2013). Plant based products undergone lactic acid fermentation are gaining consumer interest as potential alternate to dairy for their extended health benefits in preventing diseases. Over the years, plant products global market as alternate to dairy and meat-based foods is expected to reach a value of 26 billion USD by 2024 (Tangyu et al. 2019).

Plant-based foods are rarely consumed raw and are processed to increase product palatability and nutriture. Industrially explicated processing may be minimal to drastic such as simple soaking to fermentation and cooking to extrusion cooking (Fardet 2017). Together with dehydration and salting, fermentation is one amongst the most promising techniques of food preservation in various cultures. Though the

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technique is practiced at large in rural settings to ensure household food security, but it has also gained interests of large-scale manufacturers for scale up production at industrial level.

Historically, human has used at least 3000 plant species as food while around 150 plant species have been cultivated at commercial scale. In the context of human requirements for plant-based protein, only a few plant groups have gained popularity at commercial scale that includes cereal grains and legumes (Young and Pellett 1994). Global estimates of world protein supplies suggest plants to provide up to 65% of the global edible protein supply while cereals contribute a major share of this supply (FAO 1991).

Proteins are essential dietary nutrients however their nutritional quality varies with the source and other factors including digestibility, bioavailability, amino acids profile and presence of anti-nutrients factors. In a background of increasing global needs for plant-based diets, studies on plant-based diets demonstrated plant proteins as plausible source of essential amino acids (López et al. 2018). Vegetable's proteins are pride of culinary traditions in African states due to their wide acceptability as nutritive non-meat substitute and as condiments (Achi 2005). Plant protein-based food compositions are gaining popularity among the consumers due to health and lifestyle needs (Mäkinen et al. 2016). Legumes are reported to be the major source of proteins in some regions. Legumes in their fermented form are used as flavor enhancer and serve as compliment to sauces and soups.

2 Fermented Cereals and Cereal-Based Products

Despite of their deficiency in some essential amino acids, relatively low protein contents and presence of intrinsic toxicants, which hinders absorption and metabolism of important nutrients, cereals constitute a major part of the global dietary nutrients supply. In line with the recent age strategies such as genetic improvement and supplementation aimed at improving nutritional quality of cereals, fermentation has been proposed as the most economical and simple approach to improve nutritional quality and palatability of cereals (Blandino et al. 2003). Cereal's fermentation increases interaction between starch and gluten that resultantly reduces starch availability and lowers down glycemic index of the baked goods (Poutanen et al. 2009). Fermenting cereals flour has also been cited to reduce levels of flatulence factors including non-digestible oligosaccharides and resistant starches. In addition to providing optimum pH that aids in enzymatic degradation of intrinsic nutrient inhibitors like phytates and tannins, fermentation also yields improved B vitamins and mineral contents of the cereals' dough (Capozzi et al. 2012; Nkhata et al. 2018). Sourdough fermentation of conventional cereals yields a complex blend of flavouring compounds from organic acids, alcohols, aldehydes, carbonyl compounds and ketones, which anticipate remarkable appetizing effect to the edible goods (Campbell-Platt 1994).

Hundreds of fermented cereals – based foods are available as primary source of protein – energy to socio-economically vulnerable populations of developing countries. Ultimate objectives of fermenting cereals in various cultures and traditions are to improve palatability of the cereals based conventional foods and to enhance their shelf stability. Lactic acid fermentation is practiced in nearly all conventional cereals including wheat, rice, maize, millet, sorghum, rye, oat, barley. The later three including maize, sorghum and millet are used in African cuisines to prepare food like *poto-poto* (Congo), *ogi* (Nigeria and Benin), *koko* (Burkina faso), *togwa* (Tanzania), *hussuwa* (Sudan), *pozol* (Mexico), *atole agrio* (Guatemala), *injera* (Ethopia and Eritrea), *idli* (India), *burong isda* (Philipine) (Guyot 2012).

2.1 Cereals-Based Fermented Foods and Beverages

A wide variety of traditional plant-based beverages are available worldwide that include tigernut milk i.e., *Horchata* (Cortés et al. 2005), rice, malt and sugar based fermented beverage i.e., *Sikhye* and *Amazake* (Jeske et al. 2018), fermented sorghum or fermented millet – malt-based drink i.e., *Bushera., Boza* – a fermented drink made of conventional cereals including wheat, maize, millet and rye (Blandino et al. 2003) and Asia originating traditional soy milk (Mäkinen et al. 2016).

Organoleptic acceptability of plant proteins-based foods is generally ranked low; however, processing like fermentation do not merely increases nutritional properties of the fermented plant protein-based foods but also enhance sensory acceptability and palatability (Lee and Beuchat 1991; Leroy and De Vuyst 2004). Commercial milk alternatives are derived from plants including legumes, cereals, pseudo-cereals, seeds and nuts (Mäkinen et al. 2016). Fermented plant-based milk resembles animal milk for color and texture; however, their natural composition may not be compared to cow milk. Hence, commercial plant-derived milk formulations are amended with added nutrients including vitamins, minerals, and essential amino acids (Sethi et al. 2016). Fermentation increases growth of fermenting food grade microbes and enhances plant protein solubility with better amino acid composition and availability (Tangyu et al. 2019). Mixed-culture fermentation with two or more than two microbial species has emerged as a viable technique to improve nutritional and sensory qualities of fermented products. Synergistic effect of diverse microbial population on sensory attributes of plant-derived milk alternatives had been reported promising (Sieuwerts et al. 2008). Interactions between microbes during mixed-culture fermentation are mutualistic in nature. Such an interaction promotes or improves beneficial biological activities of at least one type of microbe (National Research Council 1992). An ideal example of mutualism is interaction between *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in yogurt fermentation wherein release of peptides and amino acids is ensured by proteolytic Lactobacilli strain while *Streptococcus* provides growth stimulating factors such as pyruvic acid, formic acid and folic acid to *L. delbrueckii* (Sieuwerts et al. 2008). Mixed-culture during fermentation `not merely stimulates microbial growth but also enhances production of volatile compounds and acids production. Bifidobacterium, as an example, increases protein contents of soy-based drinks. Significantly higher levels of L-lysine are reported from soybean meal fermentation with *Lactobacillus plantarum* while some other bacterial strains and yeasts have also been suggested to synthesis essential vitamins like vitamin K and B vitamins (Bentley and Meganathan 1982; LeBlanc et al. 2011, 2013).

Attractive nutritional profile of cereals favours their candidature for development of fermented functional foods. Availability of a range of micro and micronutrients in cereals create necessary environment for the growth of lactic acid bacteria and increases bio-accessibility of nutrients (Blandino et al. 2003; Endo and Dicks 2014). Incorporating malted cereals alone or in combination with hydrolytic enzymes increase bioavailability of bound nutrients such as starches and proteins (Luana et al. 2014). Lactic acid bacteria release high molecular weight polysaccharides which improve viscosity of the substrate, a key textural feature of cereal-based fermented beverages and yogurt.

Various strains of Lactic acid bacteria isolated from the African native products including Sorghum-based Nigerian Ogi and Cassava based fufu were reported as exopolysaccharides producers (Adebayo-Tayo and Onilude 2008). Promising EPS producing strains include *L. delbrueckii ssp. bulgaricus* NCFB 2772 and *Pediococcus damnosus* which had been used to attain desired level of viscosity and ropy texture in oat – based non – dairy products (Mårtensson et al. 2002).

2.1.1 Traditional Fermented Cereals-Based Beverages

Rabadi

Rabadi is a cereal – based fermented milk beverage and has high popularity in Northern states of India (Hussain et al. 2014). The product is usually made from underutilized cereals including sorghum, miller and barley by mixing flour of the listed cereals with butter milk followed by 4–6 h fermentation under sun. The recipe is diluted with water, salted, cooked and cooled prior consumption (Modha and Pal 2011). Utilization of cereals flour in combination with butter milk for developing *Rabadi* makes the product more nourishing, palatable and digestible for the end users (Gupta and Nagar 2010). Rabadi developed by fermenting pearl millet flour with butter milk for a period of ~9 h has been reported to reduce phytic acid contents of the product by 30% (Dhankher and Chauhan 1987).

Boza

Boza is a non-alcoholic traditional Turkish beverage made by lactic acid bacteria and yeast fermentation of wheat, semolina, millet, maize or rice. Boza is known to the peoples of Central Asian countries for centuries from where it was introduced to Anatolia and Europe by the immigrants. Different variants of boza including braga, busa and bouza are produced in Eastern Europe, The Balkans and Egypt, respectively. The word "boza" originated from a Persian word "buze" that means millet. It's the reason that best quality boza with distinct taste and flavour is made of millet while the one produced in Egypt is consumed as beer on account of its high alcohol contents i.e., up to 7% (Arici and Daglioglu 2002). Commercial scale production of boza follows various operations including grain milling to the size of semolina i.e., ~300–800 μm, addition of water and cooking in a steam jacketed boiler, cooked material is cooled with cold water in marble vessels. Straining of the cold material is performed to remove bran, hull or any other foreign matter. Strained matter is enriched with sugar and inoculated with starter culture, fermented boza, yogurt or sourdough and fermentation is carried out for a period of 24 h at 30 °C. Fermented boza is cooled and served refrigerated to a maximum of 3–5 days (Evliya 1990; Uylaser et al. 1998; Altay et al. 2013). The process of boza production may be summarized into five key steps which include raw material preparation, boiling, cooling and straining, addition of sugar or sweetener and fermentation (Arici and Daglioglu 2002). Microflora identification of boza indicates lactic acid bacteria including *L. plantarum*, *L. acidophilus*, *L. fermentum* and *Leuconostoc spp*. to dominate over yeasts such as *S. cerevisiae*, *Candida tropicalis*, *C. glabrata* and *Geotrichum spp.* (Gotcheva et al. 2000).

2.1.2 Traditional Fermented Cereals-Based Other Foods

Ogi

Ogi is a maize, sorghum and millet derived fermented cereals gruel and is one amongst the most common breakfast meal, and an important weaning food in West Africa (Banigo and Akinrele 1993; Amusa et al. 2005). Sorghum and millet are traditionally used as substrate for fermentation in Ogi preparation while quite different nomenclatures like *koko, furah, kamo, eko* have also been assigned to similar product with different substrates in the Coastal regions of West Africa (Blandino et al. 2003). Ogi bearing smooth texture, characteristic aroma, and sour taste similar to yogurt has varying colour profile i.e., creamy white to reddish brown and dirty grey that matches to the cereal grain used as base ingredient for Ogi's production. Ogi is traditionally prepared by steeping cereals for a period of 1–3 days to ferment in earthen ware, enameled or plastic pots. Fermented grains are wet milled and sieved to yield ogi slurry (Steinkraus 1983; Iwasaki et al. 1991). Ogi fermentation is aided with mixed microflora including lactic acid bacteria predominately *L. plantarum*, *Corynebacterium*, moulds and yeast (*Saccharomyces spp*. and *Candida Spp.*) to anticipate starch hydrolysis and unique flavours development (Caplice and Fitzgerald 1999). Conventional process of Ogi production yields substantial losses in lysine and tryptophan contents which have been proposed to be reclaimed by incorporating lysine and methionine secreting mutant strains of *Lactobacilli* and yeast for fermenting cereals (Odunfa et al. 2001), and by fortifying cereals with legumes, pulses and nuts flour. Cereals fermentation during ogi processing releases phosphorous bound phytate and improve niacin and riboflavin contents. Fortifying maize with pigeon pea at 60:40 ratio for Ogi production has been reported to yield highest levels of thiamine (1.34 mg/g), riboflavin (1.4 mg/g), niacin (6.9 mg/g) and essential amino acids including lysine (94 mg/g), leucine (110 mg/g), isoleucine (55 mg/g), tryptophan (20.4 mg/g), phenylalanine (86.23 mg/g) and valine (68.3 mg/g) (Okafor et al. 2018).

Pozol

Pozol is fermented maize derived nourishing food of Indians and Mestizo groups from South – East Mexico where this drink is the main component of the daily diet (Pérez-Armendáriz and Cardoso-Ugarte 2020). In Indian African tradition, pozol is prepared by boiling maize kernels in lime water. Kernels are dehulled, drained and ground to a coarse dough that is shaped into 5–8 cm diameter balls. Dough balls are wrapped into banana leaves and kept at ambient temperature for a period of 1–5 days. The product is served by suspending fermented dough balls in water with or without flavouring (Cañas-Urbina et al. 1993). Fermentation of pozol dough balls for a period of 1–4 days reduces pH of the dough from 7.5 to 4.1. With an estimated count of 10^9 cfu/g, lactic acid bacteria dominate pozol microflora followed by aerobic mesophiles (10^7 cfu/g), enterbacteriaceae (10^6 cfu/g), yeasts (10^6 cfu/g) and moulds (10^4 cfu/g) (Wacher et al. 1993; Nuraida et al. 1995). In recent days, pozol as a probiotic beverage is also used for the treatment of diarrhea and fever (Velázquez-López et al. 2018). Though nixtamalized maize derived pozol is a popular recipe yet modified forms of pozol produced by incorporating seasoning ingredients, roasted and milled mamey seeds, fermented seeds of cacao and pataxte are also consumed in Southern Mexico (Barros and Buenrostro 2011).

Injera

Injera is an ethnic traditional fermented food of Ethiopia where almost every individual is expected to consume injera at least once a day (Neela and Fanta 2020). Though tef is the principal cereal in Ethiopian injera yet it can be made from other cereals including sorghum, finger millet, barley and corn. Ethiopian injera is like pancake and is made from tef flour, water and starter culture. Highest consumer acceptability of injera is generally attributed to its uniformly spaced honeycomb like eyes, pleasant sour taste, softness, sponginess and rollability (Girma et al. 2013). Preparation of injera involves various operations including manual or mechanical dehulling of the grains, milling, dough development and inoculation with starter culture, and fermentation for a period of 2–3 days. Fermented dough is converted into a thick batter, poured onto a oiled pan and steamed for 2–3 min by covering the pan with a tightly fit lid (Parker et al. 1989). Freshly prepared injera can be stored up to 3 days without any significant loss in physical and sensorial attributes.

3 Fermented Legumes and Pulses

Cereals are principle dietary source of energy and supply approximately 50% of the protein needs worldwide. However, unfavorable amino acid balance of cereal grains necessitates supply of complimentary protein sources to meet essential amino acids inadequacies and optimal nutrition. Legumes as 2–3 times more protein carriers serve as potential source of energy and protein for the underprivileged populations of the tropical and sub – tropical regions where economic access to animal proteins is poor. Among edible plant grains, legumes and products derived thereof are the richest source of edible proteins (Deshpande 1992; Duranti and Gius 1997; Iqbal et al. 2006). In addition to their significant caloric and protein contents, legumes are also potential source of dietary fibers, B – vitamins and minerals for human nutrition. Proportion of edible leguminous grains consumption varies with the region. In Latin America, legumes constitute approximately 10% of the regular diet while it ranges between 30% and 50% in India. Alike leguminous grains, nuts are 100% higher protein carrier than cereals while their amino acid distribution patterns are more promising than common edible grains. Even though nuts are rich source of nutrients like essential fatty acids, amino acids and micronutrients of human health significance, higher cost of the nuts do not allow consumers to rely on such sources to meet their daily dietary needs for optimum nutrition.

The developing world is reliant upon fermentation as one amongst the major food preservation techniques and as a natural mean to enhance micronutrient supplies from plant-based products. Steinkraus (1997) suggested fermentation to anticipate a variety of important functions including (a) dietary enrichment of the fermented foods (b) organic preservation (c) biological enrichment of the plant material (substrate) with essential nutrients including protein, essential fatty acids and amino acids, minerals and vitamins (d) removal of toxicants and (e) reduced cooking time.

A range of microbiota including lactic acid bacteria, a few fungal species and yeasts are deployed in spontaneous (natural) fermentation of the pulses that may cause non-consistent changes in product quality. Such a combination of fermentation microbiota may further favor production of potential pathogens and toxins. Lactic acid bacterial cultures dominate pulses fermentation and reduce pH of the substrates to such an extent where the growth of competing microbiota including pathogens is hindered. Pulses fermentation favors reduction in substrate pH and subsequent changes in composition and contents of carbohydrates, proteins, lipids, minerals and vitamins, and enzymatic degradation of anti-nutritional compounds and concentration of macronutrients (Galati et al. 2014; Adebiyi et al. 2016). Microbiological activity of lactic acid bacteria on endogenous compounds yields production of value-added microbial metabolites including organic acids, aldehydes, ketones and alcohols (Adebo et al. 2017). Fermentation of pulses thus improves texture, nutritional quality and sensory attributes of the consumable goods.

Protein contents of pulses vary between 22% and 28% depending on cultivar, growing conditions and maturity stage (Sotelo and Adsule 1996; Roy et al. 2010).

Glutelins, globulin and albumin constitute major part of the pulses' storage protein (Duranti and Gius 1997; Roy et al. 2010). Fermentation of pulses yields bioactive peptides and hydrolysates bearing multifunctional properties i.e., antioxidant, antimicrobial, anti-proliferative and angiotensin converting enzyme activities (Zambrowicz et al. 2012). A study by Xiao et al. (2015) reported fermentation of chickpea to increase true protein and essential amino acid concentration while solid-state fermentation hydrolysates of chick-pea have also been observed as carrier of low molecular mass proteins. Extent of protein hydrolysis and nature of the protein further determine functionalities of pulses protein that include digestibility, fats absorption capacity, emulsification, water absorption capacity (Jung et al. 2005; Lee et al. 2008; Tavano 2013; Xiao et al. 2015).

Fermented pulses and pulses – based products are ubiquitously available in developing countries as healthy meal, snack and spices, and its amongst the reasons that a range of pulses based fermented products including *tempeh*, *dawadawa*, *dhokla* are developed at industrial level in recent era.

Despite its significance, commercialization of fermented pulses and products has not gained enough success due to lack of appropriate processing technology and techniques, and affordability of the microbiological cultures desired in development of safe and shelf-stable consumer goods.

3.1 Pulses Yogurt

Pulses – based yogurts market is tremendously growing due to their relatively high protein contents and reduced risks of allergenicity as being linked with plant – based yogurts of soy and coconut origin (Boeck et al. 2021). Development of pulses – based yogurt with good sensory attributes demands inactivation of lipoxygenase activity via heating. Roasting of cowpea and mung bean prior dehulling and soaking had been reported to generate yogurt with reduced beany flavours (Rao et al. 1988). Certain pulses of wild origin like lupin seed are too bitter for human consumption. Acceptability of lupin yogurts had been improved by soaking lupin seeds in 0.5% boiling sodium bicarbonate solution. Dehulling and grinding of the treated lupin seeds do not merely reduce astringency and beany flavour of the lupin yogurt but also anticipate elimination of quinolizidinic alkaloids and a significant improvement in product proteins contents. Fermentation of lupin to develop yogurt (Jiménez-Martínez et al. 2003). Legume proteins are generally reported to generate soft gels than their counter part milk proteins. Developing pulses – based yogurt by fermenting flours with heteropolysaccharides producing strains such as *L. rhamnosus* and *L. plantarum* improve yogurt gel structure and reduces risks of odorous compounds generation (Li et al. 2014). Recent studies also suggest value-addition of conventional yogurts by incorporating lentil flour to improve nutritional, textural and storage stability properties of the fermented good (Haq et al. 2019).

3.1.1 Non-dairy Soy Yogurt

Yogurt is a widely consumable highly palatable traditional dairy product made by fermenting cow milk with starter lactic acid bacteria (Tamime and Marshall 1997). Contrarily to its good nutritional composition, cow milk associated health risks including allergenicity and high saturated fat contents have increased consumers demand for vegetarians' non-dairy alternatives (Debruyne 2006). Soybean and its products are reported to anticipate reduced risk of allergenicity and have anti-cholesterolemic, anti-atherogenic and hypolipidemic properties suggesting soymilk and its products as healthier alternatives to cow milk (Onuegbu et al. 2011; Chen et al. 2017).

Soy yogurt, also known as sogurt is prepared with a variety of lactobacillus strains including *L. delbrueckii.*, *L. plantarum.*, *L. acidophilus*, *L. casei*. Earlier, sogurt had been reported beany in aroma, stringent in taste and slight sandy in texture when compared with yogurt (Cheng et al. 1990). However, later studies suggested improved sensory properties of sogurt by deploying mixed cultures in fermentation process. Mixing *Bifidobacterium breve* with *L. acidophilus* and *S. thermophilus* were found to mask undesirable sensory effects in sogurt cultured with *B. breve* alone (Chang et al. 2010). In a study by Cheng et al. (1990), fermenting soy milk-based formulation with *L. casei* and *S. thermophilus* was reported to produce yellower and firm sorgut with sandy texture. Careful adjustment of soymilk solids and sugars increase acceptability score of fermented soy-based products including sogurt. Enriching soymilk with glucose and sucrose to 8% solid contents increases titrable acidity, reduces syneresis and improve overall sensory acceptability of sogurt (Estévez et al. 2010). Physico-chemical, textural and sensory attributes of sogurt may also be improved by using germinated soybean seeds. A study by Yang and Li (2010) reported significant improvement in textural and sensory characteristics of sogurt prepared from germinated soybeans (3 cm hypocotyls). Germination further demonstrated reduction in pH while increased acidity and lower degree of hardness were also observed in sogurt. A crucial role of natural sweeteners i.e., fructose, sucrose, fructose/sucrose and fruits flavor has been suggested to formulate soy protein fortified yogurts. Soy flavor and its astringency markedly reduce with increasing concentration of sweeteners between 6% and 8% while fruits flavors when incorporated in soy protein augmented yogurts significantly mask soy aroma and astringency (Drake et al. 2001).

Soy based products have gained tremendous attention of vegetarians and aged communities, however its low vitamin contents, more specifically the vitamin B12 anticipate risks for the development of vitamin B12 deficiency in vegetarians and elderlies. Co-fermentation of fructose and glycerol in soy-yogurt with *L. reuteri* enabling enhanced production of vitamin B12 upto 18 μg/100 ml has been suggested as a viable solution to vitamin B12 deficiency (Gu et al. 2015). Mounting evidence suggests therapeutic properties of soy yogurt, isoflavone – supplemented soy yogurt, *Spirulina platensis* fortified soy yogurts and synbiotic soy yogurts against dyslipidemia and atherosclerosis (Cavallini et al. 2009; Rossi et al. 2008; Sengupta et al. 2018, 2019). Soy-yogurts fortified with *Spirulina platensis* restores impaired antioxidant defense in dyslipidemic animal models and decreases atherogenic index (Sengupta et al. 2018). Soy based foods including soy milk and soy yogurt are major sources of phytoestrogens i.e., isoflavone that anticipate important role in breast cancer genesis and its progression. A prospective study on isoflavone based foods and survival of breast cancer patients suggested positive effect of soy foods consumption with an average isoflavone intake above 17 mg per day on higher survival rate in breast cancer patients (Zhang et al. 2012).

3.2 Legumes-Based Fermented Beverages and Milk Analogues

Milk analogues or non-dairy milk substitutes are plant derived water extracts and are widely demanded by the consumers for reasons including lifestyle, sustainability, and other health related concerns. Global market of plant-derived milk analogues is a multi-billion business and is expected to grow to 26 billion USD by 2023 (Bloomberg Surveillance 2015; Tangyu et al. 2019). Fermented milk market is worldwide largest fermented food market that worth more than 46 billion Euro (Marsh et al. 2014).

In addition to their protein dense profile, pulses are considered as abundant source of functional dietary fibers and or prebiotics including fructooligosaccharides, galactooligosaccharides, and resistant starches (Wongputtisin et al. 2015). Based on their health promising composition and high nutritional value, pulses and legumes serve as cost-effective alternatives to milk-based beverages for impoverished and milk sensitive communities. Recently, yogurt like beverage bearing comparable physicochemical properties to that of conventional yogurt has been developed from cow pea extracts (Aguilar-Raymundo and Velez-Ruiz 2019). The product was further ranked higher than milk yogurt based on its functional benefits. Neuroprotective effect of *L. plantarum* M-6 fermented Chickpea milk carrying high contents of GABA were reported by Li et al. (2016) suggesting chickpea milk as more health promising to natural yogurts.

3.2.1 Non-dairy Soy Milk

Soy-milk fermentation with lactic acid bacteria offers improved flavor and texture to the product and preserves soy-milk quality. Utilization of soy stachyose and raffinose by appropriate selection of fermenting organisms decreases flatulence tendency of soymilk. Fermenting soymilk oligosaccharides thus increases product digestibility and overall acceptability (Mital and Steinkraus 1979). Inoculation of soymilk with bacterial cultures of *Enterococcus faecium* and *Lactobacillus jugurti* (1:1) was reported to anticipate best sensory and physicochemical properties (Rossi et al. 1999). Simultaneous soymilk fermentation with *Bifidobacterium infantis* 14,603 and *Streptococcus thermophiles* 14,085 was reported to reduce saponins and phytate contents, and enhanced antitumor cell proliferation activity against HT-29 and Caco-2 cells (Lai et al. 2013). Consuming fermented soymilk has a probiotic effect on human intestinal tract ecosystem. A study on consumption of fermented soymilk (250 ml twice a day) by 28 healthy human subjects for a period of 2 weeks was reported to significantly increase ratio of *Bifidobacterium* spp. and *Lactobacillus* spp. to pathogens (Cheng et al. 2005). Natural fermentation of soymilk had been reported to increase protein, iron, calcium, magnesium and zinc contents suggesting fermented soymilk a suitable analog to cow milk (Obadina et al. 2013).

3.2.2 Traditional Pulses-Based Fermented Products

Miso

Miso is a soybean based fermented paste and has an established place in Japanese cuisines as an essential seasoning. The product is more like soy sauce in its taste and aroma however it is made with soybean, salt, and fermentation starter i.e., Koji. Japan only produces around 600,000 tons of Miso per annum from more than 1500 commercial processing units. Historically, Miso is one amongst most widely consumed traditional soybean based fermented food of Japan. Miso's production was supposed to be introduced from China and is inspired from Jan – a rice or soybean based Chinese origin fermented food. Different variants of Miso have been reported worldwide depending on type and nature of ingredients, the ratio of soybean, salt and koji starter, and aging duration (Minamiyama and Okada 2003). A major share of miso production is used as soup ingredient while a variety of miso-like products are used as seasoning in different cultures. Looking into the raw ingredients of miso that include rice, barley and soybean, the product is generally classified into its three variants namely rice miso, barley miso and soybean miso, respectively (Ebine 2004). The most common form of miso is the rice miso while other types include soybean and barley miso which varies from each other based on type of starters like rice koji, soybean and barley koji starters. A complex combination of miso flavor originates from a variety of microflora including fungi, bacteria and yeast which anticipate fermentation under high concentration of sodium chloride (Kobayashi and Sugawara 1999).

Tempeh

Tempeh is a soybean based fermented product and a national specialty of Indonesian cuisines. Product is originally developed through *Rhizopus* sp. mediated fermentation of the soybeans, hence referred as a mold-modified fermented product (Hachmeister and Fung 1993). Tempeh production process has been reported with moderate variations while the principal activities include dehulling of the seeds, soaking, cooking, inoculation with Rhizopus spp., fermentation and caking (Babu et al. 2009; Bavia et al. 2012). Food features of tempeh that make a characteristic difference include its unique nutritional profile, sensory attributes and consumer preference choice as meat-analog. Fermentation is the key process in tempeh production that brings significant physico-chemical changes to the product quality including mushroom like aroma and nutty flavor, higher protein contents with a balanced amount of essential amino acids, improved product texture and digestibility (Nout and Kiers 2004). Nutrient dense composition of tempeh and its bioactive compounds favor reduction in risk of various health ailments i.e., obesity, cardiovascular diseases, cancers, and osteoporosis in menopause (Deping 2001; Cassidy et al. 2006; Wati et al. 2020). Tempeh is one amongst the most common source of isoflavones while its concentration varies with processing operations like frying. A study by Haron et al. (2009) reported 205 mg per 100 g total isoflavone including daidzein (26 mg/100 g) and genestein (aglycones) (25 mg/100 g) in raw tempeh. Isoflavones hold weak phytoestrogenic antioxidant activity and have ability to bound single oxygen. Antioxidant activity of tempeh isoflavones had been suggested to improve brain cholinergic activity, reduce neuroinflammation and reverse memory impairment (Ahmad et al. 2014). *In vivo* modeling of low molecular mass isoflavones like genistein, daidzein and orobol have been found to inhibit angiogenesis (Kiriakidis et al. 2005).

Dawadawa

Dawadawa is a traditional high protein-based condiment of African origin and is widely used in seasoning soups and stews. Dawadawa is locust bean derived fermented non-conventional and low-cost source of high-quality protein for food insecured populations of African countries. The product is generously used as meat analog and instance source of calories by the underprivileged families from Gambia to Cameroun (Campbell-Platt 1980; Dakwa et al. 2005). Soybeans as plentiful source of protein, fats and an array of minerals and vitamins have also been tested as potential replacer to the locust bean for dawadawa production (Dakwa et al. 2005). Pure microbial cultures are rarely used in traditional African fermented foods. *Bacillus subtilis*, *Leuconostoc dextranicus* and *L. mesenteroides* are the predominant microorganisms involved in fermentation of traditional dawadawa (Antai and Ibrahim 1986).

Dhokla and Idli

Originating from steamed fermented Bengal gram flour and wheat semolina, dhokla is used as a traditional condiment in breakfast foods of India. Although there is least to no difference in recent age recipes of dhokla and Idli, dhokla is prepared by soaking rice and bengalgram dhal, separate grinding of soaked grains, preparation of composite batter, spontaneous over-night fermentation and steaming (Sharma et al. 2018). Maize dhokla – a different recipe, prepared by mixing maize semolina with Bengal gram dhal in a ratio of 3:1(w/w) have also been reported superior in taste, texture and overall acceptability (Shobha et al. 2020). *Lactobacillus fermentum*, *L. mesenteroides*, *Pichia silvicola*, *Streptococcus faecalis*, *Torulopsis pullulans and T. candida* are the key microbiota associated with Bengal gram – rice batter fermentation. Development of characteristic dhokla flavor is attributed to lactic acid bacteria while yeast anticipate batter volume, sponginess to the cake and increased level of folic acid (Ray et al. 2016).

Idli is pulses and cereals based traditional fermented breakfast food of India. The product is prepared by steaming fermented composite batter of black gram and rice. Nutritional composition of Idli reveals it to be a good source of protein and vitamins, more specifically the B – vitamins. Compared with the unfermented rice and black gram, fermented composition, the *Idli,* has a better nutritional profile and is a preferred choice to treat protein energy malnutrition (Reddy et al. 1982). In order to secure desirable textural properties, optimum ratio of black gram dhal to rice with 14 h fermentation has been reported as 1.575: 3 (w/w) (Durgadevi and Shetty 2012).

Soy Sauce

Soy sauce (Shoyu – Japanese) is soybean and wheat derived famous liquid condiment of East Asian countries (Kobayashi et al. 2004). Traditional oriental fermented soy sauce is a light brown to black liquid with distinct salted and umami taste (Steinkraus 1983; Yokotsuka 1986). More than 90% of the Soy sauce is koikuchi type i.e., preparation carrying equal amount of fermented sauce and hydrolysate of defatted soybeans and roasted wheat (Yokotsuka 1961). Japanese Shoyu production involves two stage fermentation process namely koji and moromi. Briefly, the process includes de-fattening and roasting of the raw material, koji development, brining, mashing, fermentation, pressing, refining of the raw soy sauce and pasteurization of the shoyu (Yokotsuka 1986). Principle ingredients of Japanese soy sauce include equal amount of wheat and soybean, and salt while Chinese soy sauce production involves low contents of wheat as compared to soybean (Wanakhachornkrai and Lertsiri 2003). Microbiota preferred for soy sauce production includes *Aspergillus oryzae*, *A. sojae* (koji mold), lactic acid bacteria like *Padiococcus halophilus* and yeasts including *T. versatilis* and *T. etchellsii* (Sugiyama 1984).

Sufu

China originating *sufu* or *furu* is a soybean derived cheese – like fermented product that bears appealing flavouring and creamy consistency that eases its application with breakfast cereals. Historically, Sufu's production date back to 220–265 AD while present day production technology of *sufu* involves solid-state fermentation of soybean curd i.e., *tofu* followed by aging in salt and alcohol derived brine (Wang and Du 1998). Over 300,000 metric tons of *sufu* products are manufactured in China which are used as appetizer and to season bland tasting recipes like breakfast rice and steam bread (Han et al. 2001). Patterns of amino acid i.e., essential in *sufu* (red & white) has been reported comparable with those of cow milk and egg. In a study by Han et al. (2004), free amino acid contents of red & white *sufu* increased from 28 to 88 mg/g and 33 to 104 mg/g, respectively 80 days aging period. The study reported *sufu* to hold a fairly constant ratio of each amino acid and relatively larger concentration of amino acids including alanine, aspartic acid, lysine, leucine, glutamic acid and phenylalanine. In addition to their promising protein profile, appreciable levels of certain other health promoting biomolecules including isoflavone {(0.58–2.2 mg/g dry matter (DM))}, phytosterols (0.73–2.72 mg/g DM), amino butyric acid {(GABA) (7.46–57.95 mg/g DM)}, and soyasaponins (10.89–23.35 mg/g DM) have also been identified in *sufu* developed with different microbiological culture (Xie et al. 2018). Correlation studies on fermentation microflora community and final characteristics of *sufu* revealed Lactococcus as one amongst the strongest flavour influencing microflora that favours production of esters and acids (Huang et al. 2018).

4 Fermented Nuts & Their Products

Nuts are considerable source of essential macro and micronutrients which include essential fatty acids, proteins, vitamins and minerals. A few species among nuts provide more than 10% of the recommended daily allowances of essential nutrients like niacin, thiamine, zinc and P from a 40 g serving (USDA 2010). Total protein contents in nuts are relatively higher than conventional cereals thus making them plausible source of plant proteins. Additionally, nuts proteins have low lysine to arginine ratio that has an inverse relation with the risk of hypercholesterolemia development (Brufau et al. 2006). USDA nutrient data base declares a broad range of protein contents i.e., 7.9–26.1 g/100 g in raw nuts including almonds, Brazil nuts, cashews, hazelnuts, peanuts, pecan, pistachios, walnuts, pine nuts and macadamia nuts. Contrary to their high protein contents, nuts proteins have low biological activity when compared with complete proteins (Brufau et al. 2006).

An inverse relation has been reported between nuts consumption and risk of cancer related and all-cause mortality and cardiovascular diseases (Bao et al. 2013; Damasceno et al. 2013; Estruch et al. 2013). Fermentation of fruit nuts like almond, hazelnut, walnut, pistachio and macademia to increase concentration of short chain fatty acid (SCFA) by 1.9–2.8 times (Schlörmann et al. 2016). In another study by Lux et al. (2012), two to three folds higher concentrations of SCFA were reported in *in vitro* fermented nuts. In vitro digestion and fermentation of nuts was thus reported to promote chemoprevention against colon cancer by increasing chemopreventive SCFA production, inhibiting oxidative stress and reducing tumor producing deoxycholic acid contents.

Nuts derived fermented products have also been reported to anticipate health promoting properties against a variety of disorders. Almond milk fermented with *Lactobacillus reuteri* and *Streptococcus thermophilus* at in instance is considered as a potential substitute to cow milk for cow milk allergic and lactose – intolerant individuals (Bernat et al. 2015a, b). In a recent study by Sánchez-Bravo et al. (2020), pistachio – based fermented beverage was developed wherein the product was reported as an excellent substrate for the survival and growth of lactic acid bacteria while the product has high umami intensity and pistachio flavor. Fermented peanut flour has also been reported as a novel type of probiotic food. In a study by Wang et al. (2007), the researchers suggested peanuts flour fermentation with Lactobacillus plantarum P9 strain for a period of 72 h at 37 °C to not merely increase the crude protein contents of the product but also increased the degree of protein hydrolysis. Further, feeding fermented peanut flour to mice model was also suggested to significantly increase fecal count of lactobacilli when compared with the normal control.

4.1 Fermented Almond Milk

Almonds are one amongst the highly accepted fruit nuts and are used in a variety of food products on account of their remarkable nutritional and health promoting attributes. With their essential nutrients' dense composition, incorporating almonds in daily foods not merely improve nutritional characteristics of daily diet but also anticipate desirable sensory attributes. Although fats contribute 50% of the almond weight, higher concentrations of monounsaturated fatty acids and α – tocopherol reduce serum low density lipoprotein concentration and inhibit LDL oxidation (Chen et al. 2006). Proteins constitute approximately 20–25% of the dried fruit weight while amandin is the single dominating storage protein among others that also anticipates characteristic water-soluble properties (Sathe et al. 2002). Fermenting almond milk with *L. reuteri* and *Streptococcus thermophilus* was reported to add versatile health promoting properties to the product without attributing distinct sensorial changes (Bernat et al. 2015a). In another study by (Kannan et al. 2021), fermented almond tea was reported to act as prophylactic drink against diabetes and was found more potent than fermented almond milk alone.

4.2 Fermented Seeds and Their Products

Industrial processing of fruits and vegetables generates a high amount of waste that includes seeds, stones, peel, and other inedible fractions. A considerable majority of world population rely on plant proteins including those of oil seeds, nuts, fruits and vegetables to satisfy daily dietary protein requirements. Melon seeds are suggested as rich carriers of fats (13–37%), protein (15–36%), carbohydrates (6–28%) and dietary fibers (7–44%) (Silva et al. 2020). Such a promising nutritional composition of melon seeds encourages their utilization to develop innovative health promotive food solutions. Complete utilization of the seeds of edible plants as potential protein carriers can help in recovering plausible amount of concentrated protein products including protein flours (50% protein), protein concentrate (70% protein) and protein isolates holding more than 95% protein (Oreopoulou and Tzia 2007). Referred to the given ranges of protein products, defatted flour of pumpkin was reported to contain 55.4% protein (Lazos 1986) and hence may simply be classified as a protein flour. Seed kernels of watermelon, pumpkin and paprika have also been suggested as rich sources of good quality protein carrying plausible amounts of lysine and other essential amino acids (El-Adawy and Taha 2001). Existing set of data on nutritional composition of seeds including some oil seeds suggest high concentration of proteins and balanced range of amino acids as pre-requisites for their consideration to develop plant protein-based health solutions (Oreopoulou and Tzia 2007). Seeds of melon (*Cucumis melo* hybrid 'ChunLi') were reported as rich source of arginine, glutamic and aspartic acid, however the seeds were deficient in lysine and methionine (Mian-hao and Yansong 2007). Seeds protein of another cultivar of melon (*Curcumis melo* cv. 'Pele de Sapo') were reported deficient in methionine, threonine, lysine and valine (de Mello et al. 2001). Fermentation of melon seeds aimed at improving nutrients delivering properties of the seeds, the technique also reduces the burden of intrinsic toxicants like phytates, oxalates and saponins from 18.6–2.7 mg/g, 2.1–0.3 mg/g, 6.8–3.4 mg/g, respectively (Ibukun and Anyasi 2013).

4.2.1 Ogiri Egusi

Ogiri is an important condiment of Nigerian origin and is produced by fermenting watermelon seeds. Conventionally, the product is prepared by boiling the de-husked watermelon seeds to a soft texture. Pulpy melon seeds are wrapped in banana leaves and cooked again for 2–3 h. Oil and water contents of the seeds are drained off from the wrapped seed pulp. The wrapped pulp contents are placed in earthen ware air-tight jars and fermented under low oxygen exposure for 5 days. Prior its use as condiment, leaves wrapped fermented mash is smoked over charcoal heat for 2 h. The dried mash is then ground to powder for subsequent use as a condiment (Odunfa 1981). Microbiota frequently isolated at various stages of Ogiri fermentation include *Proteus*, *Pediococcus*, *Klebsiella*, *Bacillus* and *Escherichia*. *Ogiri egusi* on account of their functional characteristics as thickener, source of protein and a flavoring ingredient is frequently used in preparing soup (Abaelu et al. 1990).

4.2.2 Seeds Protein Concentrate

Unconventional edible plant seeds have gained significant popularity for their consideration as economical and environmentally sustainable sources of quality proteins. The seeds of some non-conventional vegetables like *Cucumeropsis mannii* have been suggested to yield protein concentrate (~82% proteins) with good functional properties (Bassogog et al. 2020). Earlier, pumpkin seeds fermentation and subsequent alkaline extraction to yield protein concentrates (61.5–70.8% protein) was reported to enhance protein digestibility of the concentrates by reducing total phenol and phytic acid contents (Giami and Isichei 1999). Fermentation was also observed to increase foam volume and reduce foam stability over a 2 h period. Albumin and globulin are the major protein fractions of the raw fluted pumpkin seed which increase to a level of 35% (albumin) and 31% (globulin) of the total extractable protein on fermentation. Further, 5 days fermentation of the fluted pumpkin seeds improves overall protein contents and their digestibility and reduces seeds phytic acid contents (Giami 2004) (Table 7.1).

Table 7.1 Application of plant proteins for developing value – added fermented foods

Product	Raw material	Starter culture	References
Probiotic drink	Oat	*Lactobacillus plantarum* B28	Angelov et al. (2006)
Fermented milk	Soya milk enriched with isoflavone aglycones	*Lactobacillus rhamnosus* CRL981	Marazza et al. (2012)
Fermented beverage	Walnut milk	Kefir grains	Cui et al. (2013)
Synbiotic beverage	Aqueous extracts of Soybean and Qunioa	*Lactobacillus casei* LC-1	Bianchi et al. (2015)
Fermented milk	Mung bean supplemented with sucrose	*Lactobacillus plantarum* B1-6	Wu et al. (2015)
Probiotic drink	Cereals (barley, millet) and pulses (moth bean)	*Lactobacillus acidophilus*	Chavan et al. (2018)
Probiotic beverage	Chickpea	Mixed culture of *Lactobacillus acidophilus, Lactobacillus bulgaricus and Streptococcus thermophilus*	Wang et al. (2018)
Probiotic beverage	Chickpea	*Lactobacillus plantarum* 299v	Skrzypczak et al. (2019)
Drinking yogurt	Chickpea	Mixed culture of *Lactobacillus delbrueckii, Lactobacillus bulgaricus and Streptococcus thermophilus*	Aguilar-Raymundo and Velez-Ruiz (2019)
Coconut flan	Coconut milk	*Lactobacillus paracasei* & *Bifidobacterium lactis*	Corrêa et al. (2008)
Fermented puree	Chestnut	*Lactobacillus rhamnosus* and *Lactobacillus casei*	Blaiotta et al. (2012)
Sweet and Sour flour	Cassava	Mixed starter culture (*Lactobacillus cellobiosus*, *Streptococcus lactis*, and *Corynebacterium spp*. and *Pichia membranaefaciens*)	George et al. (1995)
Iru – condiment of Sub-Saharan Africa origin Tempeh	Bambara nut	*Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*	Fadahunsi and Olubunmi (2010), Fadahunsi and Sanni (2010)
Cheese Bread (fermented starch)	Cassava flour	*Rhizopus spp.*	Escouto and Cereda (2000), Begum et al. (2011)

(continued)

Table 7.1 (continued)

Product	Raw material	Starter culture	References
Protein concentrate	Chickpea	*Pediococcus spp*.	Xing et al. (2020)
Protein concentrate	Pea	*Lactobacillus plantarum* NRRL B-4496	Çabuk et al. (2018)
Fermented flour and pasta	Pigeon pea	Natural and or indigenous microflora of pigeon pea	Torres et al. (2006)
Vegtal and algae protein enriched sausages	Beans, lentils, broad beans, Chlorella and Spirulina	Natural and or indigenous microflora	Thirumdas et al. (2018)

5 Conclusion

Substitution of animal protein with complete and balanced proteins of plant origin is gaining popularity. In recent era, fermentation in a preview of local food heritage has emerged as a promising strategy to ensure adequate utilization of foods of plant origin by improving nutritional quality, nutrients digestibility and bioavailability, and consumers palatability. Even though fermenting cereals, pulses, legumes, nuts, tubers, and roots have a historical background in different cultures of the world, yet lesser is known on advances in exploiting fermenting plant proteins for producing protein-based health solutions, and to mitigate food and nutritional security. Sustainable production and adequate consumption of fermented plant proteins and peptides can reduce the burden on animal proteins and anticipate protective effects against an array of chronic health ailments. Opportunities exist on biotechnological exploitation of non-conventional sources of plant proteins like seeds of edible plants to produce complete protein solutions for health promotion and well-being.

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Chapter 8
Pulse-Based Yogurt: Physicochemical, Microbial and Sensory Characteristics

Julian Kaskens and Annamalai Manickavasagan

1 Introduction

Pulses are a category of legumes belonging to the *Leguminosae* family, which are rich in protein, and predominantly found in the form of a dry grain (Singh 2017). According to the Food and Agriculture Organization (FAO), there are 16 types of pulses: adzuki bean, bambara groundnut, broad bean, chickpea, common bean, cowpea, hyacinth beans, lentils, lima bean, lupin, moth bean, mung bean, mungo bean, pea, pigeon pea and rice bean (FAO 2017). Each pulse type varies in its cultivation practices and physicochemical characteristics. In general, they have a high protein content ranging from 19.87 g/100 g to 36.17 g/100 g, fat ranging from 0.53 g/100 g to 9.74 g/100 g, carbohydrate ranging from 40.37 g/100 g to 65.22 g/100 g (Table 8.1).

Apart from protein content, the pulses have higher protein delivery efficiency compared to meat products. For example, broad beans have a rate of nearly 60 g of protein/MJ of energy, whereas chicken, beef, pork, and fish rate less than 10 g of protein/MJ of energy (Sabaté and Soret 2014). Also, protein concentration in pulses rate similarly to meat alternatives while also having more efficient protein delivery (Sabaté and Soret 2014).

There is a huge demand and trend for plant-based foods in the recent years. In general, plant-based diets have been estimated to reduce CO_2, CH_4, and N_2O emissions by 17%, 24%, and 21% respectively, while comparing to their meat counter parts (Sabaté and Soret 2014). There are lots of plant-based protein rich foods including yogurt are being developed and marketed.

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Table 8.1 Macronutrient composition of pulses (raw-boiled without salt)

Pulse type	Protein g/100 g	Fat g/100 g	Carbohydrate, by difference g/100 g	Source
Adzuki Bean	19.87	0.53	62.90	USDA (2019)
Bambara Groundnut	23.59	6.51	64.43	Azman Halimi et al. (2019)
Broad Bean	26.12	1.53	58.29	USDA (2019)
Chickpea	20.47	6.04	62.95	USDA (2019)
Common Bean	23.58	0.83	60.01	USDA (2019)
Cowpea	23.52	1.26	60.03	USDA (2019)
Hyacinth Beans	23.90	1.69	60.74	USDA (2019)
Lentils	24.63	1.06	63.35	USDA (2019)
Lima Bean	21.46	0.69	63.38	USDA (2019)
Lupin	36.17	9.74	40.37	USDA (2019)
Moth Bean	21.31	1.14	65.22	Kamani et al. (2020)
Mung Bean	23.86	1.15	62.62	USDA (2019)
Mungo Bean	25.21	1.64	58.99	USDA (2019)
Pea	23.12	3.89	61.63	USDA (2019)
Pigeon Pea	21.70	1.49	62.78	USDA (2019)
Rice bean	25.57	2.77	55.64	Katoch (2013)

According to the U.S. Food & Drug Administration (FDA), traditional dairy yogurt is produced by introducing cultured lactic acid bacteria (LAB) to milk to promote the production of lactic acid (U.S. Food & Drug Administration 2020). This includes the use of primarily *lactobacillus bulgaricus* and *streptococcus thermophilus* in various types of dairy milk. Lactic acid coagulates the proteins within dairy milk to produce the characteristic texture and acidic flavour associated with yogurt. The nutrient composition in the final product is based upon the feedstock used for fermentation, which typically varies with the percentage of milk fat used.

Due to lactose intolerance and the rise of plant-based based diets, many individuals are seeking alternatives for dairy-based yogurt. In recent years, this consumer trend has prompted the developments of other popular alternatives such as soy, oat, and nut based dairy products. These alternatives have their own challenges and limitations. A portion of the population still has allergies to soy and nut-based products, including those derived from almond and peanut milks, preventing them from being universal options. Oat-based products are limited nutritionally, since they do not meet the protein content of their dairy and soy counterparts despite their ability to offer a good sensory experience.

In order to closely match the nutritional profile of dairy yogurt, pulses have been studied as a viable alternative. The protein contents of many commonly used pulses are similar to yogurt with the additional benefit of having low fat content. They can be tolerated well by the general population, are more environmentally sustainable than dairy-based products, and produce a similar sensory experience. In 2016, General Mills filed a patent for legume-based dairy products including the addition of fermentable cultures to form yogurt-based products (US Patent No. 172,570, 2016).

Although, the potential of several pulse type has been investigated for yogurt production, the results are scattered. Therefore, the objective of this chapter was to review the process of pulse-based yogurt production and their characteristics in comparison with dairy yogurt. Processing techniques, nutritional, sensory, microbial, and rheological information is presented, to provide an insight into the challenges of competing against dairy-based yogurt. This review also highlights the deficiencies and opportunities in research related to production and consumption of pulse-based yogurt.

2 Search Criteria

Electronic databases: Google Scholar, OMNI, were searched without date limitations. Journal articles were selected based on relation to the 16 pulse categories and containing the phrases "yogurt", "yogurt-like", "gel emulsion", and "fermented beverage". Articles analyzing the addition of pulses to dairy products were omitted and not directly compared to strictly pulse-based yogurts.

2.1 Analyzed Pulses

For the scope of this paper, 16 categories of pulses were researched: adzuki bean, bambara groundnut, broad bean, chickpea, common bean, cowpea, hyacinth beans, lentils, lima bean, lupin, moth bean, mung bean, mungo bean, pea, pigeon pea, and rice bean. The nutritional contents of these pulses are outlined in Table 8.1, according to the published USDA database with the exception of bambara groundnut and moth bean (USDA 2019). The scope of the analyzed pulses is based on FAO's classification (FAO 2017). Information regarding subspecies of a given pulse category will be grouped according to the general classification shown in Table 8.1.

2.2 Comparison Criteria

It is noteworthy that the studies reviewed for a single pulse category differ in methodology. This means that methods used to produce a yogurt-like product out of the same feedstock may be slightly different. Because there are limitations to the available information for each pulse category, each of these studies may not be equally comprehensive. To avoid confusion or misrepresentation, the general methodology is stated in conjunction with the resulting properties. The most common methods are described in detail in this section.

2.3 Utilized Feedstocks

Based on the existing literature, there have been three common feedstocks to produce pulse-based milk and yogurt. This includes dried flours extracted from raw pulses, raw pulse seeds, and protein isolates produced from raw pulses. Each of these ingredients produces final products with unique properties and nutritional profiles. Hence, the source of raw material is included for all analyzed criteria.

3 Yogurt Production and Processing Using Pulses

3.1 Heat Treatment and Fermentation

The purpose of heat treatment and fermentation (HTF) is to increase the availability of nutrients and develop the positive sensory characteristics, such as removing "beany" flavours (Lopes et al. 2020). This first step involves the soaking of raw seeds in warm or cold water, although higher temperatures have been shown to shorten the required soaking times (Pan and Tangratanavalee 2003). Following the soaking process, cooked seeds are added to boiling water to soften the outer hull and ease the grinding process. The ground seeds produce a slurry which is filtered through muslin or cheese cloth to produce the final milk product and exclude insoluble solids (Ma et al. 2015). Samples are often then pasteurized and cooled to prepare for fermentation and yogurt production (Mohamed et al. 2019). The fermentation time and temperature are dependent upon the strains of lactic acid bacteria used. Table 8.2 summarizes microbial characteristics of different fermented pulse products. In the fermentation stage, it is also common to add other substrates for the bacterial culture to utilize lactic acid production, as shown by Falade et al. (2015). After fermentation, the samples undergo a pasteurization step to deactivate any leftover microbial cells. There are variations in methodology with time and temperature being the main variables during the soaking and boiling stages.

Table 8.2 Microbial properties of pulse-based yogurt

Pulse type	Raw material	Bacteria strain	Fermentation process		Microbial count log units (cfu/mL)		pH		Source
			Temperature (°C)	Time (h)	Culture	Final product	Before fermentation	After fermentation	
Dairy product									
Milk, whole	Raw milk	*S. thermophilus and L. delbrueckii subsp. bulgaricus*	42	4	–	8.5	6.5	4.5	Zare et al. (2011)
Bambara Groundnut	Raw beans	*Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarus subsp. thermophilus*	42	6	6.0	7.5	6.6	5.0	Falade et al. (2015)
Broad Bean	Raw beans	*L. bulgaricus and S. thermophilus*	43	6	–	–	6.4	4.6	Chandra-Hioe et al. (2016), Sundholm (2019)
Chickpea	Raw beans	on-GMO maltodextrin, Streptocpolicy thermophils, Lactobacillus bulgaricus, Lactobacillus acidophilus	42	16	4.0	6.0	6.8	4.9	Wang et al. (2018)
Cowpea	Raw beans	(Lactobacillus acidophilus La-5 + Bifidobacterium animalis Bp-12 + Streptococcus thermophilus	45	14	3.1	7.1	–	–	Aduol et al. (2020)
Cowpea	Raw beans	Streptococcus thermophilus + Lactobacillus bulgaricus subs. debulgaricus	45	14	4.7	6.7	–	–	Aduol et al. (2020)
Cowpea	Raw beans	(Lactobacillus rhamnosus GR-1 + Streptococcus thermophilus	45	14	4.5	7.4	–	–	Aduol et al. (2020)
Lupin	Raw seeds	S thermophilus and L delbrueckii ssp. bulgaricus	45	8	3.6	8.0	6.6	4.5	Jiménez-Martínez et al. (2003)

(continued)

Table 8.2 (continued)

Pulse type	Raw material	Bacteria strain	Fermentation process		Microbial count log units (cfu/mL)		pH		Source
			Temperature (°C)	Time (h)	Culture	Final product	Before fermentation	After fermentation	
Mung Bean	Raw seeds	L. plantarum B1–6	36	5	7.1	8.4	–	–	Han Wu et al. (2015)
Pea	Pea protein powder	Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus	43	18	–	–	6.6	4.7	Klost and Drusch (2019)

3.2 Novel Processing Methods

Some of the novel processing methods that have been proven for yogurt production, but not necessarily pulse-yogurt production are given below. They represent opportunities for further research and development into innovative approaches that have not been thoroughly explored. This includes the use of pulse electric field processing (PEF), ultrasonic processing, microwave processing, and high-pressure processing (HPP).

3.2.1 Pulse Electric Field Processing

PEF processing is primarily concerned with reducing microbial activity in very short periods of time (Sobrino-López and Martín-Belloso 2010). In this approach, electric pulses are sent through the yogurt liquid media to destabilize the microbial cells (Wouters et al. 2001). It has also been shown that PEF in conjunction with mild heat treatment can produce much lower aerobic bacteria counts than the heat-treated samples (Yeom et al. 2004). This treatment has the potential to increase the stability and long-term storage conditions of pulse-based yogurt.

3.2.2 High Intensity Ultrasonication

In this treatment method, ultrasonic waves are passed through the product in the frequency range of 20 kHz, generating high temperatures and pressures (Demirdöven and Baysal 2008). This has many benefits when it comes to dairy products, including efficient homogenization and reducing fat globule size (Chandrapala et al. 2011). Moreover, despite changes in secondary protein structure, dairy products retained key functional properties following ultrasonic processing (Chandrapala et al. 2011). Studies involving the analysis of yogurt products specifically showed an increase in both water-holding capacity and viscosity as well as a decrease in fermentation time (Wu et al. 2000). Although the protein structure in pulses is inherently different than whey proteins, it is possible that ultrasonification could have similar benefits such as improved viscosity and water-holding capacity. In cold set soy gels, large increases in gel strength, water holding capacity, and denser structures have been noted following 40 min of treatment (Hu et al. 2013; Zhang et al. 2016). It is however important to note the need for optimization of amplitude and wave frequency for a particular protein structure. For example, one study on soy with differing ultrasonic wave parameters produced a negative impact on gel stability (Arzeni et al. 2012). While this method has not been widely researched for pulse-based gels, through optimization and further testing, ultrasonication can potentially improve the structure and stability of pulse-based yogurts.

3.2.3 Microwave Processing

Microwave processing involves the use of electromagnetic waves in the range of 915 MHz to 2.45 GHz for food processing (Kubo et al. 2020). Microwave treatment for roughly 80 s at 210 W has been shown to produce soy gels with properties comparable to 10 min of heat-treated samples (Liu and Kuo 2011). However, in the same study it was noted there were negative effects experienced to the microstructure due to lack of uniformity (Liu and Kuo 2011). As with other novel methods, this would require more investigation to determine the efficacy in pulse-based gels specifically and at varying MW times and intensities.

3.2.4 High Pressure Processing

High pressure processing (HPP) has been explored as an alternative to plant-based yogurt production (Sim et al. 2020). The benefit of HPP can be attributed to the altering protein structure in plant-based protein gels (Queirós et al. 2018). It has also been shown that HPP can form strong plant-based protein gel structures without additional ingredients in a reduced period of time compared to heat treatment coupled with fermentation (Queirós et al. 2018).

However, this method is still relatively at preliminary stage. Sim et al. (2020) provided an in-depth analysis into a variety of pulse protein isolates utilizing this method. Protein isolates were added to water in 12% (w/w) concentrations to achieve minimum gelation concentration and shear mixed at 20,000 rpm (Sim et al. 2020). The samples were then introduced into a HPP chamber and were subjected to 600 MPa of pressure for 5 min (Buerman et al. 2020; Sim and Moraru 2020). In dairy applications, HPP has been shown to improve the texture and water holding capacity of yogurt samples in comparison to fermented samples (Trujillo et al. 2002). This is an impactful observation as it allows for HPP to be used as an alternative to fermentation processes which often take long periods of time. In relation to potential pulse processing, this would reduce the time for production, however the sensory aspects really to a fermented product such as the flavour and characteristic texture would need to be addressed.

4 Nutritional Properties of Pulse-Based Milks

The important aspects for consumer acceptability are the structure and stability of yogurt products. Like dairy yogurt, this is done through the implementation of LAB to coagulate the proteins in the pulse-milk. While this is usually done with naturally occurring sugars in milk such as lactose, a similar result can be achieved through the addition of sugar or starches in pulses to similar efficacy. In other studies, pulses have also been used to improve the stability of dairy yogurt due to their high starch content (Hussein et al. 2020).

Table 8.3 Nutritional properties of pulse-based milk

Pulse type	Raw material	Protein g/100 g	Fat g/100 g	Carbohydrate g/100 g	Treatment method	Source
Dairy product						
Milk, whole	Raw milk	3.27	3.20	4.63	–	USDA (2019)
Milk, 2%	Raw milk	3.36	1.90	4.90	–	USDA (2019)
Milk, 1%	Raw milk	3.38	0.95	5.18	–	USDA (2019)
Milk, no-fat	Raw milk	3.43	0.08	4.92	–	USDA (2019)
Bambara Groundnut	Bambara groundnut flour	1.80	–	4.20	HTF[a]	Pahane et al. (2017)
Broad Bean	Broad bean protein isolate	12.00	0.80	5.20	HPP[b]	Sim et al. (2020)
Chickpea	Whole chickpea seeds	1.21	0.33	3.10	HTF	Wang et al. (2018)
Chickpea	Whole chickpea seeds	1.30	–	9.01	HTF	Lopes et al. (2020)
Chickpea	Whole chickpea seeds	2.10	0.44	3.39	HTF	Rincon et al. (2020)
Cowpea	Whole cowpea seeds	3.23	2.11	0.09	HTF	Sanni et al. (1999)
Cowpea	Whole cowpea seeds	1.66	0.40	5.20	HTF	Aduol et al. (2020)
Lentil	Lentil protein isolate	12.00	1.00	7.30	HPP	Sim et al. (2020)
Lima Bean	Whole Lima Beans	3.00	0.97	–	HTF	Agim-Ezenwaka et al. (2020)
Lupin	Whole Lupin Seeds	5.80	2.94	0.92	HTF	Jiménez-Martínez et al. (2003)
Mung Bean	Mung bean protein isolate	12.00	<0.10	<0.90	HPP	Sim et al. (2020)
Pea	Pea protein isolate	12.00	1.00	7.90	HPP	Sim et al. (2020)
Pigeon Pea	Whole Pigeon Peas	9.55	0.56	3.70	HTF	Yusuf (2017)

[a]*HTF* heat treatment & fermentation
[b]*HPP* high pressure processing

The methodology for processing these pulses is consistent among the literature and well researched to obtain acceptable sensory profiles as well as maximizing nutrient recovery. However, these have largely not been proven industrially (add Reference). Table 8.3 summarizes the nutritional properties of pulse-based milks in comparison with dairy products. In general, the macronutrients of pulse-based milk are comparable to dairy milks and are better tolerated by most of the population without the drawback of potential allergens.

Drawings parallels to dairy yogurt production, many procedures involve the process of creating a pulse "milk". The process to yield these pulse milks differs, involving heat treatment or novel processes to minimize negative sensory characteristics associated with bean substrates. This process is also important for establishing a feedstock for culture fermentation to create a more palatable yogurt. The profiles of three basic macronutrients, i.e., protein, fat, and carbohydrates are correlated with the methodology used and often time compared with standard dairy milk. Because the definition of "milk" is not strictly defined, the source of the raw material also impacts the nutrient density of the end product greatly. Milks developed from protein isolates or concentrates produce a more protein dense profile in comparison to raw beans or flours.

Table 8.3 describes the macronutrient profile of purely pulse-based milks. Additionally, there is existing literature on adding small concentrations of dairy to fortify plant milks. An example of this includes using skim milk powder in quantities as small as 5% to enforce lactic acid production (Song and Yu 2018). Owing to the scope of this paper, those were excluded from the table.

4.1 Adzuki Bean Milk

Currently, studies are non-existing on the production of fermented adzuki bean milk analogue with a focus on the nutritional aspects. There has been a focus on analyzing the gamma aminobutyric acid (GABA) production present in adzuki bean milk (Liao et al. 2013; Song and Yu 2018; Z. Wu et al. 2021). GABA has been shown to improve many aspects of human health and is difficult to find in higher concentration in naturally occurring foods (Song and Yu 2018). However, these studies did not focus on the use of milk to create a fermented yogurt analogue or mimic a similar nutritional profile. Utilizing similar methodology mentioned in other pure pulse-based milk fermentation, adzuki bean milks have been produced through the addition of milk powder and LAB (NanWei et al. 2011).

4.2 Bambara Groundnut Milk

Bambara groundnut has several advantages over other pulses, the first being its resistance to drought conditions and ability to grow in nutrient poor soil (Gulzar and Minnaar 2017). Milk preparation follows the procedure for HTF very closely as mentioned previously in the review. Brough et al. (1993) noted that the Bambara groundnut requires heat treatment to minimize beany flavour and odour. In their study, although fermentation was not included, the remaining steps followed a similar procedure. In comparison to both cowpea and soy milks bambara groundnut showed a lower overall viscosity but higher sensory profile ratings (Brough et al. 1993). Pahane et al. also noted about the lack of studies specifically on the nutritional profile of both Bambara groundnut milk and yogurt. In their study, they produced bambara flour based on raw nuts and added to water at a later date (Pahane et al. 2017). Introduction of LAB showed a positive increase in protein content for a total of 1.8 g, which is slightly lower than 2.04 g reported by a previous study comparing bambara and soybean milks (Pahane et al. 2017; Poulter and Caygill 1980). Overall, it has been consistently reported that bambara groundnut lacks the protein content present in soy as seen in Table 8.3, but Pahane et al. (2017) also mentioned the impact that pre-treatment methods can have on overall nutritional profile of pulse-based milks. Because of the limited number of research papers comparing different processing methods of Bambara milk, it could be worth investigating the nutritional properties produced through alternative methods.

4.3 Chickpea Milk

Chickpea milk is advantageous due to its high protein content and lack of allergens in comparison to soy dairy analogues (Cabanillas et al. 2018). In a comparative study where processes conditions were identical for both soy and chickpea, protein contents of the resulting milk were 1.21 g/100 g and 2.09 g/100 g respectively (Wang et al. 2018). It also received similar sensory ratings to the soy counterpart, except in terms of colour (Wang et al. 2018). Under similar preparation steps on both chickpea seeds and sprouts, similar protein contents in the range of 1.0–1.5 g/100 g of milk product were obtained (Lopes et al. 2020). One of the limiting factors in the use of chickpea milk seen in both studies is the characteristic bean flavour and yellow appearance. To mitigate these factors, it's possible to combine other plant products and mitigate losses in nutritional content. One example is the use of coconut milk to alter the colour and taste of pulse-milks (Rincon et al. 2020). In this study, the chickpea milk has a protein, lipid, and carbohydrate contents of 2.1 g/100 g, 0.39 g/100 g, and 3.39 g/100 g, respectively (Rincon et al. 2020). Small additions of coconut extract up to 20% yielded protein contents as high as 1.96 g/100 g of product and a higher fat content of 1.74 g/100 g (Rincon et al. 2020). This exhibited properties closer to 2% milk with higher protein content than shown by Wang et al. (2018). The discrepancies seen in the nutritional content of these samples could be due to raw material quality differences as well as minor deviations in sample preparation. Rincon et al. (2020) used a pressurized container during the cooking step but also did not ferment their beverage. Wang et al. (2018) used lower cooking temperatures under atmospheric pressure but did inoculate their beverage.

4.4 Cowpea Milk

Cowpeas are popular in many parts of Africa. Diets consisting of large quantities of pulses are also shown to cause flatulence and discomfort. However, fermented products have been shown to minimize the antinutritional components responsible for gastrointestinal flatulence (Madodé et al. 2013). In two different studies following similar treatment methods, protein contents of 3.23 g/100 g and 1.55 g/100 g were produced from the fermentation of cowpea milk (Aduol et al. 2020; Sanni et al. 1999). However, Aduol et al. (2020) showed a more robust analysis then Sanni et al. (1999) on the effect of different strains of LAB on the nutritional profile of final cowpea milk. Although the differences between LAB strains did not change the nutrient composition significantly (Aduol et al. 2020).

4.5 Lima Bean Milk

Lima beans have been shown to produce milks of high sensory preference in comparison to many other pulses, including lupins, lentils, soybeans, and broad beans (Sosulski et al. 1978). Although in a comparative study against, bambara,lima bean yogurt, lime bean's rated lower in sensory characteristics (Agim-Ezenwaka et al. 2020). Nutritionally, in the same study done by Agim-Ezenwaka et al. (2020), Bambara groundnut and lima bean were found to have similar protein, fat, and carbohydrate levels when treated using heart treatment and fermentation.

4.6 Lupin Milk

Lupins are unique in the very high protein and fat content they exhibit as seen in Table 8.1. This makes them a very good candidate to achieve a protein rich milk analogue when pre-treated similarly to other pulses. There are few studies focusing on the nutritional aspects of lupin-based dairy milk and yogurt analogues. In a study done preparing milk from raw lupin seeds, the resulting product exceeded both milk and soy protein contents at 5.8 g/100 g of milk in comparison to 2.62 g/100 g and 3.91 g/100 g within the same study (Jiménez-Martínez et al. 2003). This study shows the potential of lupin as a feedstock, it would be valuable to further test different treatment methods and raw material samples to further investigate the nutritional potential.

4.7 Pigeon Pea Milk

Pigeon pea is unique as its consumption is largely concentrated in South Asia and Africa, which limits the amount of research done on its potential as a viable vegetable protein source. As seen in Table 8.1, It has one of the highest protein contents, comparable to a more commonly used pulse such as the broad bean. In the absence of studies analyzing the nutritional aspects of pigeon pea milk, the only published study reported a very high protein content of 9.46 g/100 g in relation to other pulses (Yusuf 2017). One possible explanation can be the lack of filtering the solids through a cheese cloth and instead diluting the resulting pulse paste after soaking the seeds. This would dramatically increase the total solids content and therefore the protein in the final milk solution. Similar to other studies, the filtering step reduces the beany flavour and improves the overall sensory properties of the final product.

4.8 Broad Bean Milk

Broad beans or more commonly known as faba/fava beans, have been studied as a dairy substitute in a limited number of papers. A study by Sim et al. (2020) utilized a more novel approach to producing pulse-based milks and yogurts through high-pressure processing. In this case, the "milk" was broad bean protein isolate that was shear mixed into solution in order to obtain a suitable liquid for gelation (Sim et al. 2020). The protein content was fixed at 12 g/100 g of solution (Sim et al. 2020). Similarly, lentil, mung bean, and pea milks were analyzed under the same study parameters. Comparing them nutritionally, mung beans presented the lowest fat and carbohydrate contents.

4.9 Other Pulse Milks

At the time of writing, published literatures on milk analogue products for common bean, hyacinth, moth bean, mung bean, and rice bean are non-existing. Similar to the comparison of pulse milk nutrition, yogurts are compared on the basis of their primary macronutrients. The source of raw material and process involved would affect the nutritional profile, as seen in Table 8.3. For comparison purposes, dairy yogurt is included as well.

5 Nutritional Content of Pulse-Based Yogurt

5.1 *Adzuki Bean Yogurt*

At the time of writing, no study on pure adzuki bean yogurt has been reported. However, NanWei et al. (2011) have developed a fermented set yogurt combining adzuki bean milk with added milk powder prior to inoculation (NanWei et al. 2011). However, the study was excluded from Table 8.4 because it did not make the criteria of being 100% plant based.

Table 8.4 Nutritional properties of pulse-based yogurt

Pulse type	Raw material	Protein g/100 g	Fat g/100 g	Carbohydrate g/100 g	Treatment method	Source
Dairy product						
Yogurt (whole), King Cheese Inc.	Raw milk	4.41	3.08	5.73	–	USDA (2019)
Yogurt (nonfat), Tops Markets, LLC	Raw milk	5.73	0.00	7.93	–	USDA (2019)
Bambara Groundnut	Bambara groundnut flour	2.60	–	3.10	HTF[a]	Pahane et al. (2017)
	Raw Bambara groundnut[a]	5.70	1.40	75.30	HTF + foam mat drying	Hardy and Jideani (2020)
Broad Bean	Broad bean protein isolate	12.00	0.80	5.20	HPP[b]	Sim et al. (2020)
Chickpea	Chickpea protein isolate	12.00	0.20	6.10	HPP	Sim et al. (2020)
Common Bean	Raw Black Bean	6.12	1.86	5.16	HTF	Lim et al. (2019)
Lentil	Lentil protein isolate	12.00	1.00	7.30	HPP	Sim et al. (2020)
Lupin	Raw lupin seeds	4.70	0.88	8.68	HTF	Jiménez-Martínez et al. (2003)
Mung Bean	Mung bean protein isolate	12.00	<0.10	<0.90	HPP	Sim et al. (2020)
Pea	Pea protein isolate	12.00	1.00	7.90	HPP	Sim et al. (2020)
Pigeon Pea	Whole pigeon peas	5.02	1.25	11.26	HTF	

[a]*HTF* heat treatment & fermentation
[b]*HPP* high pressure processing

5.2 Bambara Groundnut Yogurt

The Bambara groundnut has been mentioned previously for its resilience to environmental conditions. However, there have also been several successful attempts at taking raw Bambara groundnut milk and creating a yogurt-style product from it. In the first paper, this involved the process of heat treating and fermentation described above to allow for structure formation through lactic acid production, an extra step was taken to dry the blanched Bambara nuts and turn them into a flour (Pahane et al. 2017). The overall solids content was found to be low in the resulting yogurt although fermentation assisted in increasing protein content (Pahane et al. 2017). Ultimately, the final product contained 2.6 g of protein per 100 g of yogurt, which is considerably lower than both conventional dairy yogurt at 4.41 g/100 g and soy yogurt at 4.12 g/100 g as seen in Table 8.4. An attempt to create a powdered Bambara yogurt product yielded a protein content of 5.7 g/100 g of product but this figure would likely be significantly lower once mixed in water (Hardy and Jideani 2020). Hardy and Jideani (2020) created a dried product could extend the shelf life of pulse-based yogurts. While the end product still does not match the protein content of dairy yogurt, it's possible that a dried product could be supplemented with protein isolate to enrich the protein level.

5.3 Common Bean Yogurt

Common beans represent a large family of pulses and opportunity for development of yogurt analogue products. Currently, there are few published articles outlining the nutritional aspect of a common bean yogurt. One study analyzed and compared the nutritional composition of soy, black bean (*Phaseolus vulgaris*) and traditional kefir, which is similar to yogurt but slightly different in consistency (Lim et al. 2019). Their sample preparation procedure was similar to other pulse-based yogurts but differed in the addition of kefir water grains and glucose; however samples prepared without kefir grains were also produced. The resulting black bean yogurt exhibited high protein content at 6.12 g/100 g, fat content of 1.86 g/100 g, and carbohydrate content of 5.16 g/100 g (Lim et al. 2019). In comparison to whole fat yogurt (Table 8.4), the product exceeded in protein content and had less fat but comparable levels of carbohydrates. Further research on different types of common beans and raw feedstocks following similar methodology would be worthy in analyzing the nutritional profile of the resulting products.

5.4 Lupin Yogurt

Lupin yogurt in theory should provide the highest protein content, especially considering the results shown for the raw milk protein content in Table 8.3. Still, due to the novelty of the concept, few studies have analyzed the nutritional content of a lupin-based yogurt. However, lupin yogurt has exhibited very high protein content that are comparable to dairy yogurt (Jiménez-Martínez et al. 2003). With a protein content of 4.7 g/100 g of yogurt, it is slightly higher than whole dairy yogurt at 4.41 g/100 g (Table 8.4). In this study, the final yogurt had higher carbohydrate content of 8.68 g/100 g compared to 5.73 g/100 g in dairy yogurt.

5.5 Pigeon Pea Yogurt

Pigeon pea yogurt is still largely unexplored, lacking much formal literature. However, in a published master's project, the concept was explored in detail, focusing primarily on the stability of pigeon pea yogurt (Yusuf 2017). The preparation methodology did follow a similar heat treatment and fermentation as proven in many other pulse-based yogurt studies (Yusuf 2017). The nutritional content of the yogurt exceeded both soy and dairy counterparts, with a protein content of 5.02 g/100 g, fat content of 1.25 g/100 g, and a large carbohydrate content of 11.26 g/100 g (Yusuf 2017). While these figures are impressive, one reasoning for this is the lack of a filtration step, which led to an increase in total solids in the final solution.

5.6 Other Pulse Yogurts

For the broad bean, chickpea, lentil, mung bean, and pea, the only research paper analyzing their nutritional contents were done using a standardized 12% protein concentrate for each feedstock (Sim et al. 2020). This makes it difficult to compare them on the basis of nutritional content alone but does still provide some information for other sections and it is useful because the methodology is standardized for the different samples.

At the time of writing, adzuki bean, cowpea, hyacinth bean, lima bean, moth bean, mungo bean, and rice bean have not been extensively researched on the nutritional content of resulting yogurt-analogue products. However, some of these have still been tested for their mechanical and microbial characteristics.

6 Microbial Properties

The pH of the solution prior to fermentation and following the resulting yogurt are included in Table 8.2. The microbial counts and utilized bacteria culture are also included. To measure the significance of these values, a control milk sample is also included to draw comparison between feedstocks. The most used choice was differing strains of LAB.

To match the characteristics of dairy yogurt, optimal fermentation process is critical, especially the dselection of bacterial strains, such as *Streptococcus salivarious ssp. Thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (de Brabandere and de Baerdemaeker 1999). These bacterial cultures ferment lactose into lactic acid, which coagulates the dairy proteins. As a result of the acidity of the fermented product can impact the structure, taste, and aroma of the yogurt. Hence, pH is a good indicator of product acceptability (Soukoulis et al. 2007).

Similarly, in pulse-yogurt, pulse milks are inoculated with LAB to achieve the similar product characteristics. As seen in Table 8.2, in general, target pH is around 4.6–4.9 and fermentation conditions include temperatures from 36 to 45 °C and times from 5 to 18 h. Although these conditions are a function of the bacteria used for the fermentation process. Comparing this to the dairy equivalent, caseinate particles destabilize from a pH of 4.6–4.7, forming the complete structural component of yogurt (Rasic and Kurmann 1978). In general, fermentation occurs at a temperature range of 40–46 °C depending on the source of dairy and for a duration of around 8 h (Aldaw Ibrahim et al. 2019). In a study determining the microbial characteristics of soy and Bambara yogurts, both products had similar stability and pH over storage time (Falade et al. 2015). Pulse-yogurt performs similarly to dairy yogurt when inoculated with LAB and meet sensory criteria. The growth of microbial counts, decrease in pH, and development of titratable acids shows a positive response to initial fermentation. However, more work can still be done to determine the microbial characteristics over prolonged periods of shelf life, with more studies where storage temperatures should be taken into consideration (Falade et al. 2015).

7 Rheological Properties

The rheological properties of dairy yogurt are extremely important towards consumer acceptability as well as the stability of commercial yogurt products. Increases in protein content in yogurt have been shown to increase viscosity and storage modulus (G′), which are important when measuring the gel structure formed in a yogurt product (Prajapati et al. 2016). Additionally, syneresis is a critical measurement for the separation of liquid from the gel matrix, which is another component of consumer acceptability and stability characteristic of yogurt (Delikanli and Ozcan 2017). In the simulation of dairy products using pulses, these measurements and

Table 8.5 Rheological properties of pulse yogurt

Pulse type	Raw material	Viscosity (cP)	Syneresis (%)	Storage modulus (Pa)	Water holding capacity (%)	Treatment method	Source
Dairy product							
Yogurt (whole)	Raw milk	5000	26	100	52	–	Akalın et al. (2012), Alhejaili et al. (2019), Yu et al. (2016)
Yogurt (skim)	Raw milk	40,000	–	1200	–	HPP[a]	Sim et al. (2020)
Bambara Groundnut	Bambara groundnut flour	698	–	–	–	HTF[b]	Falade et al. (2015)
Broad Bean	Broad bean protein isolate	100,000	–	800		HPP	Sim et al. (2020)
Chickpea	Chickpea protein isolate	175,000	–	2600		HPP	Sim et al. (2020)
Common Bean	Raw Black Bean	2165	–	–	–	HTF	Lim et al. (2019)
Lentil	Lentil protein isolate	150,000	–	2600		HPP	Sim et al. (2020)
Mung Bean	Mung bean protein isolate	20,000	–	100		HPP	Sim et al. (2020)
Pea	Pea protein isolate	75,000	–	600		HPP	Sim et al. (2020)

[a]*HPP* high pressure processing
[b]*HTF* heat treatment & fermentation

important to quantify the relative differences in rheological properties. Comparisons of rheological properties of pulse and dairy yogurts are summarized in Table 8.5.

Cold stored plain soy and Bambara groundnut yogurts showed a peak viscosity of 900 and 772.3 cP measured at 600 rev/s and 4 °C respectively after 9 days, but reported decreases at room temperature (Falade et al. 2015). Comparing this product to the dairy equivalent, a viscosity of 5000 cP has been shown to produce by fresh dairy yogurt (Alhejaili et al. 2019) While the differences are significant, the bambara yogurt still has a similar viscosity as the soy yogurt, and the bambara yogurt increased slightly during storage, indicating stability. In a study of specific pretreatment methods to raw bambara groundnuts, it was found that roasting following germination provided the best mechanical properties and stability suitable for large-scale production, while also reducing anti-nutritional properties (Salami et al. 2020).

A comprehensive study done on the effects of adding chickpea extract to dairy yogurt also analyzed rheological properties of pure chickpea-based yogurt as well (Aguilar-Raymundo and Vélez-Ruiz 2019). It was found that after a 22 days of storage at 4 °C, the chickpea samples had 71.85% syneresis while the control dairy sample showed just 22.8% syneresis (Aguilar-Raymundo and Vélez-Ruiz 2019). In addition, chickpea yogurt exhibited non-Newtonian behaviour and a small decrease in density from 1033 kg/m^3 to 1021 kg/m^3, as compared to dairy yogurt from 1109 kg/m^3 to 1064 kg/m^3 (Aguilar-Raymundo and Vélez-Ruiz 2019). The low stability of chickpea yogurt can be seen in this study from the rapid increase in syneresis over storage time, even in a refrigerated setting.

Common bean yogurt has been researched as a kefir yogurt replacement, using fermented black bean milk to produce yogurts that exhibit very high viscosities (Lim et al. 2019). When comparing soy and black bean kefir yogurts fermented at 20 °C, they produced viscosities of 1125.33 and 2247.67 cP, respectively (Lim et al. 2019). This is significant when comparing these values back to the viscosity of dairy yogurt which is in the range of roughly 2000 cP, although the soy kefir was similar to the results of Falade et al. (2015).

Analyzing the effects of fermentation on lentil yogurt, viscosity readings before and after having been measured 3.7 Pa.s and 4.23 Pa.s respectively (Pontonio et al. 2020). These values correspond to over 4000 cP post-fermentation, which is very significant compared to the Bambara groundnut and common bean. In this study, it could be due to the use of lentil flour in order to produce the yogurt, the low pH indicates significant microbial activity. It's also known that the increase of protein from fermentation could contribute to an increase in structure and therefore viscosity.

The shear stress-shear rate relationship of lupin yogurt at 2 °C behaves similarly to dairy yogurt but decreases rapidly as temperature increases (Jiménez-Martínez et al. 2003). This could relate to structural differences in casein proteins compared to proteins in lupin milk, producing different behaviour to shear stresses, which was reported to be seen visually as the samples were less gelled at higher temperatures (Jiménez-Martínez et al. 2003). The hardness, cohesiveness, springiness, and gumminess of lupin yogurt have been compared with dairy yogurt (Mohamed et al. 2019), showing that the springiness value of the lupin sample was comparable with the dairy sample but decreased rapidly during storage, with lupin yogurt exhibiting 0.1 mm, while dairy yogurt 1.5 mm (Mohamed et al. 2019). A similar trend was seen for all rheological measurements, citing the stability of the lupin yogurt to be greatly inferior to that of milk, as is common with the other pulses presented previously.

Addressing this stability issue, pea protein concentrates have been used to form gels and evaluate methods to improve gel stability (Klost and Drusch 2019). The addition of oil and fibre was tested to see the resulting potential increases in viscosity and effects on stability. The results showed that the protein content contributed the most towards structure formation (Klost and Drusch 2019). Pea protein concentrate formed a weak gel with a maximum complex viscosity of 3.7 Pa.s, although the stability over storage life was not reported (Klost and Drusch 2019).

Sim et al. (2020) evaluated and compared the properties of many pulse yogurts, including those derived from mung bean, chickpea, pea, lentil, and common bean, as well as a whole milk control. It was determined that the pulses exhibiting the highest gel strength were chickpea and lentil protein, however, common beans showed viscoelastic properties closest to that of whole milk yogurt (Sim et al. 2020). The viscosity of the final yogurt samples, pea, mung bean, and common bean displayed viscosities that were similar to the whole milk yogurt, while lentil and chickpea had higher viscosity values (Sim et al. 2020). While this study utilizes high pressure processing instead of fermentation, it is valuable in understanding the differences between the structures formed by the proteins of the various pulses. While the protein concentration remains are similar, they produce vastly different rheological properties. Pulse yogurts show a more significant drop-off in mechanical properties suggesting that it would be advantageous to have a higher initial viscosity.

8 Sensory Properties

Sensory attributes in all published articles involved a direct comparison to a dairy control sample. In order to understand the impact of the sensory ratings, they have been compared to the relative difference of their dairy control. The analyzed attributes were consistent among all sensory studies in Table 8.6.

The sensory results for studies comparing pulse yogurt to dairy yogurt are shown in Table 8.6. Although the focus of research recently has been concentrated more on proof of concept, some researchers have done work to compare actual acceptability of pulse-based yogurts as well. Unfortunately, no large-scale studies have been done to compare a wide range of pulse yogurts under the same parameters. However, all reported studies in Table 8.6 were with a dairy control, which can give some indications on general acceptability relative to each other. To make some definitive statement on the acceptability, a more comprehensive tasting study would be needed with identical preparation steps.

8.1 Bambara Groundnut

The Bambara groundnut yogurt was rated overall as a 5.03 compared to a 5.77 of conventional dairy yogurt, shown in Table 8.4. However, in the same study by Falade et al., when soy yogurt was prepared similarly to the Bambara, it was rated only 4.3. The common comments dealt primarily with the distaste for the bean flavour that was evident in the pulse yogurts.

Table 8.6 Sensory properties of pulse-based yogurt

Pulse type	Raw material	Rating scale	Aroma	Colour	Taste	Consistency	Overall	Source
Dairy product								
Yogurt (3.5% fat)	Raw milk	5 points	0.0	5.0	4.9	5.0	5.0	Ina et al. (2015)
Yogurt (1.5% fat)	Raw milk	5 points	0.0	5.0	4.1	4.3	4.5	Ina et al. (2015)
Yogurt (0.1% fat)	Raw milk	5 points	1.6	3.7	3.2	2.8	2.7	Ina et al. (2015)
Bambara Groundnut	Raw Bambara seed	7 points	5.1 (5.6)[a]	5.4 (5.4)	5.3 (5.4)	4.8 (5.1)	5.0 (5.8)	Falade et al. (2015)
Cowpea	Raw cowpea seed	10 points	–	4.9 (8.4)	3.7 (7.4)	4.4 (7.7)	4.0 (7.9)	Sanni et al. (1999)
Lima Bean	Raw lima bean seeds	9 points	5.5 (6.7)	5.9 (6.2)	4.9 (7.1)	6.6 (6.7)	5.2 (6.8)	Aduol et al. (2020)
Lupin	Raw lupin seeds	7 points	5.5 (6.2)	5.5 (6.2)	5.3 (6.4)	4.7 (5.9)	5.8 (6.2)	Jiménez-Martínez et al. (2003)
Mung Bean	Mung bean protein isolate	5 points	–	–	2.7 (3.9)	3.1 (3.9)	2.5 (3.6)	RAO et al. (2007)

[a]Control (dairy yogurt)

8.2 Chickpea

Chickpea extract has been used to fortify dairy yogurt but also to create chickpea-based yogurt, with varying acceptability (Aguilar-Raymundo and Vélez-Ruiz 2019). However, sensory testing was not completed on the pure chickpea yogurt. The addition of chickpea milk to conventional milk in small quantities did not significantly reduce sensory ratings (Aguilar-Raymundo and Vélez-Ruiz 2019). In another study, comparing chickpea and soy yogurt directly, it was found that unfermented products were very comparable (Wang et al. 2018). However, when the products were fermented, chickpea rated considerably lower in both appearance and flavour, citing the lack of homogeneity as part of the concern (Wang et al. 2018).

8.3 Cowpea

Cowpea yogurt performed very poorly in comparison to dairy yogurt as shown in Table 8.6. The particular attribute of concern was the flavour, which was cited as a result to unfamiliarity to the flavour profile of a pulse-based yogurt (Sanni et al.

1999). Within the same study, it was also seen that the addition of flavours increased consumer acceptability as shown by the strawberry flavoured cowpea yogurt receiving a rating of 5.7, but also had the potential of reducing the structure of the yogurt (Sanni et al. 1999). In another study comparing cowpea, mung bean, and dairy yogurt, it was shown that panelists preferred cowpea the least, rating it 2.33 on a 5-point scale (RAO et al. 2007). By comparison, dairy was rated at 3.57 and mung bean at 2.52 (RAO et al. 2007).

8.4 Lima Bean

The sensory ratings of lima bean were compared to Bambara, soy and dairy yogurt within the same study. The results showed that all the pulse yogurts displayed a lower acceptability than dairy but Bambara was rated as the highest out of the three pulse options at 5.8 compared to 5.6 of soy and 5.2 of lima yogurt on a 9-point scale (Aduol et al. 2020). Primarily complaints were cited towards the beany flavour produced from the samples, which were most significant in the lima bean sample, reflected by the flavour rating of 4.85 while bamara and dairy were rated at 6.7 and 7.1 respectively (Aduol et al. 2020).

8.5 Lupin

Plain lupin yogurt was shown to have very little flavour; the ratings in Table 8.6 reflect a strawberry flavoured lupin yogurt against a strawberry flavoured dairy yogurt (Jiménez-Martínez et al. 2003). In this scenario, lupin yogurt rated favourably in comparison to the dairy yogurt at 5.8 and 6.2 respectively (Jiménez-Martínez et al. 2003). The heat treatment and fermentation that were carried out on the raw lupin seeds assisted in removing off-flavours that typically are associated with pulse yogurts, when in turn gave it a very plain profile. The combination with flavour additives greatly increased the acceptability (Jiménez-Martínez et al. 2003). Similarly, in another study, the sensory aspects of plain lupin yogurt was rated 2.84 compared to 4.36 of dairy yogurt on a 5-point scale (Ertaş et al. 2014). This example further illustrates the need for assessing the flavour components in pulse-yogurts in order to increase their consumer acceptability. However, if plain yogurt is being used as an additive, it may be acceptable on its own due to the neutral flavour profile it can provide.

9 Conclusion

The development of plant-based yogurts have seen many innovations in recent years. The demand for vegan substitutes and allergen-free products is growing within the commercial market at a rapid pace. Although there are already existing solutions that have been shown to be viable, they come with their own limitations and challenges. There is still a demand for a protein rich, fermented, plant-based yogurt and as seen in this review, considerable work has already been done into the research of fermented pulse-based yogurts. Many studies in this area are novel in nature and occurred in the last few years; however, many pulses still remain largely unresearched. Processing methods such as heat treatment and fermentation have shown promise in establishing sensory characteristics that match dairy yogurt very well but need further optimization to meet nutritional profiles. Methodology to produce products with stable mechanical properties is challenging and it remains a key factor for establishing acceptable consumer products. Many of these concepts are still yet to be established on a commercial or industrial scale.

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Chapter 9
Plant Protein Based Beverages

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1 Plant Based Proteins

Protein based food products and beverages are recognized as an essential part of diet due to their nutritive value. Principally proteins are indispensable macronutrients source made up of essential amino acids (Adenekan et al. 2018). Adequate human nutrition depends on a variety of protein sources as its nutritional value varies in terms of its source, composition, digestibility and its bioavailability factor. From prehistoric times, animals are considered as prime rich source of proteins consumed globally. Continuous growing population, prevailing protein malnutrition and global food security challenges continue to be a major concern in many states all around the world. Additionally, recent trends of consumer dietary habits and consumer knowledge related to food nutrition, demand innovative plant based protein food and beverage products with high bioavailability. Factors mainly contributing towards changing trend from animal to plant proteins are their reported nutritional benefits alongwith their role in disease prevention and health promotion. It is a demanding challenge that encourages scientists to explore alternative protein sources to ensure world food security (Qin et al. 2018).

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Plant-based beverages (PBB) have stand over as a growing globally culinary trend in the food sector. These are utilized as substitute for cow's milk and as an alternative to juices or other beverages. In addition, lactose intolerance, cow's milk allergy, heart disease (caused by high cholesterol levels), as well as vegetarian, vegan, and flexitarian diets have contributed to the increased consumption. Food industries are investing in the creation of new non-dairy products as a result of influence of demand. According to many studies, the nutritional properties of PBB can vary according to the raw material used, the type of processing and the chemicals used in fortification. These variables may alter particle size, rheology, stability, colour, and macro or micro-nutrient composition (Nawaz et al. 2020).

Oftenly there are four parts to the prospective plant-based protein source beverages: Nuts (almond, walnut & hazelnut) Legume (pea, chickpea, lentil & soy) Seeds (hemp, sesame seed, pumpkin & watermelon seeds) and Cereals (oat, rice, millet & barley). Nut or cereals based beverages such as soya, almond and rice based beverages are the most popular plant-based replacements to milk but additional substitutes either directly produced from the traditional edible plant extraction, or fermented, are gaining popularity as the market expands (Qamar et al. 2020). Among plant-based alternatives to milk, the most popular are soya, almond- and rice-based beverages, but other substitutes, either directly obtained from traditional edible plant extraction, either fermented, are gaining interest as the market is rapidly expanding. Health benefits of nut based beverages comprises of high amount of fibers, phytol-chemicals or vitamins along with their relatively low caloric amount (Manousi and Zachariadis 2019). Thus, such items are broadly consumed nowadays. Profiling of volatile compounds is directly linked with flavor of food. Aldehydes, ketones, alcohols, alkanes or terpenes are the usual catagory of volatiles that is present in nuts (Silva et al. 2020).

Beverages containing legumes and seeds provide a handy way to overcome the hurdles to legume and seed consumption and represent a huge business opportunity for the health food industry (Jeske et al. 2018). Legume or seed based beverages also contain a variety of isoflavones, phytosterols, polyphenols, and prebiotic oligosaccharides, which provide nutritional advantages over dairy products. However, there are still processing issues with legume-based beverages, particularly in terms of colloidal product stability, flavour stability, microbiological and chemical shelf life, anti-nutrients, and mineral composition (Roselló-Soto et al. 2019).

Plant proteins can be extracted by using chemical extraction techniques such as solvent extraction with alkaline, acidic, neutral and with alkaline aqueous mix solutions. These solvents are employed to solubilize proteins which further depend on conditions employed and plant used for extraction (Qin et al. 2018).

Various evidences supported that the use of the alkaline extraction technique as it resulted in improved protein yield. Another important reason is that alkali can effectively break disulphide cross-linkages and easily ionize neutral and acidic amino acids that in turn improved overall protein solubility (Sari et al. 2015).

2 Plant Protein Based Beverages

2.1 Nut and Legume Protein Based Beverage

2.1.1 Beverages Derived from Nut Protein

Commonly three nuts (*i.e.* walnuts, pistachio and almonds) are highly recommended to be used in beverages due to their high nutritional balance. Other than these varieties, hazelnuts are also recommended as these are excellent source of phenolic compounds such as, sinapic acid, quercetin, p-hydroxy benzoic, gallic acid and epicatechin (Sadia Qamar et al. 2019). Among nut based beverage, almond milk is one of the important product to be researched. Its serving of 100 gram gives >20% daily intake of riboflavin, vitamin E, niacin, iron, calcium, phosphorus, zinc, manganese and magnesium. The protein content of almond milk is reported as 1.70 g/100 mL (Alozie Yetunde and Udofia 2015).

Extraction of proteins from plant sources performed by using alkaline organic solvents like alcohol, hexane, ethanol and isopropanol. The purpose behind using a solvent is the solubilization of storage proteins from different plant base materials, especially in cereals and legumes (Capellini et al. 2017; Hojilla-Evangelista et al. 2017). Solvents like chloroform are used to extract fat free protein from high lipid containing produce (Wang et al. 2007). The resulting protein yield varies depending on certain factors like solvent type, solvent purity (with or without water) and heating temperature. Various databases reported higher protein extraction rate when ethanol is used as an extraction solvent with improved hydration level and at specific high temperature.

The alkaline extraction method followed by iso-electric precipitation is another important technique commonly employed in protein extraction from leguminous plants (Freitas et al. 2000). As leguminous and pulse proteins are more soluble at alkaline pH than at acidic pH. In this extraction technique, sample pH is adjusted in specific alkaline (8–11) range; where extract is allowed to solubilize at continuously increasing temperature for a set period of time. After that filtration is performed to separate impurities and pH is adjusted to induce protein precipitation prior to centrifugation. At the finishing phase, the recovered protein is washed to remove salts and residues, followed by neutralization and drying before storage.

2.1.2 Stability of Protein

Stability of protein is a complex topic to deal with as it changes with temperature, pressure and pH fluctuation. Thermal and HHP treatments at pressure of 62, 103 and 172 MPa was given by M-110P model, to check effect on viscosity, particle size denaturation and stability of protein of almond and hazelnut beverage. Samples were heated in water bath at low heat treatment of 85 °C for 30 min followed by high heat treatment in autoclave at 121 °C for 15 min. CLSM (Confocal Laser

Scanning Microscopy) was suggested to obtain results. As the temperature increase viscosity also increase and particle size reduced due to homogenization with enhanced clarity and surface charge. When pH was neutral around 8.0 then heavy weight molecular protein of SPI (>94,000 KDa) aggregates. Somehow, at 2.0 and 11 PH values, heavy weight from 7S or 11S globulins protein was aggregate (Bernat et al. 2015).

Acid Extraction is a solvent extraction technique similar to the alkaline extraction method except that primarily extraction steps are performed in acidic conditions (Boye et al. 2010). This extraction method is commonly employed for the extraction of pulse proteins as they show more solubility at low pH (<4). In this way, initially, proteins can be easily solubilized prior to precipitation by iso-electric precipitation (IEP) or refrigeration.

Pulse plant proteins can easily be extracted with an aqueous solvent even without acid precipitation. The previous database reported protein extraction from dry beans by using water twice as an extraction solvent (Martin et al. 1995). In a similar research study, protein from pulses (chickpea, lentils, mung beans and fava beans) was extracted by blending seeds with aqueous solvent followed by centrifugation (Cai et al. 2001). In this extraction method whole process was repeated to improve protein yield.

Micellization is commonly known as salt extraction different than the solvent extraction process. Micellization (MI) is based on the specific phenomenon of salting in and out of food proteins. In this process, the salt solution of specific ionic strength is employed for the extraction of proteins. After extraction solution is diluted to induce protein precipitation followed by centrifugation. After centrifugation, the extract is dialyzed using distilled water and subjected to freeze-drying in order to attain a final fraction (Liu et al. 2010; Stone et al. 2015).

2.1.3 Health Benefits

Comparing tiger nut based beverage with any other soft drink will conclude that nut based beverages are not just healthy but also are refreshing. It lowers down LDL (Low Density Lipoprotein) and enhances HDL (High Density Lipoprotein). It has some anti-oxidant properties as well because it contains natural antioxidant (*i.e.* vitamin E) which can control coronary cardiac aliments. As it contains huge amount of fiber, so it is ideal in controlling obesity. Without sugar it is thought to control diabetes as it has increase content of arginine which release insulin. It can highly recommend for patients facing problem with digestive system, diarrhea and flatulence, as it gives some enzymes for digestive tract such as amylase, catalase and lipase. It provides 100 cal/100 g energy values that make it an excellent energy drink. Another significant point is that there is no lactose or gluten content present in it (Gambo and Dau 2014).

Fermented almond milk with addition of various probiotics improves uptake of iron by epithelial cells of intestine. Moreover, consumption of this type of product could lower down the range of intolerances and allergies derived from the intake of cow's milk. Beverages based on nuts are very good source for antioxidants and vitamins. Beverage made of Hazelnut contains significant source for phytochemicals, especially lipid-soluble phenolic or natural anti-oxidant *i.e.* vitamin E.

2.1.4 Commercial Products

In Spain, Tiger nuts are used for the production of 'horchata' (40–55 million liters/year), a soft drink that intake almost all population parts, including children (especially significant because of their lower body weight). It is available primarily as a refreshing drink, also recognized as a nutritional drink because of its low caloric content (Arranz et al. 2006).

Talking about the health benefits of almond milk, first it is the excellent solution for children's that suffer from intolerances or allergies. Other than this, almond contain high amount of MUFA (Monounsaturated Fatty Acid) that is significant for weight control (Vanga and Raghavan 2018).

The Peanut Soy Milk (PSM) was developed to replace animal milk for vegetarians. Now it is prepared by addition of probiotics strains including *Lactobacillus acidophilus*, *Lactobacillus rhamnosus, Lactobacillus bulgaricus*, and *Lactobacillus lactis* and *Saccharomyces cerevisiae* in order to ferment soy and peanut by nurturing yield of probiotics and then fermented drink stored at refrigeration temperature (Agrahar-Murugkar et al. 2020).

Almond milk prepared by UHPH (Ultra High Pressure Homogenization) method including or deducting lecithin at 200–300 MPa to investigate its favorable as well as harmful effects on nutritional, physical and bio-functional characteristics. This technique increases particle size to almost three fold without changing amount of vitamin B1 and B2 (Briviba et al. 2016). Almond seed contains around 188 various protein checked by 2-phase electrophoresis technique. Among these 188 types of protein, (Li and He 2004) amandin is the prime protein that account for almost 65–70% soluble protein (Grundy et al. 2016). Another study has shown the preparation of pistachio milk by utilizing small and un-split nuts with ratio of 1:5 (Nut: Hot water) involving steps of soak, mill, centrifuge, homogenization, clarification, pasteurize procedure. Pistachio milk processed at pH of 8.5 was observed as stable emulsion with protein of almost 4.0% (Shakerardekani et al. 2013). Major protein present in pistachio is globulin (66%) followed by albumin (25%), glutelin (7.3%) and prolamin (2%) (Kashaninejad and Tabil 2011).

On the other hand, walnut beverage emulsion has gain attraction in market. The prime proteins in walnuts is glutelin (70.11%) while other minor proteins include albumin (6.81%), globulin (17.57%) and prolamin (5.33%) (Sze-Tao and Sathe 2000). But environmental stress conditions such as pH and thaw treatment lead to destruction and destabilization of its proteins. A study is conducted to determine the impact of heat sterilization, pH value and freeze/thaw on oxidative and physical stability of beverage emulsion derived through walnuts by utilizing different emulsifiers. Results indicate that assorted emulsifiers, leads to notable influence on physical stability (Liu et al. 2016). A study is conducted on tiger nut based beverage both roasted and non-roasted. Acceptability was determined under sensory evaluation conducted by 40 panelists. Tiger nut is highly being utilized due to its high amount of starch and protein content (Sanful 2019). It is reported that tiger nut is one of the efficient sources of protein contains up to 8% protein content (Sanful 2019; Abodunrin and Belewu 2008). Another demanding beverage is Hazelnut milk beverage prepared by the process of High-Pressure Homogenization (HPH). Research database conducted to investigate its rheological properties and behavior under HPH treatment. Results indicate that hazelnut proteins' solubility increases as pressure of homogenization (P_H) increased, but after pressure of 75 MPa no effect was indicated on solubility (Gul et al. 2017). Cavcava varieties contains maximum amount of protein that is around 20.8%. Its protein composition is based on non-essential amino acid like aspartic and glutamic acid (Köksal et al. 2006).

2.2 Legume Protein Based Beverage

Legumes are world's most important source of food. The properties related to protein are that they give desirable functional characteristics like water binding, gelling and some emulsification characteristics (Graça et al. 2016). One of the recent convenient legumes are being denoted as drinks or beverages for example Germans brand consisting milk substitute based on lupin and brand of USA consisting milk substitute based on pea. Main legumes that are used in beverages are soy, chickpea, lupin, pea, and cow pea (Nawaz et al. 2020).

Among legume based drinks, soy based beverage is most significant and is widely used as animal milk protein replacer. Due to its demand, different variations of soy beverages are attainable. Some of these types are solid concentration (*i.e.* light, rich and dairy based beverage), fortification (regular, enriched and blended soy based beverage) and formulation (flavored, sweetened and regular soy-milk substitute) (Kumar et al. 2016). On normal basis soy-milk has 0.5% of ash contents, 2.9% of total carbohydrate, 3.5% of protein and 2.0% of fat in it (Ikya et al. 2013). Other than soy, lupin legumes are getting high demand in market primarily due to the fact it is rich source of protein that is used by both humans and animals. Four major domesticated species of lupins are *L. luteus*, *L. albus*, *L. mutabilis* and *L. angustifolius.* Comparing these types, L. *mutabilis* contains highest amount of protein (*i.e.* 44%). Its drinks are Miso, Tempeh and some dairy fermented beverages (Mahmood et al. 2014).

Legume like cowpea is very significant source of protein content which is 2–3 times higher than cereals and tubers. Beverages based on cowpea legume are not yet commercialized due to its problems related to its immisibility. All the processing operations reduce stability of emulsion therefore is not suitable at commercial market scale (Onyesom et al. 2005).

Beverages based on chickpea legumes are taking place in the market scale, but very little work is being done on its production. Four steps were performed for its processing which include soak, blend, boil and filtration of liquid to separate out its solid residue. One of the researchers stated that in the production of plant beverages form chickpea is the best alternative to soy (Wang et al. 2018). Horse bean which is also called as Fava or Faba bean is very traditional legume crop. Now Fava beans are being raised as valued plant, so that it can be used as milk alternative as it has high amount of protein in it (Gugger et al. 2016).

Cereals are a rich source of many metabolically active and storage proteins that facilitate biosynthesis of functional proteins. Cereals are recognized as the world's most important food groups and are widely consumed as a staple food. In some regions, they are consumed as seeds like rice, barley, oats and maize while mostly used as flour like wheat and maize flour. Some cereals are also consumed in form of flakes like barley, oats and maize due to their nutritive value. Cereal proteins are mostly found as storage proteins in the form of albumins and globulins and metabolic active forms such as enzymes (protease inhibitors). Storage proteins function as building blocks in protein biosynthesis during the process of seed germination.

Cereal proteins provide adequate source of energy, about 6–15% of whole grain and have several biological properties. Regular consumption of cereal grains not only plays a significant role in health promotion but also control various chronic diseases associated with oxidative stress. Evidence reported that its hydrolyzed proteins and peptides have significant antioxidant, anti-inflammatory and anti-cancerous activities (Esfandi et al. 2019). Epidemiological studies confirmed that the increased consumption of whole grains reduced the risk of diabetes (Murtaugh et al. 2003), cardiovascular disorders (Cho et al. 2013), obesity (Hajihashemi et al. 2014) and hypertension (Borneo and Leon 2012).

2.2.1 Stability of Protein

Evidence on thermal stability comparison between chickpea isolate (CPI), lentil protein isolate (LPI) and pea protein isolate (PPI), illustrate that CPI is more thermally stable as compared to LPI and PPI. Effect of secondary hydrophilic emulsifier and primary lipophilic emulsifier (caseinate sodium) on stability of protein for Faba bean protein isolates emulsion. It can be concluded that prepared emulsions from un-modified beans of Faba protein isolates is stable. It is stated that increasing concentration of protein, temperature pH can significantly improve emulsion viscosity and protein aggregation. Around pH of 8, protein with high molecular weight of soy protein isolates aggregate (Ladjal-Ettoumi et al. 2016).

2.2.2 Health Benefits Related to Legume-Based Protein Products

Cowpea contains many active ingredients including phenolic compounds that has functional characteristics like antidiabetic, antihypertensive and hypocholestrolemic by decreasing LDL and increasing HDL which helps in decreasing cardiovascular diseases. Its protein isolates and peptides have anticancer property (Jayathilake et al. 2018). Utilization of soy nourishments is expanding a direct result of announced gainful impacts on sustenance and wellbeing. These impacts incorporate bringing down of plasma cholesterol, counteraction of malignant growth, diabetes, and heftiness, and insurance against kidney sickness (Friedman and Brandon 2001). Utilization of soy protein appears to reliably bring down blood LDL cholesterol in hypolipidaemic subjects. Despite the fact that soy protein or ISF emphatically sway biomarkers of prostate malignant growth, their potential benefits have not been validated in clinical preliminaries. The impacts of soy protein and ISF in alleviating menopause manifestations and counteraction of bosom malignant growth are not clear. Future investigations should give more consideration to identification of the bioactive segments in soy and clarification of the sub-atomic components (Chatterjee et al. 2018).

2.3 Seeds Protein-Based Beverages

Seeds are of enormous economical and biological significance due to their rich nutritional profile containing oils, protein and starch. The most common commercial food application of seed is the development of value-added food commodities like seed isolate as a functional ingredient of food products and nutritional supplements.

2.3.1 Types and Nutritional Benefits of Seed Protein Beverages

In food processing prospective seeds like hemp seeds are considered one of the most valuable and profitable seeds due to their protein reserves. They normally contain about 75% legumin protein and 37% albumin. These proteins from seed sources usually do not contain protease inhibitors and have good digestibility characteristics.

Seeds from pumpkin and melon are used in development of various snacks (El-Adawy and Taha 2001). Isolates from these seeds are also used as a source protein in traditional beverages. Similarly, sesame seeds are considered to be one of the cheapest protein sources with high biological value. These seeds are of commercial importance due to their nutty flavor and high oil content. Sesame seeds contributes up to 90.7% share in global edible oil production (Bamigboye et al. 2010).

Protein profile of any food system *i.e.* structural configuration and amount, play a significant role in designing novel food and beverage formulations. Malomo and Aluko (2015) worked to establish structural profile of hem seed as a source of salt soluble globulins and water-soluble albumin through electrophoresis and intrinsic fluorescence. Results revealed that globulins have higher aromatic and hydrophobic content in comparison to albumin. Research database found approximately 65 amino acids (23- short-chained peptides) in the protein hydrolysate of Hemseed (Girgih et al. 2014).

2.3.2 Health Benefits of Seed Protein Beverages

In globular fraction of sesame seed arginine and lysine ratio is about 67% which is comparable to casein, thus making it ideal for cholesterol metabolism. Bioactive profile of sesame seeds comprises sesaminol glycoside lignan glycosides and seasamolinol. Bioactive sesame protein has anti-oxidative stress, neuro-preventive and anti-cancer, hypolipidemic and hypoglycemic potential (Das and Bhattacharjee 2015). These seeds have diverse phytochemical profiles comprising of phenolic antioxidants, fiber and protein content. Products with a high percentage of sesame are usually rich in protein and low in carbohydrate sugars making them ideal for diabetics.

According to another study, 1 g of HPI protein isolate contains about 51.8 mg valine, 14.5 mg methonine, 43.3 mg lysine, tyrosine 38.2 mg, phenylalanine 49.6 mg, isoleucine 69 mg, cystine 17 mg, aspartic acid 98 mg, threonine 47.6 mg, alanine 47 mg, histidine 29.3 mg, proline 47.2 mg, glutamic acid 168.1 mg and arginine 103.2 mg (Qamar et al. 2020). Food and agricultural organization reported that the essential amino acid profile of HPI is sufficient to cope with malnutrition issues among children. Seed protein harbor bioactive peptides with strong antioxidant potential. These seed peptides are commonly used in formulations designed to treat hypertension and cardiovascular aliments (Lu et al. 2010).

2.3.3 Commercial Beverages

Sesame and pumpkin seed protein milk: To prepare sesame seeds are soaked in water for about 8 h, followed by wet milling of seed. Extracts are then sieved to get residue free sesame seed milk, used to fulfill daily protein requirements. A similar method is followed to prepare pumpkin seed milk, having similar health benefits (Hassan et al. 2012).

Sübye: Sübye is a traditional cold beverage and consists of melon seeds, sugar and water. Subtype production is important from an economical and environmental point of view since melon seeds as a food waste utilization, are used as its raw material (Sabancí et al. 2014).

Hemp infused protein sports drinks: Protein sports drink infused with hemp protein is one of the most complete and comprehensive sports drinks on the market (Galaz 2019). These sports drink is designed for athletes both during and immediately after intense workouts and competition. During extreme levels of muscular and physiological stress, the body demands additional protein, carbohydrates, amino acids and healthy fatty acids in order to feed muscles and allow athletes to maintain peak performance levels for extended periods of time (Van Nieuwenhoven et al. 2005).

2.3.4 Cereal Protein-Based Beverages

The most common cereal grains used to design commercial beverages are sorghum, rice, barley, and oat; members of monocotyledonous grass. Cereals are rich in carbohydrates, fats and proteins, crucial for human growth and development. They also fulfill the daily caloric requirements of the body; out of which 50% requirement is covered by proteins (Zhou et al. 2013).

Edible seeds of legumes like dry peas, lentils, beans and chickpeas are a good source of protein. Interest in the utilization of pulse proteins and their constituents for the development of innovative food formulations is a new prevailing trend, especially in developed countries. The major reason behind this concept is consumer dietary preferences associated with knowledge about the nutritional benefits of leguminous foods. Evidence reported that pulse legumes comprise 17–30% protein mainly albumins and globulins. They are also rich with amino acids such as lysine, leucine, aspartic acid, glutamic acid and arginine. One important property of pulses is that when used in combination with protein based cereals and other foods rich in sulfur-containing amino-acids they provide a balanced essential amino acid profile. In this way, pulses are ideal alternative to animal proteins as they have promising nutritional and functional protein characteristics (Christudas et al. 2020).

Recent evidence related to the bioactive properties of pulse proteins and peptides gained recognition in domain of nutraceutical foods for their potential health benefits and controlling or reducing the onset of chronic diseases. Various research databases reported health promising benefits of pulse proteins (Tharanathan and Mahadevamma 2003). They play a substantial role in cardiovascular disease prevention (Hu 2003) and reduction of its associated risk factors like blood pressure, LDL cholesterol levels, platelet activity, obesity and cell inflammations (Mudryi et al. 2014). Pulse proteins and its other numerous nutritive and non-nutritive constituents have significant anticancerous activities (Dahl et al. 2012).

2.3.5 Health Benefits of Cereal Proteins Beverages

Cereals are of prime importance due to their strong nutritional profile. Among cereals, maize is one of the most grown crops worldwide. Maize is rich source of energy dense proteins, with diverse amino acid profile. Cereals are prime protein source in under privileged countries, where meat protein is scarce. In addition to nutritional source cereal proteins have numerous health beneficial, functional and bioactive characteristics. Bioactive cereal peptides have physiological effects like anti-inflammatory, anti-oxidant, anti-hypertensive, anti-diabetic, anti-inflammatory and anti-cancer actions (Korhonen and Pihlanto 2006). Cereal based foods and beverages are effective in controlling diabetes mellitus, carcinomas and coronary heart diseases. 100 g sorghum contain 18.5 g total protein (Montonen et al. 2003).

2.4 Commercial Products

Rice milk: Rice milk is prepared by soaking rice for 2 h in water. Afterwards, water is drained and rice are cooked in water in 1:3 for about half hour. Slurry is then blended to make rice milk used as a protein beverage for muscle building (Hassan et al. 2012).

Millet milk: Millet milk is prepared by soaking millet grains in water for 12 h, then the water is drained. Soaked grains are milled with water in 1:1, prepared slurry is then filtered and sediments are removed by settling the solution for 24 h. Mixture obtained is used as millet milk (Hassan et al. 2012).

Oat-milk with plant protein: The protein-rich oat-based beverages are available in flavorful varieties like Double Chocolate, Matcha Chai, Rose and many more. The functional beverages are about sharing more than protein with the body, as each variety is crafted to help with a specific benefit like boosting the metabolism or providing energy or restoration. While the Double Chocolate Organic Oat-Milk with plant protein takes the form of an oat milk product with almond protein, the other varieties explore combinations like oat milk with almonds and pumpkin seeds. Organic Golden Turmeric Oat Milk with Almond & Pumpkin Seed Protein also contains activated medium chained triglycerides to make the product suitable as a breakfast replacement (Greither 2011).

Barley infusion: Coffee substitute (contains no caffeine), obtained from toasted and ground grains, lyophilized in pods prepared form espresso machines; barley coffee is very popular in Europe particularly in Italy.

Barley water: A drink that is made by boiling whole or pearled barley and then flavoring with various fruits. It is a flavorful drink that is enjoyed similar to soft drinks with healthy properties; barley water is used as a dairy substitute for drinks such as smoothies or hot chocolate, or to replace milk on breakfast cereals.

Malted barley beverage: There are also various malted beverages available, often in the form of “malty milk” in which malt extract is blended with milk.

3 Plant Protein Blend Beverage Innovations

With advanced research in food science and nutrition now plant protein based products are widely recognized for their improved nutritional value and health benefits. Various research databases reported that plant protein products especially beverages and drinks are now used as a replaceable source of animal protein drink source (Jain and Goomer 2020).

Recent evidence suggests that plant based protein beverages consumption is not only nutritionally beneficial but also have a pleiotropic impact that gives new ways to innovate the beverage industry. Researchers are now focusing on innovations based on the development of non-dairy fermented beverages that are recognized as a potential carrier of bioactive components. These components play a significant role as probiotics and prebiotics to improve gut microbiota that in turn improve individual health status (Valero-Cases et al. 2020).

Functional food development pertains to probiotic beverages development due to its nutritional and functional role in promoting health by improving microbial flora of the intestine. Development of sprouted cereal based probiotic drinks is growing scientific evidence to innovate beverage industry that helps in promoting health such as fermented wheat based probiotic beverage (Sharma et al. 2014).

Brown rice based probiotic beverage is now recognized widely acceptable non-dairy probiotic source as it acts as a good substrate for the growth of probiotic bacteria and its prebiotic role by non-digestible components (Majumder 2020).

A recent research database suggested an innovative fermented cereal based beverage produced by co-culturing fermentation of lactic acid bacteria and yeasts. Resultant product Boza has significant nutritional value and a valuable source of probiotic (Arslan-Tontul and Erbas 2020).

Fruit based probiotic beverages that have more iron uptake potential are introduced by ProViva® introduced by one of the international brands, Skane, Sweden. It is nutritionally improved product with improved iron (Fe) content and widely accepted by consumers (Neutraceuticals World 2008).

As non-dairy probiotic market is now gaining increased importance with increasing consumer awareness and customer demand. Vegetable based drinks (Lambo et al. 2005; Rakin et al. 2007) are cheap and valuable source of probiotics. Soya based beverages mostly soya milk consumed widely as a non-dairy beverage source now reinnovated as B-vitamin enriched fermented soya drink on functional food development concept (Zhu et al. 2020).

4 Consumer Perception of Plant Protein Drinks

The two most significant factors driving the food industries are health and consumer convenience. In the dairy industry, the probiotic health-improving microbes have become a significant ingredient in order to offer healthy foods for consumers, since the last two decades (Menrad 2003). In certain cases, consumers have a perception of healthy food as tasteless foods, as they perceive that healthy foods can only be processed by compromising their sensory pleasure (Tuorila and Cardello 2002). It may also lead to the unacceptability of probiotic drinks as the consumer may judge these drinks as less attractive compared to non-probiotic drinks. Various research databases reported that taste is considered as the significant driving factor for food selection, while nutrition remains the secondary factor (Tuorila and Cardello 2002). But today's consumer tends to buy non-dairy based probiotic drinks that are not only more nutritious beverages but also with free of food allergens causes intolerance issues. Therefore, industries are also focusing on developing nutritionally enriched innovative foods and beverages to meet consumer preferences. Evidence reported that soy-based nondairy products are rich sources of proteins and are also safe for consumers having lactose intolerance. Non-dairy beverages have been in huge demand especially by vegetarian and lactose-intolerant consumers (Farnworth et al. 2007).

Ultrafiltration or membrane separation is frequently employed in the pulse protein extraction method as an alternate to isoelectric precipitation (IP). In this technique, alkaline or acid treated extract is further subjected to ultrafiltration to obtain extracted protein concentrate. In ultrafiltration or diafiltration membranes of specific molecular weight and mesh size are carefully selected to obtain desired proteins (Fredrikson et al. 2001).

One of the limiting factors in utilization of extracted plant based proteins is their limited solubility in aqueous and other organic solvents. Therefore, to extend its use, extraction process assisted by hydrolyzing enzymes in specified conditions is used. Various peptidases and carbohydrate hydrolyzing enzymes are employed in alkaline or acidic conditions which improve protein solubilization (Sari et al. 2013; Fetzer et al. 2018).

Recent research evidence suggests that plant based proteins are now extensively used as a substitute to animal protein (Sha and Xiong 2020). Evidence confirmed that animal proteins pose severe risks associated with climate change, losses in biodiversity and human health like chronic and cardiovascular disorders (Alemayehu et al. 2015). There are several reasons behind this changing trend as plant proteins are economical, easily accessible and more acceptable to consumers. Another major reason is that plant proteins are safer than animal proteins as they are least affected by microbial hazards, thus, posing lesser risk to human health (Sa et al. 2020).

Recent research focusing to innovate industry with economical and safe plant based food products that can address both environmental and social issues globally (Sa et al. 2019). Plant proteins products are not only an important dietary source for a major segment of world's population where animal protein is unavailable but also for those regions where the limitation is self-imposed because of religious beliefs and social habits (Boye et al. 2010). Therefore, plant based protein products especially beverages are now extensively used and recognized as an important source of essential amino acids and also considered as environment friendly due to its reduced hazardous waste input (Sa et al. 2020).

5 Sources and Nutritional Benefits of Plant Based Proteins

Plant based protein rich food products and beverages demand is increasing globally, therefore, to meet such increasing demands researchers investigate various protein rich plant sources. Multiple research databases investigate various protein plant sources majorly cereals, legumes and seed crops (Table 9.1), their utilization for

Table 9.1 Sources and nutritional benefits of plant protein beverages

Plant protein source	Beverage	Nutritional benefits	Reference
Cereal and grain proteins	Wheat protein base probiotic drink	Rich protein source Improved probiotic source to promote health	Sharma et al. (2014)
	Rice and Millet milk beverages Oat (fermented) Probiotic drink Oat and Barley concentrates	Rich source of protein, vitamins, and minerals Millet rich source of Fe Beta-glucan source Low Glycemic index important for diabetes Beta-glucan rich Probiotic source	Hassan et al. (2012) Angelov et al. (2006) Lambo et al. (2005)
Leguminous proteins (Milk substitute)	Peas and bean protein source products Protein rich beverages drinks	Source of amino acids Reduced risk of metabolic syndrome	Ahnen et al. (2019)
	Soya beverages Chickpea beverages Lupin (Tempeh), and pea protein source beverages	Rich source of protein Whey, B amylase, Lectins, Globulins Good source of Phenolics	Nishinari et al. (2014) Gugger et al. (2016)
Nuts and seed proteins	Almond proteins Almond milk And their Fortified products with rice, pea protein Walnut beverage emulsion Hazel nut beverages	Provide improved protein content Energy source Good source of proteins and vitamins & minerals (Ca, Fe, B1, V-E) Good nutritional lever Lower risks of cancer Coronary artery disease Total mortality Rich source of phytochemicals, Phenolics (lipid soluble) Vitamin E source	Chalupa-Krebzdak et al. (2018) Alozie Yetunde and Udofia (2015) Salome et al. (2020)
Soya bean proteins	Soya milk Probiotic drink Soya milk Soya beverages	Reduced total cholesterol content in Hypercholesterolemia Bioactive components rich source Rich with Phytochemicals like phenolics and carotenoids Vitamins source especially V-E, Source of fatty acid and dietary fiber	Pratiwi et al. (2019) Ahnen et al. (2019)

development of nutritive products, drinks/beverages and confirmed their significant impact on human nutrition.

Globally, food industries have been developing innovative non-dairy beverages such as probiotic drinks with an appropriate ratio of fruit juices and soy-extract to meet consumer requirement with balance taste and nutrition (Champagne and

Gardner 2008) that have more acceptability. Researches have depicted almost double utilization of soy-based beverages in US markets since 2000, thus touching the sales of almost 100 million US$ per year (Beverage Marketing Corp. of New York 2005). Reportedly, the sales of soy-products have been significantly raised from 300 million US$ to approximately four Billion US$ during 1992–2008. This sale figure shows the demand and acceptability of non-dairy protein rich beverages in consumers (Granato et al. 2020).

Many oilseed plants are now used extensively as a source of dietary nutrients due to their health benefits. Soybeans, canola/rapeseed, sunflower, safflower, peanuts, corn, flaxseed, cottonseed, and chia seeds all are well known potential protein sources for human consumption. Among these, soybean is one of the important oilseed plants that is recognized for its high nutritional value. Evidence reported that it is an inexpensive alternative to animal protein as it has high protein content about 35–40% protein on a dry weight basis (Sha and Xiong 2020). The amino acid profile of soy-protein is comparable to animal protein except for few sulfur-containing amino acids (methionine and cysteine) (Xu et al. 2020). Therefore, soy protein is now being extensively used as a replacement for animal proteins and is considered safer. Soy and rapeseed have well balanced amino acid profile whereas sunflower has improved amino acid bioavailability (Arrutia et al. 2020). Hemp seeds especially dehulled and defatted are also a prominent protein source. Hemp seed protein is predominantly concentrated with major proteins globulin and albumin that are further characterized for their exceptionally high-level amino acids arginine and glutamic acid (Leonard et al. 2020).

Oilseed plants are not only dietary protein sources but also known for their nutritional and health-promoting properties. Evidence reported that soy proteins have significant potential to lower serum cholesterol levels by modulating low-density lipoproteins (LDL) receptors of the liver (Arnoldi 2020). It also helps in lowering glucose levels in diabetic patients when supplemented there food with soy flour. Hemp seed proteins are recognized for their bioactive peptides having significant antioxidant potential (Malomo et al. 2014). Help protein amino acids also help in the regulatory functions of human organs and metabolism (Wu et al. 2009).

6 Conclusion

Various plant sources based on their protein value are now extensively used in the food processing sector as replaceable sources of animal protein groups. Food industry never faced such demands and challenges that it is facing now in every aspect. With changes in dietary patterns and increased consumer knowledge this sector faces multiple challenges of making functional food from a variety of sources regarding nutritional value and possible health risks. Plant based food products are one of the contemporary innovations to meet food challenges and to provide solutions for increased demand of protein nutrition. Plant based protein products, especially beverages are now extensively available in the market to meet consumer demand. Among these cereal beverages like soya beverages (soymilk and drinks), rice, millet beverages, fermented oat probiotics and leguminous protein beverages like tempeh, lupin, chickpea beverages, and pulse protein beverages are now demanding. Now the main focus of food scientists and processors is to replace milk and milk beverages by plants based protein beverages. Various probiotic beverages and non-dairy beverages are on the way to innovate the food sector.

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Chapter 10
Plant-Based Protein Films and Coatings

Loong-Tak Lim

1 Introduction

Dried seeds from legume are rich in protein. Through physical and chemical treatments, these proteins can be converted into coherent films and coatings. Films are referring to as standalone continuous structures with sufficient mechanical strength and flexibility for handling, which can be further converted into the final product. On the other hand, coatings are thin continuous structures formed by depositing coating-forming solution on a substrate surface (e.g., food, packaging material), which upon solidification, becomes an integral part of the substrate. Through processing and formulation optimization, these protein films/coatings can be engineered with optimal material properties (e.g., mechanical, barrier, thermal) useful to protect and control unwanted mass transport processes in food products. Moreover, edible films and coating are versatile carriers of bioactive ingredients (e.g., antimicrobial, antioxidant and nutraceutical) for the development of innovative products with enhanced functionality, safety and/or quality (Debeaufort et al. 1998; Krochta 1997).

Proteins are polypeptides made up of amino acid monomeric residues derived from approximately 20 different amino acids, which can be classified as nonpolar with uncharged side chain (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine tryptophan, methionine), uncharged with polar side chain (e.g., serine, threonine, cystine, tyrosine, asparagine, glutamine), and charged side chain (e.g., aspartic acid, glutamic acid, histidine, lysine, arginine) (Belitz et al. 2009). Depending on the amino acid sequence that forms the primary structure, the polypeptide backbone can fold into various secondary structures (e.g., α-helices, β-sheets, bends, loops). Further intermolecular interactions of the amino acid side chains result in tertiary and quaternary molecular conformations. By disrupting

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these molecular structures through physicochemical treatments and exploiting the reactivity of specific amino acid reside chains (e.g., glutamic acid, aspartic acid, lysine), proteins can be converted into coherent films and coatings with desirable functional properties for edible and non-edible applications.

Plant protein films and coatings are typically prepared from commercially available protein concentrates or isolates. The production of protein concentrate involves milling and air-classification process that separate legumes into two fractions, i.e., the light/fine protein concentrate fraction and the heavy/coarse starch concentrate fractions. The purity of the protein concentrate ranges from 38% to 65%, with the remaining portions being polysaccharide, sugars, ash, and so on. To prepare protein isolate with a protein content of greater than 90% level, alkaline extraction and isoelectric point precipitation methods are employed. This wet process involves first solubilizing the protein in the concentrate or flour under alkaline condition (pH 8–11), followed by centrifugation. The pH is then adjusted to the isoelectric point (pH 4–5) to precipitate the protein. Other methods of protein isolate preparation include acid extraction, salt micellization, ultrafiltration/diafiltration (Klupšaitė and Juodeikienė 2015). Due to their high protein purity, isolates tend to produce more transparent and consistent film/coating products than those derived from the protein concentrates.

Many plant proteins are globulins with compact molecular structures. During film/coating formation, these proteins must be denatured to disrupt their secondary, tertiary, and quaternary structures. The unfolded protein structures allow for polypeptide chain-chain entanglement, hydrogen bonding, hydrophobic interaction, and formation of new intermolecular crosslinks. These molecular phenomena are essential for producing coherent film and coating structures. By and large, protein films are formed by either wet or dry methods. In the wet method, also known as solvent casting, protein is solubilized in a solvent, which is then cast/spray on a surface followed by solvent evaporation to form a solidified film. While this method is simple and readily adaptable in a laboratory setting, scale up of the batch process is challenging. On the other hand, the dry method involves plasticizing the protein, along with functional additives, through mechanical shearing/mixing at elevated temperature. The molten polymer is extruded continuously through a slit die or compressed batch-wise between two heated plates to form the final film product. Coating is formed typically by wet processes wherein the coating-forming solution is applied directly onto a substrate surface (e.g., food, packaging) by using various technologies (e.g., spraying, dipping, enrobing, brushing). Subsequent evaporation of solvent forms a continuous coating structure.

Inherently, protein films and coatings are hydrophilic; the sorption of moisture can substantially weaken their mechanical and barrier properties, especially when exposed to elevated relative humidity conditions. To address this issue, composite films and coatings are formed by the incorporating impermeable filler particles and/or blending with other polymers that are relatively hydrophobic (Robertson 2013). For edible film and coating applications, these materials must be compatible with the food product, exhibit optimal stability against deteriorative processes (e.g., biochemical, physical and microbial), and must not cause undesirable sensory issues.

This chapter provides an overview on the formulation and processing methods for the production of films and coatings derived from proteins from plant sources. Material properties and selected applications of protein films and coatings are discussed.

2 Wet Processing of Protein Film

2.1 Solvent Casting

The simplest film manufacturing technique is solvent casting. The method involves solubilizing the protein, along with functional components (e.g., plasticizer, preservative, bioactives), in a compatible solvent to form the film-forming solution. For edible applications, the solvents are limited to those that are approved for food applications. Typical solvents are water, organic acids (e.g., acetic acid, lactic acid), alcohols (e.g., ethanol, isopropanol) and mixtures of different solvents (Mellinas et al. 2016). To form a coherent and strong film, pulse proteins must be denatured to unfold their polypeptide chains essential for inducing polymer chain-chain interactions, such as the formation of new disulfide linkages through sulfhydryl-disulfide interchange reaction, hydrogen bonding, hydrophobic interaction, and electrostatic attraction (Fukushima and Van Buren 1970a, b; Quinn et al. 2003; Guerrero et al. 2014). To this end, typical approaches include heating, pH adjustment, and incorporation of chemical denaturants (Krochta 1997; Jensen et al. 2015).

To form film, the polymer solution is poured onto a levelled rimed surface and allowed the solvent to evaporate to yield solidified films. For viscous film-forming solutions or gels, the use of a draw down instrument will be needed to mechanically spread the solutions/gels cross the casting surface to achieve a consistent final film thickness. Most draw down instrument has a provision to adjust the gap width between the applicator and the substrate surface, in order to deposit a desirable amount of film-forming solution/gel for achieving the target thickness. Viscous polymer solutions tend to entrap air bubbles. The removal of bubbles from the film-forming solution is important to prevent the inclusions of voids in the final film matrix, which can act as a stress-concentrator during tensile load, thereby compromising the mechanical properties of the films. Bubbles in the film-forming solution can be minimized by using gentler mixing to reduce the incorporation of air into the film-forming solution, degassing using vacuum/sonication, or centrifugation.

The surface characteristics of the casting surface must be optimal to provide adequate film adhesion to prevent delamination/curling during the solvent evaporation process, while allowing the removal of the film without causing physical damages. Besides the amount of film-forming solution applied, the film thickness can be controlled by manipulating the total solutes present in a given volume of the film-forming solution, i.e., higher polymer concentration produces a thicker film and reduces the time required for solvent evaporation, as compared to diluted solutions. The rate of the formation of film is determined by the volatility of the solvent; film-forming solutions prepared from a solvent of higher vapor pressure will form faster than those prepared from that of a lower vapor pressure. Since the vapor pressure of water increases with increasing temperature, elevated temperature conditions will speed up the film-forming process. At a given polymer concentration, different proteins can exhibit different thicknesses upon film forming, which can be attributed to the different cohesive energy density and free volume of the film matrices, depending on the formulations used.

Since protein films and coatings are hydrophilic, they tend to absorb significant amount of water and swell when exposed to elevated relative humidity (RH), thereby affecting their physical and barrier properties. At low water activity, water molecules form double hydrogen bonds with two C=O groups of the polyamide backbones, which can be considered as firmly bound water. As water activity increases, less tightly bound water molecules form bridges between the hydrogen-bonded carbonyl groups and the N-H groups. As moisture content increases further, the water molecules inserted between the polymer chains can disrupt the hydrogen bond bridges, causing an increase in free volume of the film matrix and thereby producing more sites for capillary condensating and forming water clusters (Puffr and Sebenda 1967; Lim et al. 1998a). Besides interacting with the polyamide backbones, water molecules can also hydrogen bond with polar amino acid residues of the protein. These polymer-water interactions disrupt the intermolecular hydrogen bonds, thereby resulting in an increased chain mobility and swelling of the film matrix. This effect must be considered during the end use application of protein films. For example, water vapor permeability (WVP) – an index that normalizes the effect of film thickness and partial pressure driving force of water vapor transmission – of protein films tends to increase substantially with increasing RH due to material swelling that increases the polymer matrix free volume and enhances the diffusion of water molecules. Hence, barrier properties must be characterized over a range of RH to better predict the end use performance. When evaluating the barrier properties of hydrophilic film specimens that exhibit high water vapor transmission rates, such as by using the ASTM E96 method (ASTM, 1994), it is important to be aware of the presence of water vapor partial pressure gradient in the stagnant air that exists in the test cup, which can introduce substantial measurement errors (Kamper and Fennema 1984; McHugh et al. 1993; Debeaufort et al. 1994).

Due to the high cohesive energy density of dried protein matrices, neat protein films/coatings are brittle; the incorporation of compatible plasticizers is needed impart film flexibility essential for end-use handling (Kester and Fennema 1986). Typically, plasticizers are small molecular weight compounds added to modulate polypeptide chain-chain interactions. They can be envisioned as “lubricant” that facilitates polymer chain slippage to increase film flexibility. Water soluble plasticizers are often used in protein film/coating formulations to reduce intermolecular hydrogen bonding, such as polyols [e.g., glycerol, sorbitol, poly(ethylene glycol)], mono-/di-/oligo-saccharides, lipids (e.g., monoglycerols, phospholipids), and surfactants (e.g., sodium dodecyl sulfate, glycerol monostearate) (Gennadios et al.

1993; Fairley et al. 1996; Galietta et al. 1998). In hydrophilic polyol plasticizers, their size, molecular shape, number of oxygen atoms, spacing of oxygen atoms, and water binding capacity are known to influence the efficacy of protein plasticization. The efficacy of a plasticizer can be evaluated on the basis of: (1) mole of plasticizer's oxygen atom per mole of polymer – reflects the number of the available oxygen atom in a given amount of plasticizer available to interact with one mole protein; (2) mole of plasticizer per mole of polymer – indicatives of the number of plasticizer molecules interacting with the polymer; and (3) mass of plasticize per mass of polymer – measures the mass of plasticizers required to plasticizer the polymer (Sothornvit and Krochta 2001). From a perspective of practical and cost implication, plasticizer/protein on a mass basis is most commonly used when comparing the efficacy of the protein film plasticizers. For plasticizers with similar molecular structures (e.g., PEGs), on a mass basis of plasticizer to protein, plasticizers with lower molecular weight tend to be more efficient in plasticizing the protein films than the larger molecules. However, on a mole basis of plasticizer to protein, larger plasticizer molecules tend to provide a greater interaction with the polymer (Sothornvit and Krochta 2001).

Although polyols are effective plasticizers to impart flexibility in protein films, these additives can further increase the hydrophilicity of the materials when exposed to any given water activity, thereby substantially reducing the film strength (Cho and Rhee 2002; Wang and Padua 2004; Chen and Zhang 2005). This phenomenon is undesirable in high moisture applications where mechanical strength is important. The moisture sorption behaviors of protein films vary substantially depending on the molecular structure of the protein. Figure 10.1 illustrates the moisture sorption isotherms of SPI and zein films, plasticized with different plasticizers (glycerol and oleic acid). As shown, being a prolamin, the zein films are less hydrophilic than SPI. Moreover, the use of fatty acid as a compatible plasticizer can further reduce the equilibrium moisture contents of zein film. The mechanical properties of fatty acid plasticized zein and other prolamin films, such as those derived from wheat gluten, are less influenced by the moisture from the environment and food products, as compared to films prepared from water-soluble proteins.

Another property of protein film/coating that should be considered is the migration of plasticizer. External plasticizers are known to migrate to the surface of protein films/coatings during storage, especially when they are exposed to elevated temperature and relative humidity environments. From an end-use handling and down-stream processing standpoint, the migration of plasticizer to protein film surfaces may result in blocking phenomenon, i.e., adhesion of films when stacked together. The gradual phase separation of the plasticizer from the protein matrix will also embrittle the film, which may impact their handling properties. The extent of these changes depends on the protein-plasticizer systems involved and the end-use conditions. Hernández-Muñoz at el. (2004) investigated the mechanical and water barrier properties of glutenin films, plasticized by glycerol, triethanolamine, or sorbitol, as affected by aging time for 16 weeks at 23 °C and 50% RH. They reported that the mechanical and water barrier properties of glycerol-plasticized changed considerably over time, but those plasticized with triethanolamine and sorbitol

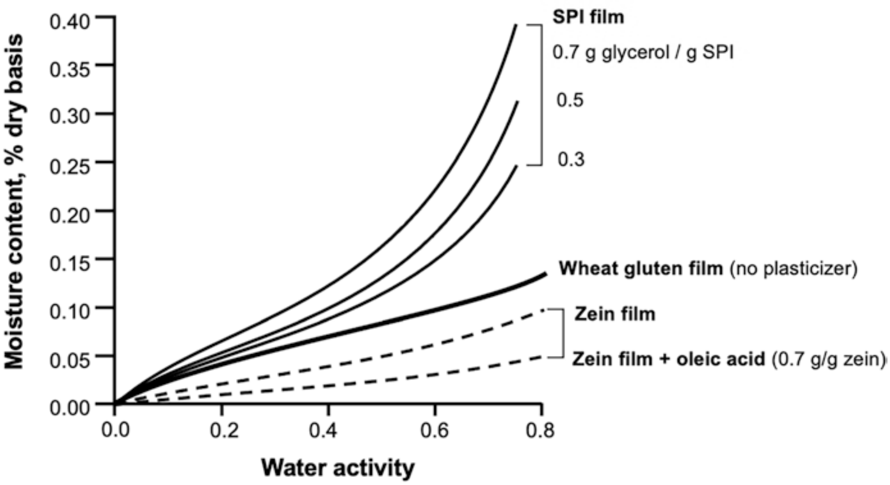

Fig. 10.1 Moisture sorption isotherms of solvent-cast soy protein isolate (SPI) films at 25 °C, as affected by glycerol contents (Cho and Rhee 2002), showing the increasing equilibrium moisture contents as the plasticizer content increases. For comparisons, moisture sorption isotherms of extruded zein film (with and without oleic acid plasticizer at 25 °C; Wang and Padua 2004) and solvent-case wheat gluten film (25 °C without plasticizer; Oliver and Meinders 2011), are included to illustrate their relatively more hydrophobic characteristics than the SPI films

remained stable during storage. Both zein and wheat gluten films exhibited similar increases in brittleness when plasticized with glycerol and/or poly(ethylene glycol) (PEG; 400 g/mol) for up to 20 d at 26 °C and 50% RH, although blending the two plasticizers could reduce the extent of increased brittleness during storage (Park et al. 1994a). Similarly, Wan et al. (2005) investigated solvent cast SPI films plasticized with glycerol, propylene glycol, PEG, sorbitol, sucrose at various ratios. They reported that a 50:50 glycerol:sorbitol blend resulted in a low water vapor permeability value while providing relatively high film flexibility and strength. On the other hand, glycerol:PEG plasticizer SPI films resulted in PEG migration to the film surface (Wan et al. 2005).

2.2 *Selected Examples of Solvent Cast Films*

One of the most explored solvent cast plant proteins is SPI. The two main fractions of protein in soy protein are the 7S and 11S proteins, which constitute about 70% of the total protein in soybeans. The 7S fraction is highly heterogeneous, comprises of mainly β-conglycinin, a sugar containing globulin with a molecular weight in the order of 150 kDa. The 11S fraction consists of glycinin – the main protein of soybeans with a molecular weight of 320–350 kDa made up of 12 subunits associated through hydrogen and disulfide bonds. The ability of soy proteins to undergo association-dissociation reactions is key to their functional and texturization properties (Klupšaitė and Juodeikienė 2015). Both 7S and 11S fractions contain cysteine amino acid residues, capable of forming disulfide bridges that are important to produce strong film and coating structures. The 11S fraction tends to produce smooth, opaque, elastic, films with high tensile strength. On the other hand, the 7S protein fraction produces translucent films with creases (Okamoto 1978). Typical procedure for forming solvent cast SPI films involves the dispersion of about 5% (w/w) protein in water, heating at 60–90 °C for 10–30 min, and then casting the resulting film-forming solutions onto surface-treated glass plate or other plastic substrates. To accelerate the drying step of the film forming process, Jensen et al. (2015) prepared SPI-cellulose composite films whereby the film-forming solution was cast on a heated glass surface maintained at 85 °C. The reduction in drying time can be beneficial in making the film casting process more commercially viable.

Glycerol is the most common plasticizer used for solvent cast SPI films, at concentration varied from 35 to 60 (% w/w protein) (Brandenburg et al. 1993; Rangavajhyala et al. 1997; Rhim et al. 1999, 2000; Park et al. 2001). Other low-molecular-weight organic acids with one or more hydroxyl groups have also been used for the preparation of a plasticizing soy protein films, including malic, lactic, citric, and tartaric acids (Cagri et al. 2001, 2004). Many of these organic acids are naturally occurring in fruits, vegetables, and fermented products, which are desirable from a consumer acceptance standpoint, although their acidic nature may impact the sensory attribute of the edible protein films. Because these plasticizers are hygroscopic, they can substantially increase the hydrophilicity of the materials, and thereby impacting their mechanical properties when exposed to elevated RH environments. This effect can be seen in Fig. 10.2; while increasing glycerol content imparts flexibility to the soy protein film (as reflected by the increased

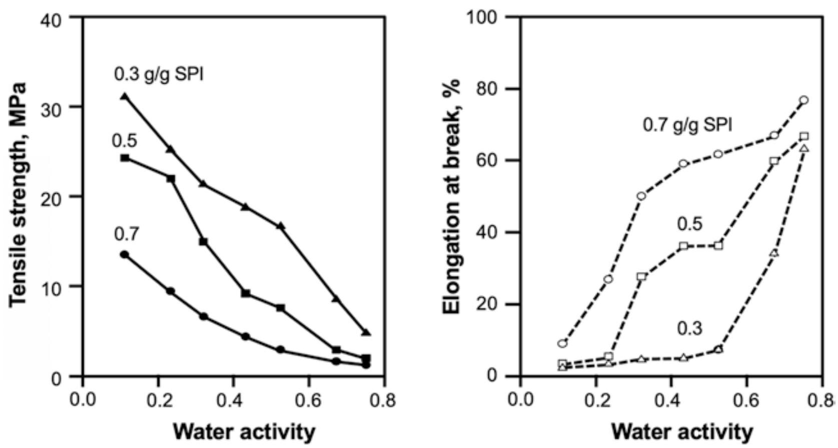

Fig. 10.2 Effects of glycerol on tensile strength and elongation at break values of solvent-cast SPI films at 25 °C. (Adapted from Cho and Rhee 2002)

elongation-at-break), a reduction of tensile strength is observed. Moreover, the added glycerol can act synergistically with water activity (a_w) in further reducing the tensile strength and increasing the elongation at break values of the SPI films (Cho and Rhee 2002).

Zein is a prolamin protein from corn that is insoluble in water due to its substantial fraction of hydrophobic amino acid residues (e.g., proline, leucine, and alanine). It is soluble in aqueous alcohol solutions, such as 70% (w/w) aqueous ethanol or aqueous isopropanol (Anderson and Lamsal 2011; Osborne 1924), which are often being used in solvent casting of zein film. Fan et al. (2018) investigated the use of zein to modify the moisture barrier properties of a fish gelatin film through exploiting the relatively hydrophobic properties of zein. Using aqueous ethanol as a solvent, they observed phase separation of the two proteins after casting, as revealed by the heterogeneous morphologies and higher opacity in the blend films. By incorporating glutaraldehyde as a crosslinking agent, they reported improved light transparency, moisture barrier properties, and mechanical strength at elevated humidity. Fourier transformed infrared (FTIR) spectroscopy confirmed the crosslinking reaction between the carbonyl group of glutaraldehyde with amino group of the polypeptides to form imine bonds via Schiff base reactions (Fan et al. 2018). Considering that glutaldehyde is not a food ingredient, its use as a crosslinker would require a demonstration of a negligible level in the food product. The use of other non-chemical based crosslinking processes may be more viable for edible applications, as discussed below.

Wheat gluten is produced by extensive washing what flour with water to remove starch and albumin. The protein is mainly consisting of gliadins and glutenins. Gliadins are the main prolamin in wheat categorized as sulfur amino rich (α/β- and γ-gliadin monomers, 30–45 kDa), sulfur amino poor (ω-gliadin monomer, 30–75 kDa), and high molecular weight gliadins (100–500 kDa). Glutenins can be classified as high (67–88 kDa) and low (30–45 kDa) molecular weight subunits (Ortolan and Steel 2017). Wheat gluten has a substantial content of cysteine amino acid residue which can polymerize via sulfhydryl-disulfide interchange reactions during heating to form a continuous network after cooling (Lindsay 1985). This phenomenon is consistent with the observations from Were et al. (1999), who studied the effect of cysteine fortification in wheat gluten-SPI blend film. They reported an increased tensile strength of the blend film due to the increased formation of disulfide bonds.

Gontard et al. (1993) prepared wheat gluten films by dissolving wheat gluten in 45% aqueous ethanol (v/v) at 7.5% (w/v) polymer concentration at 40 °C. The film-forming solution was adjusted to pH 4 using acetic acid, followed by the addition of glycerol at concentrations ranging from 0 to 33.3% of the polymer. The solutions were dried for 12 h to form transparent films. When the a_w of the films existed above a critical level, substantial deteriorations in mechanical and water barrier properties were observed. These a_w critical levels occurred >0.8, 0.7–0.8 and 0.5–0.6, respectively at 5, 30 and 50 °C. Below these critical a_w levels, the wheat gluten films had relatively strong mechanical and water vapor barrier properties. Similarly, Rocca-Smith et al. (2016) investigated wheat gluten films, prepared using a solvent casting of a film-forming solution (10 g wheat gluten, 2.5 g glycerol, 40 g deionized water, 50 g absolute ethanol) adjusted to pH 4 using hydrochloric acid. The film-forming dispersion was sonicated, heated at 70 °C for 15 min, cast on a poly(vinyl chloride)-coated plate, and then dried at 25 °C for 20 h. They reported that the reduced pH favored wheat gluten solubilization to form transparent films, as compared to elevated pH (>8) condition that resulted in brown and opaque films. The Young's modulus, tensile strength, and elongation at break values remained stable across the 0.1 and 0.4 a_w range. However, above 0.5 a_w level, modulus and tensile strength decreased substantially while an increase in elongation at break was observed. They attributed the a_w-dependent mechanical behavior to the depression of glass transition temperature (T_g) of the polymer matrix as a_w increased.

Pea protein is mainly consisting of globulins (>80% of total proteins). The major globulin is 11S legumin made up of six subunits with a molar mass of 350–400 kDa. Vicilin (7S) is the second major globulin fraction, which is a trimer with a molar mass of approximately 150 kDa (Gatehouse et al. 1982). Gueguen et al. (1998) was among the first to report the solvent casting of pea protein isolate (PPI) films. Similar to SPI solvent casting reported by other researchers, their approach involved the preparation of film-forming solution at a concentration of 13% (w/w) in 0.1 M NaOH solution at pH 12.5, followed by casting on a polyacrylamide coated glass plate. The film was dried at 60 °C for 1 h. They evaluated various polyol plasticizers, including ethylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, and glycerol at a relative high level (100% of PPI). They reported that PPI film plasticized with glycerol was the weakest (75% elongation; 0.5 MPa tensile strength), while that plasticized with ethylene glycol was the strongest (152% elongation; 2.2 MPa tensile strength). Unfortunately, ethylene glycol and the oligomers tested are toxic, and hence not suitable for edible applications. More recently, Azevedo et al. (2020) prepared PPI film using a modified approach, by stirring the film-forming solution prepared in water for 12 h without pH adjustment. Glycerol was added at 3% (w/w of PPI) and heated at 90 °C for 10 min, cooled, and degassed in vacuum followed by casting on a plexiglass plate. The elongation at break and tensile strength values of the resulting PPI film were 169% and 25 MPa, respectively. The substantial improvement in physical properties observed by Azevedo et al. (2020) could be attributed to the reduction in glycerol content, omission of alkaline treatment, and the extended hydration duration (12 h) during the preparation of the film-forming solution.

Valenzuela et al. (2015) investigated the effects of edible quinoa protein film, blended with antimicrobial chitosan polymer, on the preservation of refrigerated strawberry. The quinoa protein was extracted by from quinoa flour by solubilizing the protein at pH 8 using 1 M NaOH, followed by blending with chitosan prepared in 2% w/v citric acid solution. The resulting polymer solution was adjusted to pH 3 using citric acid and followed by dispersing sunflower oil into the polymer solution with the aid of an emulsifier (Tween 80). Strawberry fruits were coated with the formulated solution by dipping. The researchers reported that the CO_2 emission rate by the fruits was reduced by 60% compared to the uncoated strawberries. Moreover, the coated fruits had significant lower incidence of mold and yeast growth than the control samples. Reportedly, the coating treatment retained color and sensorial quality of the fruits (Valenzuela et al. 2015).

3 Dry Processing of Protein Film

3.1 Extrusion

Unlike wet processing, protein films prepared from dry processing do not involve the use of a solvent. Here, dry protein and additives (e.g., plasticizer, filler) are introduced into an extruder, in which the mixture is sheared and heated to form a molten mass. Prior to film extrusion, the dry ingredients may be compounded with plasticizer and other additives into pellets, using a mild mixing condition with an operating temperature of around 100–120 °C, as compared to higher processing temperatures typically encountered in a film extruder, ranging from 120 to 160 °C in various zones of the equipment (Chan et al. 2014; Chen and Zhang 2005; Rouilly et al. 2006; Zhang et al. 2001).

Unlike the batchwise solvent casting process, extrusion is a continuous process with a higher production throughput. The extruder system can be configured as single- or twin-screw, with the latter having a greater mixing capability. A typical extrusion process involves feeding the ingredient mixture into the hopper located at the feed throat of the barrel (Fig. 10.3). The ingredients are continuously being fed forward by the spiral flights of the rotating screw. As the screw pitch and the flight depth decrease through the transition zone of the extruder, the combined pumping force of the incoming materials and the decreasing volume generate considerable compression forces. Moreover, the rotating screw induces substantial friction

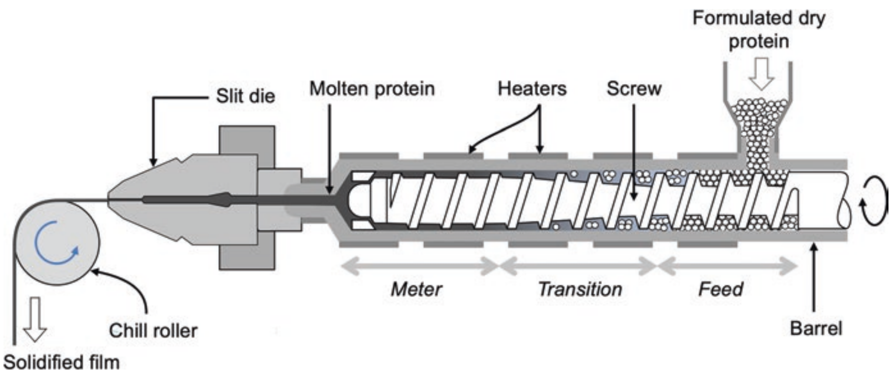

Fig. 10.3 Schematic diagram of a single-screw extruder showing the transformation of protein from solid to molten material as it moves through the feed, transition, and metering zones of the barrel. The lip die shapes the molten protein into film which is cast and quenched on a chill roller to form a solidified film

between the materials with the screw/barrel surfaces, generating heat that converts the ingredients into a thermoplastic material. Additional thermal energy is often supplied through band heaters wrapped around the barrel to optimize the temperature profile. The resulting molten material is being forced through a slit die and cooled rapidly to form a solidified film which is then spooled with a take-up roller.

During the dynamic heating process, the three-dimensional structures of proteins are altered extensively. In globular proteins, the polypeptide chains are unfolded due to the disruption of hydrogen bonding and cleavage of disulfide bonds, thereby exposing the functional amino acid residues for intermolecular interactions essential for forming a coherent network. Under the constant input of thermal and mechanical energies within the extruder, the protein undergoes glass-to-rubber transition at T_g, and eventually reaches the denaturation temperature (T_d) where the protein is substantially unfolded into an unstructured state (Fig. 10.4). For film extrusion, proteins are typically processed at a temperature above T_d to reduce the melt viscosity to facilitate the shaping of the molten material into film through the slit die (De Graaf 2000). To stabilize the dimension of the film, the extruded protein exiting the die is being cast onto a chill roller to quench the material to a temperature below T_g.

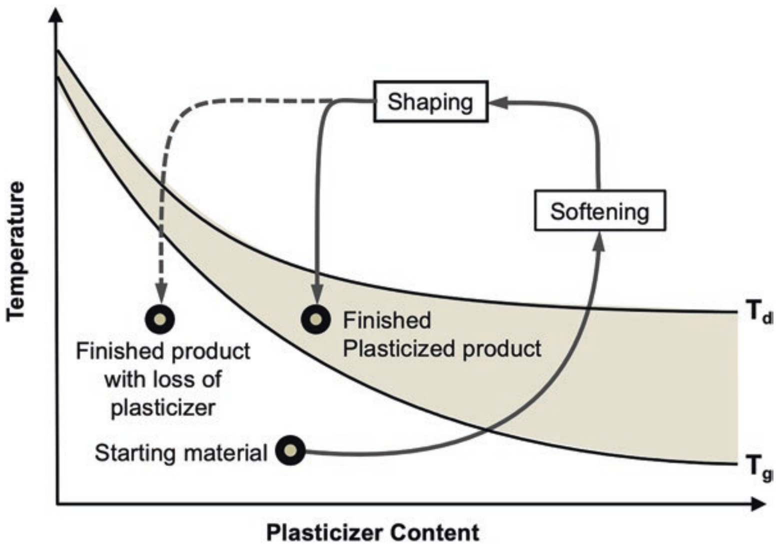

Fig. 10.4 Conceptual representation of dry film-forming process as related to glass transition (T_g) and denaturation (T_d) temperatures. The protein is extruded above T_g to convert it into a thermoplastic for shaping through the slit die. Both T_d and T_d can be depressed by the addition of denaturant and plasticizer (i.e., downward shift of the shaded region), which is beneficial to allow the extrusion processing at a lower temperature to prevent thermal degradation of protein. A loss of plasticizer due to migration to the surface or phase separation during the processing can lead to embrittlement of protein film as it's T_g elevates above the end-use temperature. (Adapted from De Graff (2000) and Zink et al. (2016))

Although proteins can be converted into thermoplastic materials through heating them above the denaturation temperature, thermal degradation tends to occur. To address this issue, a reduction in processing temperature is desirable, which can be achieved by incorporating a small quantity (several percent) of plasticizer (e.g., water, glycerol, sorbitol) to the feed formulation. As depicted in Fig. 10.4, increasing the plasticizer content would depress both the T_g and T_d, allowing the materials to be processed at a lower temperature (100–140 °C) (Zink et al. 2016; Gällstedt et al. 2011). In some extruder systems, the barrel is fitted with an inlet port near the feed throat, through which a liquid ingredient (e.g., plasticizer, crosslinker, preservative) can be continuously injected at a controlled pump rate. Judicial selection of plasticizer is critical in achieving the desirable physical properties of the resulting film. While the plasticizer will impart flexibility to the product, high plasticizer loading can substantially reduce the material's strength. Although water is an effective plasticizer in facilitating the thermal processing of protein films, it tends to evaporate after the extrusion, lead to materials embrittlement as they return to the glassy state (dotted line in Fig. 10.4). The use of compatible and less volatile plasticizers of higher molecular weight is essential to ensure long-term film flexibility. Similar to solvent case film, the plasticizer incorporated can migrate to the surface, causing material embrittlement. Considering the markedly different film-forming mechanisms involved between materials prepared from solvent-cast (excess solvent) and extrusion (minimal solvent) processes, the aging phenomena are expected to be different.

The continuous dry extrusion method is highly efficient for scaling up for industrial production. However, the extruder equipment is more costly and complex than the solvent-casting equipment that can be readily set up in most laboratories.

3.2 Compression Molding

An alternate batchwise dry processing technique is known as compression molding. Like dry extrusion, the process involves the preparation of a uniform blend of protein, plasticizer, and other additives in a mixer or extruder. The blend is then transferred in between two platens and compressed at elevated temperature (80–140 °C) and pressure (0.2–20 MPa) for a prescribed duration (5–15 min). This condition transformed the mixture into a viscoelastic melt, which upon demolding and cooling, resulting in coherent film/sheeting structures through hydrogen, ionic, hydrophobic, and covalent interactions. Plasticizer level typically ranges from 30% to 50% (w/w) on the protein basis. Plasticizers successfully deployed for compression molding of protein meal/isolate include glycerol, lactic acid, octanoic acid, palmitic acid, water, 1,4-butanediol, triethylene glycol, propylene glycol, dibutyl tartrate and so on (Hernandez-Izquierdo and Krochta 2008; Gällstedt et al. 2011).

3.3 Selected Examples of Dry-Processed Protein Films

3.3.1 Extrusion

Zhang et al. (2001) extruded SPI into sheeting by first blending SPI, water and glycerol (100, 60–90, and 20–50 parts by weight, respectively) in a high-speed mixer, followed by overnight equilibration before compounding in a co-rotating twin-screw extruder (18 mm screw; 30 length/diameter ratio; 150 rpm screw speed; 60–115 °C processing temperature). The extrudate was pelletized and then extruded into 0.35–1.5 mm sheets using a single-screw extruder (100–120 °C die temperature; 120–160 °C barrel temperature; 20–25 rpm). By incorporating two parts of $ZnSO_4$ by weight into the formulation, they reported that water absorption of the extruded sheets decreased by 30%.

Chan et al. (2014) extruded SPI composite films (0.08–0.30 mm in thickness) containing soy cellulose microfibers as a filler. The extruded SPI base film was made up of SPI, water, and glycerol blended at 100, 70 and 50 parts by weight, respectively. The film ingredients were first blended in a mixer for 15 min, followed by allowing the moisture to equilibrate for 12 h before compounding in a single screw extruder (16 mm screw diameter; 24 length/diameter ratio; 100 °C extruder barrel temperature; 120 rpm screw speed) to form extrudate strands which were then pelletized. The pellets were then extruded into film through a slit die using an extruder temperature profile ranging from 120 to 140 °C. The extrudate was cast on chill rollers maintained at 20 °C. When exposed to 43%, 60% and 84% RH, the tensile strength values of the SPI film were 7.5, 5 and 3.8, MPa respectively. Similarly, the Young's modulus values reduced with increasing RH (22, 40 and 65 MPa at 43%, 60% and 84% RH, respectively), although the elongation at break values (~220%) did not change significantly under these RH conditions. Kumar et al. (2010) prepared SPI films by using a two-step process. They first extruded the dry ingredient (85% SPI, 15% glycerol; all dry basis) in a twin-screw co-rotating extruder with screws of 25 mm diameter and 20 length to diameter ratio (L/D). The extrudate was dried in an oven at 50 °C for 48 h and then grinded into powder for solvent casting. In the second step, they dispersed the powders (4% w/v) in deionized water at room temperature and then adjusted to pH 7.5 or 9.0. The suspension was heated to 95 °C for 20 min, cooled, cast on a surface, and dried for 48 h to form films. They reported that SPI films prepared at pH 9 had stronger mechanical properties.

Rouilly et al. (2006) extruded sunflower protein isolate (SFPI) obtained from alkaline extraction and isolelectric point precipitation using sulphuric acid. SFPI, glycerol, and water at blend proportions of 100, 10–70 and 10–50 parts (by weight), respectively were mixer and allowed to condition for 12 h at 25 °C. The blends were then extruded using a single screw extrude (19 mm screw diameter; 25 L/D ratio; 1.8 compression ratio) through a slit die (0.05–1 mm thickness gaps) using die temperatures ranging from 85 to 160 °C using 20 or 200 rpm screw rotation speed. They reported that at 160 °C die temperature, 70% (w/w of SFPI) glycerol content, and

20% (w/w of SFPI) water content, the most coherent and smoothest film was obtained. The resulting optimal film had tensile strength, Young's modulus, and elongation at break values of 3.2 MPa, 17.7 MPa, and 73%, respectively. The extruded film swelled by about 186% w/w in water but resisted solubilization.

Instead of using a typical compounding technique wherein dry ingredients are mixed in a blender, Wang and Padua developed an altermnate method to prepare zein resin suitable for blown extrusion of zein films (Wang and Padua 2003, 2004). They dispersed zein, oleic acid (70% w/w of zein), and distilled monoglycerides (as an emulsifier; 5% w/w of zein) in 75% aqueous ethanol at 60–70 °C and stirred for 10 min. The solution was poured into ice-water mixture to form precipitates. The precipitates were collected and kneaded into a cohesive mass of resin for subsequent extrusion through a single-screw extruder for 3–4 passes, at room temperature, to produce an extrudate. In the final step, the extrudate was extrusion blown into film in an extruder fitted with an annular die and blowing air at relatively low extrusion temperatures (25–35 °C extruder zones; 45 °C die zone) as compared to a typical thermoplastic melt processing. After air drying at room temperature, glossy flexible films of 0.15–0.25 mm in thickness were formed. From their moisture sorption isotherms analyses, the monolayer moisture content estimated correlated closely with the monolayer value calculated from the number of polar amino acids present in zein surface. As expected, the WVP value of the extrusion blown films increased with increasing RH, although the moisture sensitivity of the oleic-acid plasticized zein film was lower as compared to that of films prepared with other hygroscopic plasticizers (Wang and Padua 2004). Oleic-acid plasticized zein films prepared using this method exhibited necking phenomenon during tensile deformation, indicative of the considerable plastic behavior of the zein films (Wang and Padua 2003). In a follow up study, the same research group employed wide-angle X-ray scattering to analyze the extrusion blown films. They observed d-spacings at 4.5 and 10Å attributed to the presence of α-helix backbone (processing-insensitive) and inter-helix packing (processing-dependent), respectively. Small angle X-ray scattering (SAXS) showed a long-range periodicity for the films, suggesting that the oleic acid and cold precipitation had promoted film structure development (Wang et al. 2005a, b).

3.3.2 Compression Molding

Wheat gluten can compression molded into films when blended with plasticizers. Pommet et al. (2005) investigated a series of plasticizers (water, butan-1-ol, octan-1-ol, triethylcitrate, tributhylcitrate, propan-1-ol, 1,8-octanediol, urea, di-/tri-ethanolamine, ethanol, glycerol, 1,4-butanediol, adipic acid, sebacic acid, citric acid, lactic acid, and octanoic acid), selected based on functional groups (alcohols, acids, esters, and amine) and hydrophobicity (different chain lengths), for gluten films prepared using compression molding. Dry or hydrated glutens, with 1.3% and 10.6% moisture contents (wet basis), respectively, were blended with the plasticizers (20–30% total weight basis) in a mixer at 60 °C (dry gluten) or 80 °C (hydrated gluten) until maximum torque was reached. The blends were compression-molded at 100 °C for 5 min or 130 °C for 15 min. On the basis of the following three criteria: (1) low melting point; (2) low volatility; and (3) sufficient hydrophilic groups to be compatible with gluten, five plasticizers were deemed to be suitable plasticizers for wheat gluten, i.e., water, glycerol, 1,4-butanediol, lactic acid, and octanoic acid (Pommet et al. 2005). As expected, the tensile properties of the gluten films were plasticizer type-dependent and the degree of cross-linking induced by the different compression molding conditions used. Moreover, the tensile strength was negatively correlated with the plasticizer content.

During compression molding, the condition used during the pre-compression mixing step will affect the subsequent compression process. Proteins, such as wheat gluten, will crosslink to form aggregation tended to occur above denaturation temperature during mixing, although competitive protein de-aggregation will also occur concurrently due to shear. On the other hand, during the compression molding process, the static condition substantially induce aggregation of protein from irreversible aggregation reactions through the formation of disulfide crosslinks. The extent of protein aggregation can be detected from the insoluble fraction of sodium dodecyl sulfate (SDS) treatment. Pommet et al. (2005) reported that after the first mixing step (before compression molding), gluten blends plasticized with water, glycerol or butanediol resulted in substantially higher aggregation values (26.6%, 31.0% and 33.9% SDS-insoluble protein; expressed in % of total proteins) than those of octanoic and lactic acids (1.9% and 7.2% SDS-insoluble protein). After compression molding at 130 °C for 15 min, the aggregate fractions for water, glycerol or butanediol were observed, to 83.4%, 83.7% and 84.7% SDS-insoluble protein), respectively due to protein crosslinking to form 3-dimensional networks. A large increase in the insoluble aggregate of octanoic acid to 69.6% was observed, while a lower insoluble aggregate fraction (31%) was observed for lactic acid, suggesting that the acidic environment might have prevented the gluten aggregation on the basis that sulphydryl/disulphide interchange reaction is not favorable under acidic condition (Morel et al. 2002; Pommet et al. 2005).

Ogale et al. (2000) studied the viscoelastic, thermal, and microstructural properties of compression molded SPI films. Similar to techniques reported in the literature, they premixed SPI with 20%, 30% or 40% of glycerol (w/w of protein) at 62 °C, either by using a roller-type intensive mixer or manually with mortar and pestle. The two varieties of SPI blends were then thermally compressed at 150 °C at 10 MPa for 2 min. Films prepared from the manually mixed protein blends were more brittle than those prepared from the intensively mixed blend, attributable to limited diffusion of glycerol from the bulk into the protein molecules, and as a result, forming local glycerol-rich domains in the film matrices. Chen and Zhang (2005) conducted a detail investigation on the effect of glycerol on compression molded SPI films. In a low-temperature-low-shear mixing (LLM) treatment, SPI and glycerol (10–50% w/w of SPI) were blended in a mortar and then mixed in a kitchen beater for 15 min, and then equilibrated at 25–30 °C for 1 week before extrusion. Alternatively, the resulting mixtures were subjected to additional mixing in a single-screw extruder (19 mm screw; 25:1 L/D; 30 screw rpm; 80–120 °C feed to exit zone temperature profile) for four times. They called this latter process as high-temperature-high-shear (HHM) treatment. The blends from LLM and HHM treatments were then compression molded at 140 °C at 20 MPa for 10 min, followed by in-mold cooling at 3 °C/min to 50 °C before demolding. From differential scanning calorimetry (DSC) analysis, two T_g's were observed for the SPI/glycerol films (Fig. 10.5b); T_{g1} values decreased from −28.5 to −65.2 °C as glycerol increased from 25% to 50%, whereas the T_{g2} values remained stable at about 44 °C, assigned to glycerol- and protein-rich domains, respectively. They proposed that the protein-rich domain is made up of compact protein chains with low compatibility with glycerol, while the glycerol-rich domain is made up of loose protein chains having good compatibility with glycerol. Based on the observation that T_{g1} values for SPI samples prepared from the HHM treatment were higher than those from of the LLM treatment, and that the T_{g2} values were lower for samples prepared from the LLM treatment than those of the HHM treatment, Chen and Zhang (2005) concluded that the HHM process has enhanced the compatibility between SPI and glycerol. At about 25% (w/w) glycerol content, microphase separation was observed, as revealed by T_{g1} transition from the DSC analysis (Fig. 10.5b) and the discontinuity of the mechanical properties (Fig. 10.5a).

Pol et al. (2002) prepared SPI-zein laminated films using a layer-by-layer compression molding method. The soy protein and zein blends were prepared separately: (1) SPI, glycerol, and water at 60:30:10 weight ratio blended in a high-shear mixer for 10 min at 60 °C to form dry free-flow powder; and (2) zein and glycerol at 50:50 weight ratio blended manually to form 1–2 mm granules. The base soy protein blend was compression molded to form the base soy layer at 150 °C with 66 kN of compression force for 2 min. The base soy layer was then laminated on

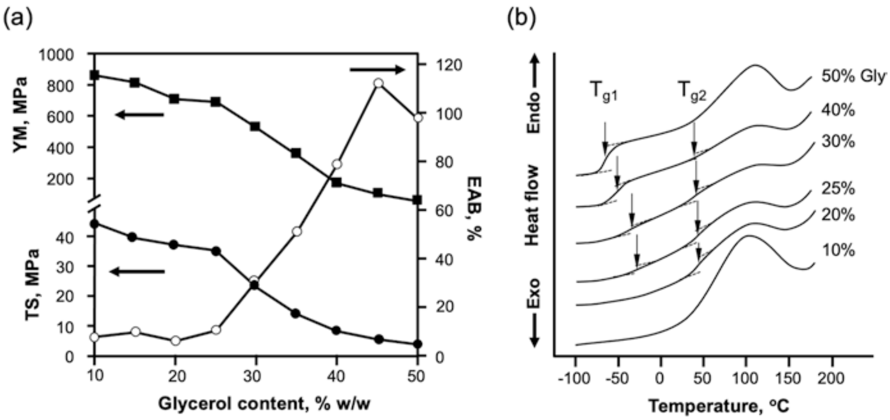

Fig. 10.5 (**a**) Changes in tensile strength, Young's modulus, and elongation-at-break of compression molded SPI film samples, conditions in desiccator for 1 week, before testing at 13 °C. (**b**) Thermograms of SPI film of compression molded SPI films as affected by glycerol content (w/w of SPI), showing the presence of two glass transition temperatures (T_{g1} and T_{g2}). YM Young's modules, TS tensile strength, EAB elongation at break, Gly glycerol. (Adapted from Chen and Zhang 2005)

one or both sides by dispensing the zein blend on the surface to be laminated and compression molded using the same condition except at a lower molding temperature of 125 °C. The relatively hydrophobic nature of the zein layer reduced the WVP values of the laminates, preserving the oxygen barrier properties of the base soy film layer. Tensile properties revealed the ductile behavior of the soy films, but a brittle behavior for the double-coat laminates (Pol et al. 2002).

4 Protein Coatings

For coating application, the protein solution is applied directly to the substrate surfaces by various techniques (e.g., spraying, dipping, enrobing, brushing), followed by allowing the solvent to evaporate under controlled conditions (e.g., natural vs force air convection, elevated temperature, reduced atmospheric pressure) for forming a solid coating. The protein layer must exhibit optimal cohesiveness to prevent cracking and strong adhesion to the edible substrate's surface to prevent delamination. To achieve adequate bonding, the coat-forming solution must be compatible with the substrate's surface to avoid beading during the coating process. The compatibility between the two materials can be evaluated by measuring the surface contact angle of the coating solution on the target substrate surface; low contact angle implies good compatibility and vice versa. In general, thin coating is less susceptible to runoff and drip issues during the form process, thereby forming more robust protein layer than the thick coating.

Another relatively new approach for depositing protein layer onto a substrate is by using a process known as electrospinning. In this electrohydrodynamic method, the protein solution is electrostatically charged at an electrically conductive spinneret, causing the solution to eject towards an electrically grounded target substrate. As the polymer jet takes flight in the air, the evaporation of the solvent, along with the Coulombic repulsion along the polymer jet that induces considerable bending instability, resulting in a continuous solidified ultrafine fiber laid down as nonwoven on the substrate (Fig. 10.6a) (Lim et al. 2019). This laboratory setup can be scale-up to the industrial level using different configurations. One high production throughput variant is called free-surface electrospinning. Here, the protein solution is deposited on the surface of an electrostatically charged wire electrode, from which numerous jets are generated (Fig. 10.6b). Many plant proteins have been successfully electrospun into nonwoven membrane made up of fibers of tunable morphologies with diameter ranging from hundreds of nanometers to several microns in diameter (Fig. 10.7).

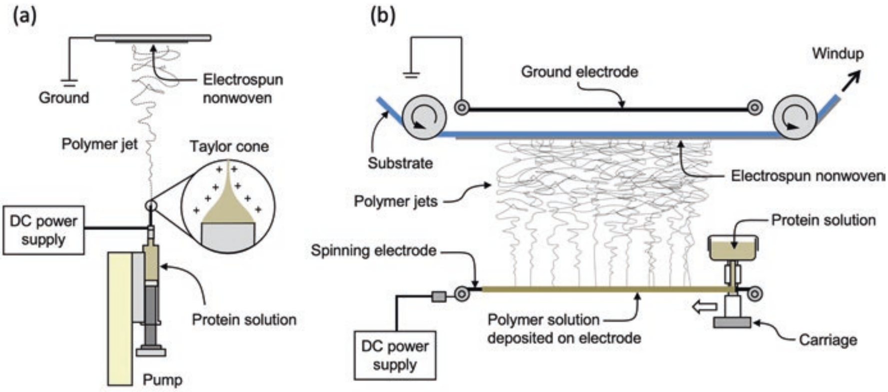

Fig. 10.6 Schematic representations of key components of equipment for electrospinning of protein nonwovens by using: (**a**) the lab-scale single-spinneret; and (**b**) and industrial-scale high throughput free surface techniques. Many variants of electrospinning setup have been developed for the production of electrospun nonwovens which can be found in the literature (Lukas et al. 2008; Zhou et al. 2009; Luo et al. 2012)

4.1 Selected Protein Coating Examples

When applied to the surface of fresh fruits and vegetables, protein coatings can be beneficial to reduce CO_2, O_2 and moisture mass transfer phenomena. Fruits and vegetables have different maximal CO_2 and minimal O_2 tolerances, ranging from 10 to 20% and 3 to 5%, respectively (Robertson 2013). Coatings with optimal permselectivity value ($\beta = CO_2$ permeability / O_2 permeability) and transmission rates that match with the respiration rate of fresh produce can be useful to establish an internal modified atmosphere, thereby delaying senescence (Zagory and Kader 1988). Park et al. (1994b, c) applied zein coating of various thicknesses (5–66 μm) to tomatoes at maturity turning stage. The coating-forming solutions were prepared in 95% aqueous ethanol, along with glycerol and citric acid as plasticizers. They reported that the uncoated control samples turning red in 6 d, while 12 d was observed when the fruits were coated with the zein coating of up to 25 μm. Moreover, the coated fruits substantially delayed the firmness and weight losses during storage. However, at higher coating thickness of 66 μm, the coating interfered with color development of the tomatoes, probably due to substantial reduction in internal O_2 concentration and elevated internal CO_2 concentration to the levels beyond the tolerable levels of the fruit, causing anaerobic fermentation defects.

Baysal et al. (2010) applied zein coating to intermediate moisture apricots by dipping treatment in a 10-month shelf-life study at 5 and 20 °C. The polymer solution (6.75 g zein, 1.9 mL glycerol, 40.6 mL 95% ethanol) was heated for 10–15 min heating at 70–80 °C to form the coating-forming solution. The apricots were dipped in the coating-forming solution for 30 s and allowed to dry for 1 h at 25 °C. Reportedly, the colour changes of the zein-coated samples were significantly reduced as

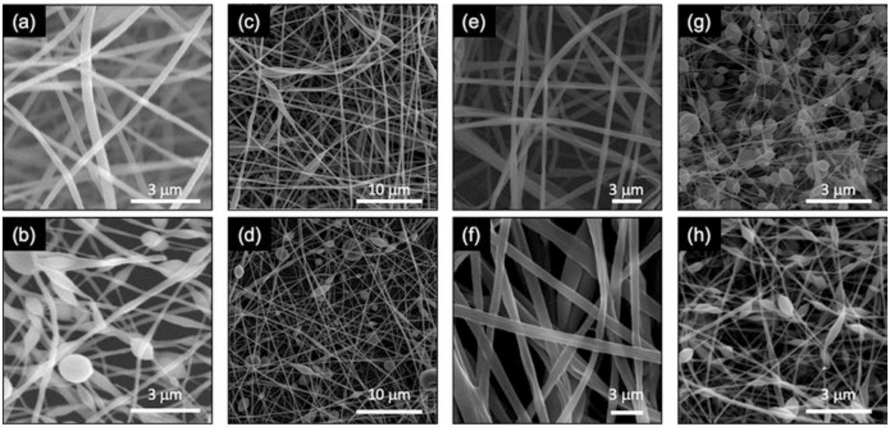

Fig. 10.7 Scanning electron micrographs of selected electrospun protein nonwovens. Micrograph (**a**) shows the morphology of SPI fibers electrospun from SPI solution (15% w/w SPI, 1% w/w Triton X-100, and 0.6% 900 kDa PEO; heated at 60 °C for 2 h) in 1% w/w sodium hydroxide solvent. Micrograph (**b**) shows the fibers electrospun from the SPI solution from (**a**) doped with 5% w/w allyl isothiocyanate, a naturally occurring antimicrobial volatile, showing the effect of the hydrophobic dopant on SPI fiber fibers. Micrographs (**c**) and (**d**) depict electrospun fibers prepared from protein concentrate extracted from microalgae biomass, using a spin dope solution prepared in 1% NaOH and glacial acetic acid solvents, respectively, at the same polymer concentrations (5% w/w microalgae protein concentrate; 0.8% w/w 900 kDa PEO). The bead-less fiber morphologies for the acidic spin dope are indicative of its higher elasticity and/or lower surface tension than the alkaline solvent. Micrograph (**e**) exhibits zein fibers, electrospun from 20% (w/w) zein solution dissolved in 70% w/w aqueous ethanol, showing ribbon morphologies. Overall diameter of the fiber increased substantially when 30% w/w fish oil was incorporated into the spin dope solution (**f**), due to the partitioning of the lipid into the fiber core. Micrograph (**g**) represents electrospun SPI fibers electrospun from 10% (w/w) SPI solution with 0.5% (w/w) 900 kDa PEO. Compared to (**a**), the reduction in protein and PEO concentrations resulted in the formation of beads due to reduced polymer chain entanglements essential to stabilize the polymer jet. Fibers show in micrograph (**h**) are electrospun form the SPI solution similar to that shown in micrograph (**g**), except that the solution was fortified with an anthocyanin-rich raspberry extract (10% w/w) and heated at 60 °C for 2 h. The added anthocyanin reduced the frequency of bead and increased the diameter of the SPI fibers. (Micrographs (**a**)/(**b**), (**e**)/(**f**) and (**g**)/(**h**) are adapted from Vega-Lugo and Lim (2009), Moomand and Lim (2014), and Wang et al. (2013), respectively, all with permission from Elsevier. Micrographs (**c**) and (**d**) are adapted from Verdugo et al. (2014) with permission from Springer)

compared to the uncoated controls. Moreover, the total viable bacteria count was significantly higher than zein film coated samples. In these prolamin-based coating systems, considering that ethanol was used as a solvent which is antimicrobial, it is conceivable that the solvent might have partially contributed to the preservative effect of the fresh produce tested by reducing the initial microbial load – an effect that has not been systematically investigated in the coating literature.

Tanada-Palmu and Grosso (2005) prepared a gluten solution for the coating of strawberries, by mixing 9% (w/v) gluten, 33% ethanol (v/v) and 1.5% glycerol (w/v) in water. The solution was adjusted to pH 10 using ammonium hydroxide, heated at 70 °C and then centrifuged. In addition, a composite coating was prepared by incorporating beeswax (0.45% w/v), 0.27% (w/v) stearic acid, and 0.27% (w/v) palmitic acid into the gluten solution formulation. Strawberries were dip-coated with these coating solutions and then air dried. Alternatively, they tested a bilayer coating by first dipping the fruits in the gluten solution, and then in a molten lipid formulation containing 4.5, 2.7 and 2.7 g of beeswax, stearic acid, and palmitic acid, respectively. Reportedly, all coating adhered well to the fruits. Wheat gluten coatings significantly extended the shelf life of strawberries and retarded fruit senescence as compared with the uncoated control. The addition of lipids to the gluten coatings showed a beneficial effect on firmness retention and reduced weight loss of the strawberries. The fruit with the gluten film also had higher firmness retention compared to the control fruit. All fruits shrank during the 16 d storage at 7–10 °C and 60–80% RH condition, except the fruits coated with the bilayer coating. However, the bilayer coating was opaque/white in appearance, which is not optimal from a consumer acceptance standpoint for this specific product. On the other hand, the neat gluten and composite coatings were transparent, and maintained the visual quality of the fruit during the storage time. Their sensory evaluation showed that the taste of the strawberries with the gluten coating was acceptable to the test panel (Tanada-Palmu and Grosso 2005).

Beside corn zein and wheat gluten, other prolamins have also been exploited as coating-forming polymer. Kafirin, a prolamin from sorghum, has been evaluated by Buchner et al. (2011) for coating of "Packham's Triumph" pears. The coating solution was prepared by dissolving kafirin in 70% (v/v) aqueous ethanol containing 1,2-propanediol (0.72% w/w) and glucono-δ- lactone (0.36% w/w) as plasticizers. The solution was stirred at 70 °C, followed by cooling. The pear fruits were coated by dipping in the coating solution for 5 s and then air-dried for 4 h at 20 °C. Although the coating substantially decreased the respiration rate and retard the progression of senescence of the pears, the researchers reported that the coating did not prevent the formation of pear skin wrinkles due to moisture loss. The incorporation of moisture barrier constituents, such as wax or triglyceride into the coating formulation, potentially could be useful in enhancing the moisture barrier properties of the coating.

By incorporating antimicrobial compounds into the coating formulation, the coating can further inhibit the growth of spoilage microorganism, thereby extending the product shelf-life. González-Estrada et al. (2017) applied limonene-fortified SPI coating in Persian lime to inhibit the growth of blue mould (*Pennicillium italicuum*). The coating-forming solution was prepared by dissolving SPI (20% w/v) in water and heated at 50 °C for 30 min, followed by the addition of glycerol (20% w/w of SPI) and stirred for 30 min. After cooling to 25 °C, limonene was added at 5% or 10% (w/w of dry SPI) levels. The lime fruits, inoculated with spore suspension of *P. italicum*, were dipped in the coating-forming solution for 10 s, and then air dried for 1 h to form surface coating. Overall, the coating did not substantially affect the weight loss of fruits as compared to the uncoated controls, although significant reductions in blue mold incidence, infection wounding, and lesion diameter were observed. Alves et al. (2017) applied SPI coating, fortified with ferulic acid – a naturally occurring antioxidant and cross-linking agent, for the preservation of fresh-cut apples. Their coating formulation was made up of 30 g/L SPI and 9.0 g/L glycerol. The solution was heated at 85 °C for 30 min. Apple slices were dipped in the coating solution for 10 s at room temperature and air dried for 10 min. Results from their shelf-life studies at 10 °C and 50% RH for 7 d showed that the lignin-fortified coating significant reduced weigh loss attributable to the ferulic-acid induced crosslinking of SPI coating. Moreover, the browning of apple was significantly delayed. Although the coating shows strong promises for shelf-life extension of fresh-cuts, the sensory aspects of the coated product must be evaluated for consumer acceptance.

Coatings can be used as a carrier of antioxidants to extend the shelf life of oxidation-sensitive food product, such as ground and tree nuts rich in polyunsaturated fatty acids. Kang et al. (2013) applied SPI-carboxymethylcellulose (CMC) edible coating, fortified with catechin to improve the lipid stability in walnut (*Juglans regia* L.) kernels. The coating solution was prepared by dispersing 7% (w/v) SPI and 3.5% (w/v) glycerol in water heated at 90 °C for 30 min, followed by the addition of CMC (2.5% w/v) to form the final coating-forming solution. To facilitate the incorporation of catechin (as an antioxidant; 0.15% w/v), the polyphenol was pre-dissolved in 70% aqueous ethanol before adding to the coating solution. Walnuts were dipped in coating solution, dried, and stored under accelerated shelf-life condition of 35 °C for 21 d. Their results showed that the catechin fortified SPI and SPI-CMC coatings reduced the peroxide value by 27% and 31%, respectively, while the thiobarbituric acid reactive substance (TBARS) value by 16% and 26%, respectively, as compared to uncoated walnut. To develop innovative use of walnut oil cake residue that is rich in proteins, Grosso et al. (2020) extracted walnut phenolics as a source of antioxidant and walnut flour (~49% protein content) from the walnut oilcake, a walnut oil industry by-product, to produce walnut protein coating to delay oxidative deterioration walnut kernels. The protein-rich walnut flour was prepared by defatting the oilcake with n-hexane, followed by ethanol/water (70:30) extraction to remove soluble carbohydrate. To prepare the coating, the walnut flour (6% w/v) was dispersed in water heated at 70 °C for 1 h. The solution was then adjusted to pH 9 and 10% (w/w of walnut flour) glycerol was added. The solution was centrifuged to remove suspended particles to obtain the final coating solution. The phenolic fraction of the walnut oilcake was extracted by evaporating the solvents of the ethanol-water (70:30 v/v) fraction, followed by purification through solvent partitioning using distilled water and ethyl acetate. The ethyl acetate-soluble fraction was subjected to rotatory vacuum evaporation treatment at a 40 °C to remove the ethyl acetate solvent, to yield the ethyl acetate-soluble polyphenols (EAP). EAP was added to the walnut protein solution at 0.1% w/w level. The walnut kernels were spray coated at 2.5% w/v coating concentration. On the 84 d of storage day, the coated samples with EAP had a lower peroxide (3.64 meq O_2/kg oil) and anisidine value (1.11), conjugated diene (15.92), and hexanal content (19.67×10^6 e.c.) than the control sample (6.23 meq O2/kg oil, 1.81, 24.65, and 122.37×10^6 e.c., respectively). Regarding consumer acceptance, the phenolic-added sample displayed a higher flavor acceptance score than the control and other treatments.

4.2 Electrospun Protein-Based Nonwovens

Among the electrospun protein fibers, zein is probably the subject of most intensive investigation (See Sect. 6). Electrospun zein fibers prepared from binary solvents consisting of a volatile and a less volatile component tend to exhibit ribbon morphologies, such as zein fibers electrospun from zein solution prepared in aqueous ethanol solvent (Moomand and Lim 2015). During the solvent evaporation, preferential evaporation of ethanol over water and diffusion of solvent into the skin are main drivers that promote the formation of a skin layer. As the core material is being depleted, the tube implodes inwards, thereby flattening the circular cross section of the fibers to form a ribbon. As the collapse continues, electrical charges tend to flow to the ribbon edges, producing a lateral force that flattens the fibers (Koombhongse et al. 2001; Arinstein and Zussman 2011). The relatively hydrophobic characteristic of zein is desirable for the encapsulation and controlled release of bioactives in aqueous environment. For example, Moomand and Lim (2014) encapsulated omega-3 fatty acids rich fish oil in electrospun zein fiber of up to 30% (w/w of zein) loading capacity at the encapsulation efficiency greater than 90%. Using aqueous ethanol as a solvent, the fish oil was mainly partitioned in the core of zein fibers, as revealed by transmission electron microscopy (TEM). The encapsulated fish oil exhibited a higher oxidative stability than the un-encapsulated counterpart, as reflected by the lower peroxide and p-anisidine values for zein-encapsulated fish oil than the free counterpart (Moomand and Lim 2014).

Electrospun zein fibers have been explored by researchers as carriers for various nutraceutical and bioactive compounds. β-Carotene, a food grade colorant and antioxidant, has been encapsulated in electrospun zein fibers of micron and submicron in diameter, resulting in a significant increase in stability when exposed to UV–vis irradiation (Fernandez et al. 2009). α-Tocopherol was entrapped within electrospun composite zein fibers (zein: PEO: tocopherol ratio: 6:5:3 w/w) with and without the addition of a soluble rice bran dietary fiber (3% w/w) (Li et al. 2016a). The solvent used was 75 wt.% ethanol in water containing Tween 80 (0.5 wt.%). The addition of the dietary fiber retained a higher amount of α-tocopherol after the exposure to heat and UV radiation than α-tocopherol encapsulated in zein fibers without the fiber fortification. In another study, α-tocopherol was encapsulated in electrospun zein:PEO:chitosan (ratio: 87.5:10:2.5 w/w) fibers with an average diameter of 450 nm, at 20 wt.% loading (Wongsasulak et al. 2014). The inclusion of α-tocopherol did not affect the fiber morphology but enhance the mucoadhesion properties of the fiber matrix. The release of α-tocopherol in simulated gastric fluid at the pH level of 1.2, in the presence of pepsin (simulate digestion), was triggered by matrix erosion. Whereas at the pH level of 2 without pepsin (simulate fasting), it was driven by swelling and diffusion of the fiber matrices. Antunes et al. (2017) prepared cyclodextrin-eucalyptus essential oil inclusion complex by a co-precipitation technique and then added to zein polymer solution prepared using aqueous 70% ethanol as a solvent. At the zein concentration of 30% (w/v) and an inclusion complex loading of 24% (w/v), the electrospun zein fibers resulted in 29% and 24% reductions in

Gram positive *Listeria monocytogenes* and *Staphylococcus aureus*, respectively. Whereas minimal inhibition effects were observed for Gram negative *Escherichia coli* and *Salmonella Typhimurium* (Antunes et al. 2017). Göksen et al. (2020) loaded 1,8 cineole-rich essential oils extracted from bay (*Laurus nobilis*) and rosemary (*Rosmarinus officinalis*) spices in electrospun zein fiber by dissolving 25% (w/v) zein in a binary solvent consisting of glacial acetic acid and ethanol (30:70 v/v). The bay or rosemary essential oils were added up to 10% (w/w of zein) into the polymer solution and stirred for 12 h. The resulting solutions were electrospun into nonwovens and applied as surface coating of cheese slices inoculated with *L. monocytogenes* and *S. aureus*. Significant reductions (~2-log) of *L. monocytogenes* and *S. aureus* were observed as compared to the uncoated control samples on after 28 d of storage at 4 °C. When the same solutions were solvent-cast as films and applied to the cheese samples, a weakening of antimicrobial efficacy against aerobic mesophilic bacteria was observed, as compared with samples coated with the electrospun zein fibers. The researchers postulated that the electrospun carrier provided a more sustained release characteristic over time than the film carrier. These observations suggested that the mass transfer phenomena of the essential oil are different in solvent-cast zein film and electrospun zein fibers.

Besides these hydrophobic bioactives, electrospun zein fibers have been explored as carriers for other hydrophilic compounds. Neo et al. (2013) encapsulated gallic acid in electrospun zein fibers with an encapsulation efficiency of nearly 100% due to strong interaction between gallic acid and zein, as revealed by calorimetry and FTIR analyses. Furthermore, the electrospinning process did not affect the antioxidant activity of the phenolic acid (Neo et al. 2013). Li et al. (2009) dispersed EGCG from tea at a polymer concentration of 20% (w/w) in an aqueous ethanol solvent, and electrospun the spin dope into continuous fibers. The zein fibers resisted the solubilization in water despite apparent swelling and plasticization after water treatment. Aging of EGCG-loaded fibers at 0% RH for at least 1 d resulted in >98% EGCG recovery. FTIR analysis revealed that drying and aging treatment caused detectable changes in protein secondary structures that enhanced the EGCG retention within the fiber matrix (Li et al. 2009).

Wheat glutenin is insoluble in water because of intramolecular disulfide bridges. Using alkaline and reductive condition (pH 10.5 and 8 M urea), Xu et al. (2014) disrupted the disulfide bridges in glutenin, followed by neutralization and washing. The alkaline treatment promoted thiol-exchange reaction while the 8 M urea solution disrupted inter- and intramolecular hydrogen bonds. A transparent wheat gluten spin dope solution was prepared by heating for 1 h in 0.3 M sodium carbonate-bicarbonate buffer. The nonwovens produced from the spin dope solution were stable in PBS buffer for up to 35 d, suggesting that they may be used as a bioactive carrier that requires long-term stability in aqueous environment and biomedical applications such tissue scaffolds (Xu et al. 2014).

Several isolates and concentrates derived from plants have been successfully electrospun into submicron fibers by researchers. Similar to solvent casting, SPI must be denatured before electrospinning. Moreover, SPI alone cannot be electrospun readily into fiber; the incorporation of a spinning aid polymer is common to facilitate the electrospinning process. For example, Vega Lugo and Lim (2008) applied a combined alkaline (1% w/w NaOH) and heat (60 °C, 2 h) treatment to prepare SPI solution (10% w/w), doped with 0.8% (w/w) PEO (900 kDa), which could be electrospun into nonwoven made up of fibers with diameters of 200–260 nm. Using a similar formulation, Wang et al. (2013) exploited electrospun SPI fibers to encapsulate an anthocyanin-rich red raspberry (*Rubus strigosus*) extract. The resulting nonwoven exhibited antibacterial activity against *Staphylococcus epidermidis*, suggesting that the bioactive nonwoven could be used for the delivery of antioxidants and antimicrobials in food. Similarly, Salas et al. (2014) electrospun soy glycinin and SPI solutions prepared in 0.1 N NaOH containing 10% (v/v) acetonitrile. The protein solutions were fortified with different amounts of lignin (22–78% w/w of protein), keeping the total protein/lignin concentration at 8% (w/w) level. PEO (400 kDa) was added at 10% (w/w of SPI) to facilitate the electrospinning process. The resulting soy composite fibers ranged from 124 to 400 nm in diameter. Based on FTIR analysis, they reported an increase in hydrogen bonding and loss of secondary structure of the proteins as the lignin concentration increased. The unfolding of the proteins and increase interaction with lignin favored the electrospinning process.

Botryococcus braunii microalgae residual biomass – a by-product from omega-3 fatty acids extraction, is rich in protein. Protein concentrate derived from the microalgae biomass was electrospun by Verdugo et al. (2014) into fibers with average diameters ranging from 192 to 770 nm, depending on the solvents used (distilled water, aqueous sodium hydroxide 1% solution, or glacial acetic acid). Regardless of the solvents tested, the incorporation of PEO as a spinning aid was needed to establish a stable electrospinning process (Verdugo et al. 2014). Similarly, Moreira et al. (2018) electrospun protein concentrate (81 wt.% protein content) extracted from spirulina microalga using a free surface wire electrospinning technique. Uniform fibers were obtained at protein concentrations of 5–10 wt.% with average diameters ranging from 118 to 452 nm when aqueous acetic acid was used as a solvent. On the other hand, 1 wt.% NaOH solvent tended to form spherical beads and beaded fibers. Like the previous studies, the additional of trace amount of PEO (0.5 wt.%) was needed to enable the electrospinning process (Moreira et al. 2018). The electrospun fibers from protein concentrate of *Spirulina sp*. have been exploited by Moreira et al. (2019) as a carrier for phycocyanin, an antioxidant extracted from microalgae biomass. The electrospun fibers increased the thermal stability of phycocyanin, while preserving its antioxidant properties.

Aceituno-Medina et al. (2014, 2015) encapsulated folic acid, ferulic acid, and quercetin in electrospun fibers of amaranth protein isolate (API) from *Amaranthus hypochondriacus* grain, by blending the isolate with pullulan. The encapsulated bioactives had increased thermal and UV stability. Similarly, Blanco-Padilla et al. (2015) encapsulated curcumin in electrospun fibers consisting of API blended with pullulan at 1:1 ratio (w/w). The fibers, with average diameters of 225–249 nm, provided curcumin encapsulation efficiencies of 73–93%. They reported a higher antioxidant activity for the encapsulated curcumin than the free curcumin during *in* *vitro* digestion, probably due to an increase in surface area of curcumin when it was dispersed in the electrospun fiber matrix (Blanco-Padilla et al. 2015).

5 Modification of Protein Film/Coating

5.1 Polymer Blending

Blending with a compatible polymer is a common approach to enhance the material properties of proteins (Doublier et al. 2000; Klein et al. 2010; Rodríguez and Pilosof 2011). To increase moisture barrier of protein films, one of the common strategies is by incorporating another polymer that is relatively more hydrophobic (e.g., wheat gluten and zein) into the film-forming solution (Were et al. 1999). Tsai and Weng (2019) exploited the hydrophobic properties of zein prolamin and oxygen/mechanical properties of whey protein isolate (WPI) to develop blended protein films, by adding zein suspension (3% w/v in water, pH 11.2) to WPI solution (3% w/v in water at pH 11.2), followed by spraying drying to form powders. The powders were subsequently dissolved in an aqueous ethanol solvent containing glycerol and heated at 80 °C for 15 min to form a uniform solution, which was then casted on a surface and dried at 50 °C. A reduction in water vapor permeability was observed in films prepared with 75% and 25% aqueous ethanol solvents, with brightness and white index of films decreased as the ethanol concentration in film forming solution increased. Increasing WPI content increased elongation-at-break, while increasing ethanol concentration had an opposite effect on film extensibility. The researchers reported an interesting observation that is noteworthy here; while zein is not water-soluble and WPI film has poor heat-sealability, the resulting zein-WPI composite films were both water soluble and heat-sealable. The unique materials properties may be related to the additional spray drying step, which is atypical of solvent casting techniques reported in the literature (Tsai and Weng 2019).

Polymer blending has been explored by researchers to modify the mechanical properties of SPI film, including the incorporation of synthetic polymers and biopolymers. For example, Ghorpade et al. (1995) incorporated PEO to SPI. They observed an increase in elongation at break of the solvent cast film from 89 to 159%, while a decrease in tensile strength from 3.9 to 1.4 MPa, as the PEO content increased from 0% to 40% (w/w of SPI). Han et al. (2015) blended SPI (7% w/v in water) with CMC (7% w/w of SPI) to produce a composite film, using glycerol as a plasticizer (50% w/w of SPI). The polymer solution was adjusted to pH 8 by using 1 N NaOH and then heated at 75 °C for 15 min before casting and dried at 25 °C, 50% RH for 24 h. The SPI-CMC film had higher water solubility and tensile strength, but lower water vapor permeability and percentage elongation, than the SPI film. However, the oxygen permeability values between the pristine SPI and SPI/CMC blend films were comparable. Chinma et al. (2012) blended soy protein concentrate (SPC) with cassava starch to form edible film by casting alkaline and thermally treated film-forming solutions (pH 10; heated at 90 °C for 5 min) with glycerol as a plasticizer. The tensile strength values of the resulting films were higher than those of low density polyethylene and comparable to high density polyethylene films. They concluded that at 90:10 cassava starch:SPC blend ratio, the low mechanical and high WVP film could be useful for coating applications of fresh produce for controlling product respiration rate. On the other hand, at 50:50 cassava starch:SPC blend ratio, the film had stronger mechanical properties and low WVP value, which are more suited for textured foods where improved mechanical strength is desirable. Bai et al. (2013) prepared SPI-gelatin blend films by solvent casting method. They prepared 5% (w/w) film forming solutions in water at 50 °C for 10 h, at different protein blend ratios. They observed that the composite film with 30% gelatin exhibited more uniform microstructural morphologies, as observed under scanning electron microscope, than blends prepared at other polymer ratios, indicating good compatibility between the two proteins at 30% (w/w) gelatin weight ratio. Moreover, the composite film containing 30% (w/w) gelatin had the most optimal barrier and mechanical properties, which correlated with the lower absorbance at 1538 cm^{-1} on FTIR spectrum as compared with neat SPI and pure gelatin films, suggesting the presence of SPI-gelatin interactions at the –NH groups of the polypeptide chains.

During dry extrusion, blending protein with a compatible polysaccharide can facilitate the film forming process. Guerrero et al. (2014) observed that incorporating polysaccharides (gum Arabic, dextran, or carboxymethylcellulose) at 6–9% (w/w) levels reduced friction and facilitated the extrusion of thermoplastic SPI through the extrusion die. A decrease in extrusion torque was observed. Their FTIR analysis revealed secondary conformational changes in the protein, suggesting that protein-polysaccharide interactions had occurred following the extrusion process (Guerero et al. 2014).

5.2 Multilayer Films

Multilayer film structures are those made up of more than two distinctive layers of polymers. The motivative of multilayer film is to leverage the unique material properties of each of the polymer to develop a composite film with barrier properties that cannot be achieved with a single-polymer alone.

Similar to nylon polymers, protein films are excellent oxygen barriers when they are dry, but the barrier properties tend to deteriorate when exposed to elevated humidity due to plasticization effect of the absorbed moisture (Robertson 2013; Lim et al. 1998b, 1999; Zhang, Lim and Tung, 2001). To exploit the oxygen barrier properties of protein film, the protein core layer can be sandwiched between outer layers of polymers that are relatively hydrophobic – a common strategy applied in high barrier food packaging film/sheeting structures. While this approach is useful to prevent the exposure of the protein core layer to environmental moisture (and hence to preserve its oxygen barrier properties), a strategy to compatibilize the protein layer with the hydrophobic substrate layer is essential to prevent layers delamination. To this end, Chang et al. (2019) prepared a PPI solution by dispersing 40 g of PPI protein in 450 mL of water with sorbitol (40 g) added as a plasticizer. NaOH (1 N, 10 mL) was added and then heated at 90 °C for 30 min. The coating solution was then applied to a corona discharge treated poly(ethylene terephthalate) (PET) film (12 μm thickness) using a roll-to-roll coating process, forming a 50 μm thick PPI coating. While corona treatment increased the surface energy of the synthetic substrate, further application of a primer layer, such as ethylene-acrylic acid, to the synthetic substrate was needed before applying the protein coating solution (Joo et al. 2018). The bilayer structure was then bonded with a nylon film (15 μm), and then finally polypropylene (PP) film (70 μm), resulting in a multilayer structure of PET/PPI/nylon/PP. The bonding between the PPI, nylon and PP layers were achieved using a liquid adhesive (100:103:15 urethane:ethyl acetate:aromatic polyisocyanate). Compared to the protein-free control (i.e., PET/nylon/PP), they observed significant reductions in oxygen and water vapor the transmission rates for the PET/PPI/nylon/PP structure, from 46.6 to 0.048 cc/m^2.d.atm and 4.22 to 2.81 g/m^2.d.atm, respectively. In the same study, they also reported considerable enhancements of barrier properties of multiple films structures laminated with whey protein isolate core layer.

Based on a similar concept, Salgado et al. (2021) attempted to improve the water barrier properties of SPI film by coating it with a layer of poly(3-hydroxybutyrate) (PHB), which is a sustainable/biodegradable polyhydroxyalkanoate derived from bacteria. The pre-formed SPI film was solvent-cast using a typical alkaline and heating treatment (pH 10.5; 60 °C for 3 h). The dried SPI film was then coated with PHB solutions prepared in chloroform solvent at different concentrations and allowed the solvent to evaporate at 15 °C. The WVP values of the PHB-coated SPI bilayer films were significantly lower ($3–4 \times 10^{-11}$ g.m/m^2.s.Pa) than that of the neat SPI film (1.1×10^{-10} g.m/m^2.s.Pa).

Although these results highlighted the potential of protein film/coating to substitute the synthetic oxygen barrier, the procedures reported above required a degassing step to remove the organic solvents from the polymer/primer/adhesive layers. The degassing step will become the production throughput bottleneck in the lamination process. Continuous co-extrusion of different polymers could be more conducive for commercial production scale-up than the solvent-based lamination process. Alternatively, bonding of multiple layers may be carried out through compression above the melting point of the polymers, followed by cooling. Fabra et al. (2013) and Busolo et al. (2009) deposited a thin layer of electrospun zein nonwoven onto biodegradable polyhydroxybutyrate-co-valerate (PHBV) and poly(lactic acid) (PLA) films, respectively, followed by hot pressing the same polymer films over the zein layer at 150 °C at 8000 psi for 2 min without the use of a binder layer. These researchers postulated that the submicron scale zein fibers in the nonwoven strengthened the interaction between the PHBV nonwoven and the PLA matrix, resulting in strong bonding at the interfaces of the polymer layers. As a result, significant reductions of WVP, limonene permeability, and oxygen permeability values by as high as one order of magnitude were observed.

5.3 Filler-Based Composites

Composites are referring to materials that are made up of two or more components with different characteristics uniformly dispersed into a continuous matrix. Composites are produced to take advantage of the properties of the constituting components to achieve ultimate material properties that cannot be obtained by conventional materials (Knight and Curliss 2002; Friedrich et al. 2005). Organic or inorganic fillers have been incorporated into protein films to produce composite of enhanced material properties, by exploiting the high strength and stiffness of the fillers. Selected fillers are discussed in this section.

Chan et al. (2014) incorporated extruded SPI composite films (0.08–0.3 mm thick) containing soy cellulose microfibers (SMF) isolated from soy pods and soy stems using a chemo-mechanical method (alkaline/acidic treatment followed by high pressure homogenization). From SEM analysis, homogenous composite films with uniform distribution of SMF were obtained up to 0.5% (w/w of SPI) level. At 1% (w/w) SMF loading and above, aggregates were observed with 2.5% (w/w) being the most prevalent. Micrographs from the cryo-fractured surfaces of the composite films did not show pulled out fibers and voids, suggesting strong fiber-SPI interfacial adhesion. At the optimal concentration of 0.25% (w/w) SMF loading, the composite films exhibited improved mechanical performance at elevated relative humidity (84%) when compared to the pristine SPI films. Using the same SMF extraction method and SPI, Jensen et al. (2015) prepared SPI-SMF composite films by a solvent casting method involving a heated casting glass plate. Among the composite films tested, the 5% (w/w of SPI) SMP loading exhibited significant enhancements in tensile strength and Young's modulus but decreased EAB as compared to the neat SPI film. Similarly, González and Igarzabal (2015) reinforced solvent cast SPI films, plasticized with 50% (w/w of SPI), by incorporating starch nanocrystal (SNC; up to 40% w/w of SPI; average particle size 35 nm;) in the film-forming solution. The cast films were homogeneous and yellowish in appearance, with increasing optical opacity as the SNC content increased, indicative of nanoparticles aggregation. A marginal but significant decrease in WVP value was observed at 40% SNC loading (3.57 g.m/Pa.s.m^2) as compared with the neat SPI film (4.3 g.m/Pa.s.m^2). The tensile strength and Young's module values increased with increasing SNC content, but a significant decrease in the elongation at break value was observed, especially above 5% loading levels, suggesting a decreased in film flexibility.

Montmorillonite (MMT) layered silicates have been studied extensively by researchers. The crystalline structure of MMT is made up of two fused silica tetrahedral sheets sandwiched with an edge-shared octahedral sheet of aluminum, iron, magnesium, or lithium hydroxides. The thickness of a single layer is about 1 nm, while the lateral dimension of the crystals can range from 30 nm to several microns. The crystalline layers are stacked regularly to provide van der Waals gaps, known as galleries. The silicate layers can be delaminated and dispersed into a polymer to give individual platelets impermeable to gases of about 1 nm in thickness (Ray and Okamoto 2003; Zeng et al. 2005). Composite materials with MMT have been developed to improve mechanical and barrier properties of protein films. For example, Dean and Yu (2005) applied ultrasonic treatment to dispersed unmodified Na-montmorillonite (Na-MMT; Cloisite Na^{+}) in water and glycerol to exfoliate the layered silicate, for the preparation of soy-protein based nanocomposite films. Enhancements of elastic modulus and tensile strength by 84% and 47% were observed compared to the neat film, but a decrease in elongation-at-break was observed. The enhanced material properties of nanocomposite films can be attributed to the presence of silicate platelets with large aspect ratios that increase the tortuous path of the permeant molecules as they diffuse through the polymer matrix (Rhim and Ng 2007). Kumar et al. (2010) prepared SPI-MMT nanocomposite films by using a combined melt extrusion and solvent casting process. The dry ingredients (70–85% SPI, 15% glycerol, 0–15% MMT; all dry basis) were first extruded in a twin-screw co-rotating extruder at barrel temperatures ranging from 70 to 130 °C. The extrudate was dried, ground into powder, and then dispersed in water at pH 7.5 or 9.0. The resulting film-forming solutions were heated to 95 °C for 20 min, cooled, and then solvent-cast into nanocomposite films. They reported that significant enhancements of tensile strength and water barrier properties were observed as the filler content increased. With the SPI-MMT powder prepared with extruder temperature ranging from 70 to 110 °C, and screw rotation speed (100 rpm), the tensile strength values of the resulting SPI-MMT films with 0, 5, 10 and 15% MMT loadings were 2.26, 6.28, 12.62 and 15.6 MPa, respectively. The WVP values of SPI-MMT films at 0, 5, 10 and 15% MMT loading were 3.8, 2.96, 2.49 and 2.17 g.mm/m^2.h.kPa, respectively. However, the elongation at break value increased from 11.85 to 64.6% as the MMT increased from 0 to 5% MMT loading level, but deceased to 23.98% and 17.8%, respectively, as the MMT loading inceased further to 10 and 15% levels. This observation suggests that there is an optimal MMT loading for the soy composite films.

Researchers have explored the use of metal oxide particles as fillers to reinforce protein films. To enhance the interaction between silicon oxide (SiO_x; 20–60 nm) nanoparticles with SPI, Liu et al. (2017) modified the surface of the nanoparticles by ultrasonic treatment and a counter-ion activation method. The former involved sonication of the nanoparticles (2% w/v) in water dosed with 10–40% (w/w of SiO_x) sodium dodecyl sulfonate, while the latter involved sonication of the SiO_x particles (2% w/v) in the presence of $CaCl_2$ (100% w/w of SiO_x), followed by the addition of sodium dodecyl sulfonate (10–40% w/w of SiO_x). The surface modified SiO_x was centrifuged, filtered, and dried to constant weight at 120 °C. The particles were then added at 0.1–0.5% loading to the SPI solution (5% w/v SPI, 2.5 w/v glycerol), heated at 80 °C for 20 min, followed by cooling. Red Fuji apples were coated with the solutions and evaluated for up to 7 weeks at room temperature. The $CaCl_2$ pre-activation method resulted in uniform dispersion of nano-SiOx particles. They reported that the optimal composite film at 10% SPI concentration, 0.3% nano-SiOx loading, and coating time of 60 s resulted in the delay of the climacteric peak of apples to the fifth week and had better physiological indices than the uncoated control group. The approach is promising for coating formulation to preserve fresh fruits.

Following a process previously developed by Sevilla and Fuertes (2009), Li et al. (2016a, b) synthetized carbon nanoparticles of average sizes ranging from 6 to 83 nm using a one-step hydrothermal carbonation process (150–180 °C; 20 min to

7 h) of precursor solutions containing glucose and poly(vinyl alcohol) dissolved in water. At 125% (w/w; dry basis of SPI) loading level, the tensile strength and elongation of the composite films, prepared by a solvent casting method at pH 9, were increased by 83% and 80%, respectively, while 48% decrease in WVP value was observed as compared to the neat SPI film. However, such composite film will likely present discoloration issues, which has not been addressed by the researchers. Recently, Wu et al. (2021) exploited diatomite of an average particle size of 29 μm as a carrier of thymol and incorporated into SPI to develop antimicrobial protein films using solvent casting method at pH 9–10. Diatomite is a naturally occurring mineral made up of fossilized skeletons of diatoms of high porosity (up to 80%) with pore sizes ranging from 50 to 200 nm. Their thermogravimetric analysis revealed that thymol was adsorbed into the diatomite pores at about 39% loading capacity. The incorporation of diatomite reduced thymol evaporative losses during the film formation and storage, thereby prolonging the antimicrobial effect of the composite film against *E. coli* for up to 100 h.

The hydrophilic nature of protein films presents a major challenge for many food applications wherein moisture barrier properties are important. To enhance the moisture barrier of protein films, composite structures with dispersed lipid phase have been developed by researchers. One approach is based on dispersing liquid lipid in the protein film-forming solution through high energy emulsification to prepare oil-in-water emulsions, which are then cast and dry to form the final composite films. With this approach, Rocca-Smith et al. (2016) investigated the effect of lipid on the function properties of wheat gluten films, prepared from the solvent casting method. They observed the dispersed lipid phase significantly decreased the equilibrium moisture content and water transmission transfer rate as compared with the unmodified wheat gluten films. Wang et al. (2014) utilized ultrasound- and microwave- assisted processes to prepare SPI containing up to 2% oleic acid and 2% steric acid with significant reduction in WVP and increased water surface contact angle values. Guerrero et al. (2011) incorporated epoxydized soybean oil and olive oil into SPI film, incorporated with 30% (w/w) glycerol and 15% gelatin by weight on dry SPI basis. However, their results did not show substantial decreases in WVP, nor increases in contact angle values. The disagreement could be due to the compression molding method (150 °C, 12 MPa, 2 min) adopted by Guerrero et al. (2011) during film-forming, whereas Wang et al. (2014) implemented the solvent casting method at 25 °C for 12 h, which might have resulted in different extent of phase separation of the lipid in the protein matrix.

5.4 *Irradiation Induced Cross-Linking*

Edible films should have adequate mechanical strength and flexibility to maintain integrity and withstand external stresses that occur during processing, handling, and storage. The mechanical strength of the protein film can be modified by high energy irradiation [ultraviolet (UV) and gamma (γ)] treatment. Moreover, irradiation may exhibit synergistic effects with coating in terms of enhancing the microbial stability of perishable food products since these radiations, applied on continuous or pulsed bases, can generate destroy the spoilage and pathogenic microorganisms (Pirozzi et al. 2020).

The effects of UV treatment on protein film have been well-documented in the literature. Gennadios et al. (1998) reported that tensile strength of SPI films treated with UV irradiation treatment (103.7 J/m^2; 0.6 mW/m^2 for 48 h) increased from 3.7 to 6.1 MPa. Tensile strength and elongation-at-break increased and decreased linearly, respectively, with increasing UV irradiation dosage up to 103.7 J/m^2, implying that the irradiated films were stronger albeit became more brittle. The efficiency of UV radiation on the mechanical properties of film depends on the amino acid composition and the molecular structure of the protein. For instance, a UV irradiation of 0.0104 J/cm^2 increased the tensile strength (65%) and decreased elongation (31%) of soy protein film, but minimal effects were observed for wheat gluten and pea protein films (Micard et al. 2000; Gueguen et al. 1998). It has been hypothesized that the aromatic amino acid residues, i.e., tyrosine and phenylalanine in soy protein might have contributed to the formation of cross-links when protein film was exposed to UV radiation.

Besides UV irradiation, researchers also employed γ-radiation to induce cross-linking in protein films. Ionizing γ-radiation affects proteins by causing conformational changes, oxidation of amino acid, formation of free radical, breakage/recombination of covalent bonds, and polymerization reactions (Cheftel et al. 1985). At a dosage range of 5–30 kGy, γ-radiation did not affect the tensile strength of soy protein film, but an increase in the tensile strength was observed for SPI-PEO composite film (52). SPI and 1:1 SPI:WPI films were crosslinked by combined γ-irradiation and thermal treatments, significantly enhanced the puncture strength and deformation. The incorporation of CMC into the film-forming solution significantly improved the barrier properties again water (Sabato et al. 2001).

Recently, combined edible coating and high intensity pulsed light (PL) treatment has been proposed for enhancing the microbial safety and extending the shelf-life of food products. In pulsed light process, the surface is exposed to successive repetition of short (100 ns to 1 ms) of high intensity flashes of polychromatic radiation at different wavelengths, including UV (180–400 nm), visible (400–700 nm), and infrared (700–1100 nm), using an inert gas (e.g., xenon, krypton) lamp, typically at an energy density of 0.01–50 J/cm^2 on the irradiated surfaces (Pirozzi et al. 2020). The lethal effect of pulsed light on microorganisms can be attributed to the UV component that disrupt the DNA. The intense visible and infrared radiations can deliver rapid increase in surface temperature to deactivate the microbial cells (Wekhof 2000; Takeshita et al. 2003, Wang et al. 2005a, b; Krishnamurthy et al. 2010). Edible coating and pulsed light treatment potentially can be coupled in a hurdle approach, with PL treatment to reduce the initial microbial load or applied to inactivate microorganisms after coating. Moreover, positive effects on the protein layer such as cross-linking reactions can improve its mechanical properties, as discussed above.

5.5 Chemical and Enzymatic Crosslinking

Tensile strength, which is the stress at the point of film failure, tends to increase with increasing crosslinking. On the other hand, the elongation-at-break, which is the corresponding strain value at failure, tend to decrease with increasing crosslinker (Nielsen 1969). Protein films can be cross-linked chemically or enzymatically to enhance their mechanical properties. Mono- and bi-functional aldehydes are useful crosslinker for forming covalent inter-/intra-molecular crosslinking of protein. The possible amino acid residues involved are lysine, tyrosine, histidine, cysteine, tryptophan and phenylalanine (Ly et al. 1998; Matsuda et al. 1999; Migneault et al. 2004). Park, Bae and Rhee (2000) exploited glutaraldehyde to chemically crosslink SPI to significantly strengthen mechanical properties (increased tensile strength and elongation at break) of cast film, as well as enhancing the foaming properties of SPI. However, GA treatment increased the yellowness of the films, which will need to be considered in applications where visual coloration are important. Considering that glutaraldehyde can exist in various forms in aqueous solutions (Migneault et al. 2004), it is important to control the reaction conditions (e.g., pH, temperature, concentration, polarity) to better control the crosslinking reactions. For example, under acidic and neutral conditions, glutaraldehyde exists as monomeric dialdehyde, cyclic hemiacetal or polymeric forms. Because Schiff bases derived from the reaction of free aldehyde and lysine amino group are unstable under acidic condition, it is more likely that the reaction with protein would involve glutaraldehyde in cyclic hemiacetal and polymeric forms. On the other hand, under alkaline condition, glutaraldehyde exists in α,β-unsaturated oligomeric form, which reacts with amine to form stable Schiff base products (Walt and Agayn 1994).

For food application, the concern of toxicity of chemical crosslinker has prompted the use of other enzymatic reaction to crosslink proteins. For example, transglutaminase catalyzes acyl-transfer reactions between glutamine and lysine residues, forming ε-(γ-glutaminyl)lysine intra- and intermolecular crosslinks (Nielsen 1995), useful for enhancing the physical properties of protein films. For globular proteins, a pretreatment is needed to cleave the disulfide bonds in order to unfold the protein in order for the enzyme to access the amino acid substrates. Yildirim and Hettiarachchy (1998) studied the properties of transglutaminase cross-linked whey protein and 11S globulin film, by using dithiothreitol. They reported that transglutaminase cross-linking decreased the solubility of the films, as well as increased the tensile and puncture strengths of whey protein and 11S globulin composite film by approximately two times of those of the control. Instead of using dithiothreitol, which is not suitable for food applications, combined thermal (~80 °C) and elevated pH (~pH 10) treatments to denature the protein, followed by adjusting the film-forming solution to optimal pH for transglutaminase reaction would be more feasible for edible applications (Lim et al. 1998b).

Were et al. (1999) reported that the puncture strength and tensile strength of SPI film increased with concomitant reduction of water vapor permeability, when gluten was added to SPI at 4:1 SPI:gluten level at pH 7.0. To solubilize the gluten, the proteins (7.5 g total) were dispersed aqueous ethanol solvent (72 mL of 95% ethanol + 48 mL water) containing glycerol (2.5 g) as a plasticizer. Cysteine (1% w/w) was added to the film-forming solution. Wheat gluten has a significant content of cysteine. The additional cysteine incorporated can undergo polymerization via sulfhydryl-disulfide interchange reaction, advantageous for forming a strong covalent network.

Micard et al. (2000) reported that aging of wheat gluten films for 5 d increased tensile strength by 75% and Young's module 314%, but the elongation-at-break was decreased by 36%. The changing material properties were attributed to the formation of disulfide bonds by thiol oxidation. Formaldehyde is an effective crosslinking agent in enhancing mechanical strength and reducing water-solubility of protein films (Galietta et al. 1998; Rhim et al. 2000). However, due to its toxicity and potential migration to food, its use is limited to non-food applications. A less toxic alternative to formaldehyde is glutaraldehyde, which is aliphatic dialdehyde with aldehyde groups at C1 and C5 positions. Recently, Fan et al. (2018) successfully compatibilized corn zein with salmon skin gelatin. They observed an increased in water barrier properties of the blend film when the hydrophobic prolamin was added to the gelatin film. However, the incompatibility between gelatin and zein polymers resulted in heterogeneous films of increased opacity due to protein phase separation. Interestingly, the incorporation of glutaraldehyde resulted in zein-gelatin zein films of improved light transparency, water resistance, and mechanical strength, even when the blend films were exposed to elevated humidity (Fan et al. 2018). This approach could be an attractive strategy to exploit the relatively prolamin to reduce the moisture-sensitivity of other plant proteins. To reduce the possible cytotoxicity of glutaldehyde, different mitigation strategies have been used, including the application of glycine or glutamic acid after cross-linking treatment, washing with saline solution, and minimize the crosslinker concentration to a trace level of less than 1% w/v (Cooke et al. 1983; Jayakrishnan and Jameela 1996; Gough et al. 2002; Li, Gao, Wang, Zhang, and Tong 2013).

Other non-covalent crosslinkers, such as divalent cations (e.g., Zn^{2+} and Ca^{2+}) can be useful for inducing electrostatic crosslinks between negatively charged groups of protein molecules, thereby decreasing the mobility of protein chains. Park et al. (2001) studied the effect of calcium salts and glucano-δ-lactone on the mechanical properties of soy protein film. The incorporation of calcium sulfate at 0.3% (w/w of soy protein) increased the tensile strength and puncture strength of soy protein film from 5.5 and 5.9 to 8.6 and 9.8 MPa, respectively. Glucono-δ-lactone 0.3% (w/w of soy protein) increased the tensile strength and puncture strength to 8.3 and 8.8 MPa, respectively. Similarly, Zhang et al. (2001) reported that the addition of $ZnSO_4$ in extruded soy protein film (100:80:30:2 w/w SPI:water:glycerol:$ZnSO_4$) significantly increased the modulus, elongation-at-break, tensile strength, and toughness of the soy protein sheet glycerol, as compared to the control. Moreover, the moisture absorption was significantly reduced by 30%. They postulated that the added Zn^{2+} can form chelating complexes with oxygen, nitrogen and sulfur groups in soy protein, in addition to forming ionic bonds with the polar amino acid residues (e.g., glutamic and aspartic acids) (Zhang et al. 2001). It is noteworthy that the "crosslinks" formed through ionic interaction are non-permanent. When exposed to other monovalent cations such as those commonly found in food (e.g., Na^+, K^+), ionic exchange could induce substantial changes in material properties depending on the ionic strength of the contacting solution.

6 Conclusion

Although protein films and coatings have been subjects of research beginning in 1970s, these versatile materials continue to generate research interest to this day. As shown in Fig. 10.8, the number of published articles with keywords on the title related to plant proteins, films, and coating continued to increase over the past two decades. The number of articles related to protein coating was approximately half of that for film. On the other hand, articles related to plant-based electrospun protein fiber did not appear until relative recently in mid 2000s, although research interest on this material has sustained a rapid growth comparable to that of protein-based coatings. Interestingly, zein seems to captivate the interest of many researchers for electrospinning; as shown in Fig. 10.8, among all the articles on plant-based electrospun materials, those based on zein represented a major fraction in the literature.

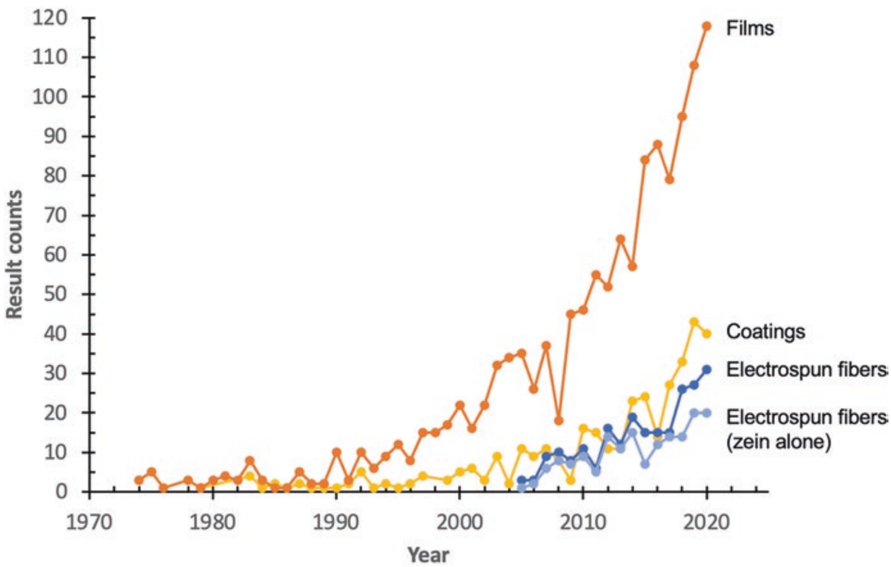

Fig. 10.8 Search counts of Web of Science's core citation indexes on published articles based on title keywords targeting plant-based proteins for films, coatings and electrospun fibers

Projecting forward, technologies development on plant protein film, coating and electrospun nonwoven are expected to continue, in view of the recent consumer preference on plant-based products. As discussed in this chapter, the manufacturing technologies for these materials are well-established and scalable to the industrial level. They exhibit material properties that can be exploited to delay food deterioration phenomena involving physicochemical, biochemical, and microbiological processes. These versatile materials can be used as one of the "toolkits" during the development of innovative products, to meet the ever-increasing consumer's demand for minimally processed, ready-to-eat/cook, and high-quality products. Moreover, as consumers are more informed than ever on the environmental impact of food production, food producers can no longer ignore food waste and packaging sustainability issues. Protein-based film/coating/nonwoven composite structures are expected to play significant roles by extending product shelf-life and reducing the reliance of non-sustainable fossil-based thermoplastics.

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Chapter 11
Sensory Qualities of Plant Protein Foods

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1 Introduction

In recent years, more plant-based protein products are entering the market than ever due to an increased interest in vegan and vegetarian diets. The rise of plant-based protein products has provided a wide variety of alternatives to animal derived foods. This not only includes meat alternatives, but also dairy, egg, and seafood alternatives. Across each of the food categories, plant-based products can be very different in terms of their ingredients because each analogue product aims to mimic a different quality attributes which present different sensorial experiences for consumers. To increase the opportunities for plant-based protein products within different food applications, establishing quality measures is important in delivering animal protein alternatives which resembles the original quality attributes. This continues to be a challenge for product development as many of the plant-based materials do not provide the desirable sensory attributes as their animal-based counterpart. Therefore, it is imperative to systematically evaluate key quality attributes throughout the development to optimize the quality.

Quality has major impact on the consumer acceptance of food products. The management of the quality starts at research development and continues after product is developed in order to keep the high standards consistent. There are many characteristics of a food product that should be considered to evaluate the overall food quality, for example appearance and aroma. In addition to the use of instrumental analysis for analyzing these physical properties, sensory analysis can play an important role too. In an ideal situation, utilizing the combination of quality and sensory analysis efficiently optimize the quality of plant-based protein foods

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throughout the product development process. The overall quality of food products can be divided into two parts: objective quality which is the physical characteristics built into the product and subjective quality which is the quality perceived by consumers (Grunert 2005). In order to maintain good product quality, objective quality can be managed by conducting bench top characterizations while subjective quality can be controlled by sensory evaluation.

When developing plant-based protein foods, initial development can utilize a variety of physicochemical characterizations to quantify different sensorial properties of the food. This helps to efficiently develop the product as the characterization will have established a fundamental understanding of the quality properties to investigate the relationships among protein ingredients, concentrations, and other product parameters. While novel plant proteins are quickly advancing in the market, the consumer perception of these quality attributes are not yet standardized. Sensory evaluation can act as the development bridge in understanding consumer acceptance of plant-based products and forecast product success within the marketplace. Establishing quality attributes for new products can be challenging as consumer's expectation of products can change from time to time due to trends, social impacts and/or other environmental factors. Consumer expectations also depend on the different variety of products that they have experienced. It is important to keep learning how product extension can be done and also how new products are viewed in the market. That is where sensory evaluation comes in to help product developers to make decisions on whether the new product can be pushed into the market or more development should be done in order to get the majority of the target consumers to buy the product. Sensory tests not only show the overall acceptability of a product, but also the attributes consumers like or dislike so that product developers can pinpoint the modifications needed to improve product quality. Consequently, by using both physical characterization and sensory evaluation methods, the overall quality for product development can be optimized. This chapter aims to describe the combination of instrumental and sensory methods to evaluate the quality attributes of plant-based food and to discuss the challenges and opportunities of using such methods. Additionally, quality attributes of various plant-based food applications are also discussed to give examples of some technical considerations within specific plant-based foods.

2 Instrumental Methods

The instrumental characterization of the functional properties of plant protein ingredients helps determine the physicochemical properties of individual ingredients and the plant-based products that can identify key quality attributes during development. Ingredient screening is important as many plant protein ingredients in the market have different functional properties which may then be best used in different applications, including structure, texture, water holding, and so on. Some plant proteins demonstrate better functional property than another, even among the same type of plant proteins because the cultivar type can change its properties. For example, the gelling property of lentil cultivars including *Ciftci* and *Kafkas* are not as strong as other cultivars (*Ali dayı* and *Fırat*) tested to form a hard gel at the same concentration (Aydemir and Yemenicioglu 2013). Therefore, it is important to understand the functionality of the selected plant-based protein ingredient used as for the property needed in the final product. Physicochemical analysis should be conducted during the development to maintain product consistency in production to screen and identify key quality attributes that later can be quantitatively tested. Furthermore, many of the physicochemical attributes provide insight into the quality acceptance metrics that can later be confirmed with sensory analysis. Below we will describe in brief different physicochemical analyses that can be applied to objectively quantify quality characteristics, including, appearance, color, mouthfeel (textural) characteristics, water and oil holding capacity, emulsifying properties and aroma.

2.1 Appearance

Color is the one of the initial cues that a consumer uses to evaluate the quality of a food product. Many plant-based protein ingredients have beige, gray, green, and/or yellowish hues that may impact the perception of the overall quality; therefore, it is important to assess the potential impact of different plant proteins by quantifying the food appearance. Color is usually measured by transmitting a standardized light to reflect the products surface. The reflectance is then quantified through a mathematical calculation using the tristimulus coordinates from the light reflectance data. Different colored plant-based materials have different abilities to absorb and scatter light, therefore resulting in different wavelengths (McClements et al. 2019). The reported colorimetric values (i.e., L*, a*, b*) can be measured numerically by utilizing a colorimeter to demonstrate the lightness (L*), green-red (a*), and blue-yellow (b*) components.

There have been some challenges with plant-based analog products where the colors of plant-based ingredients are significantly different from the traditional products that they are trying to mimic. Adjustments are often needed to formulate meat analogues to recreate meat-like colors as plant-based materials tend to be perceived differently compared to the original food products (as often times they hold a gray, beige, or yellowish tint). Samard and Ryu (2019) compared the color between a blend of soy and wheat gluten-based meat analogue to traditional meat by measuring with the colorimeter. Both raw and cooked forms of traditional meat (beef, pork and chicken) reported significant color differences for all meat and meat analog samples after they were being cooked as myoglobin in red meat is oxidized and Maillard reaction occurred during the extrusion process of the meat analog. Compared to red meats, meat analogs had significantly less redness, demonstrating that additional colorings are needed for the development of plant-based analogues for red meat application. Estévez et al. (2010) also measured the color of soymilk-based yogurt using colorimeter. Comparing to dairy yogurt, soymilk-based yogurt had a significantly lower lightness (L*) and higher redness (a*) and yellowness (b*). This might be due to the light brown color that soymilk has, showing adjustment in coloring of dairy yogurt analog is needed.

Other digital imaging methods can quantify the color appearance of food products, which are also suitable for plant-based products. Simple methods by analyzing pictures taken by camera in Adobe Photoshop is plausible (Yam and Papadakis 2004). With a high-resolution camera and proper lighting system, clear pictures can be used for color measurement and with the measurement of the software, L*a*b* values can also be obtained. DigiEye imaging system is another digital imaging system that can take colorimetrically accurate images to measure color uniformity, size and shape of food products. It measures the uniformity of the light under diffuse and directional illumination. The short-term precision of the DigiEye system is suggested to be better than those of the colorimeter (van Dalen et al. 2010).

2.2 Mouthfeel Characteristics

2.2.1 Texture

Depending on the type of food products, different instruments and methods are used to measure these texture attributes (Kohyama 2020). The instrumental methods to characterize texture are usually classified into three categories, including fundamental, empirical, and imitative methods (Nishinari et al. 2008). Fundamental methods are based on the physical properties, for example the rheology of a product, whereas empirical methods are aimed at evaluating textural experiences observed with mouthfeel. Methods are developed to mimic the mastication process of food in the mouth. A common instrument is the texture analyzer that can be used to mimic the shearing and cutting in the mouth with different attachments for various food products. For example, a blade set would be useful for cutting and shearing tests for products like hotdogs and cylinder probes can perform penetration tests. The texture profile analysis (TPA) that utilizes the texture analyzer to perform compression test can measure many texture attributes in a single experiment to help predict the quality of the food product, while only requiring a small amount of food product. The texture attributes measured include hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. As the TPA has been standardized for many food products, it has become a popular method for researchers to use.

As an example, the texture of high moisture extruded soy-based meat analog was characterized by the TPA method in a study by Lin et al. (2000) to evaluate the overall textural quality of soy protein analog with different moisture contents and cooking temperatures. In this application, by measuring the textural attributes springiness, cohesiveness, gumminess, hardness, and chewiness, the researchers could determine that different combinations of cooking temperature and moisture content would impact the quality of the texture. Specifically, they determined that the use of higher moisture content (70%) had a significantly reduced hardness, chewiness, and gumminess of the analogues.

The use of microscopy is also common to analyze plant-based meat analog texture. Scanning electron microscopy can observe the microstructure of the extruded plant protein materials used in mimicking traditional meat products. For example, with the comparison of moisture content in the extrusion process of soy proteins, microscopy images show the number of layers of proteins and how they are layered. At the same cooking temperature, the lower the moisture content, the more fibrous the extruded plant proteins are. With this information accompanying the TPA results, it was found that higher hardness and chewiness are positively correlated with the more oriented structure of the extruded plant proteins (Lin et al. 2000).

2.2.2 Rheological Properties

In plant-based semi-fluid food applications, such as dressings, sauces, and beverages, rheological properties are important as a quality indicator of mouthfeel. Often viscosity is a very important texture attribute as it is directly related to the mouthfeel of a product (Costell and Duran 2000). Viscosity is a measurement of how resistant fluids are to flow, as there is internal friction of the layers of a liquid in motion. Through the use of a viscometer, a probe is moved through a stationary fluid or the food is moved past a stationary probe (object) to measure the resistance of motion (i.e., friction) between the fluid and the probe or surface Pa s), which corresponds force (N) per unit area (m^2) divided by the rate of shear (s^{-1}). A common type is the rotational viscometer which consists of a rotating bob that is put inside the food (liquid or semi-fluid) to measure the torque needed for the rotating in the fluid in a single direction. For example, a viscometer was being used in the development of a chocolate-flavored peanut–soy beverage (Deshpande et al. 2008). The viscosity measurements determined the optimization of ingredients including the use of a stabilizer, emulsifier, oil, sweeteners, and flavoring agents. A rheometer may be needed when the viscosity of a liquid varies with flow conditions. For example, unlike Newtonian fluids, pseudoplastic foods like yogurts depend on rheological parameters like yield stress on its flowability, where its viscosity decreases with increasing shear rate. This is important to understand as different rheological properties should be characterized for different kinds of semi fluid food applications.

Jiang et al. (2020) characterized the gel properties of yogurt and tofu analogue products made with whole faba bean flour. A rheometer was used to measure the viscosity and viscoelasticity of the yogurt analogues as an indicator to textural quality. The removal of starch in faba bean yogurt analogue was reported to be weaker than the one containing starch hydrolysate in terms of firmness and viscosity due to lower storage modulus and lower loss modulus values. However, this study did not include dairy yogurt sample for direct comparison, so the difference between dairy and starch hydrolysate containing yogurt analogue was not shown. In contrast, starch hydrolysate containing tofu analogue was found in the TPA to have a lower firmness than the one that did not contain the starch hydrolysate. Therefore, through the texture measurements, the incorporation of starch hydrolysate in different analogue products was found to improve the textural quality of yogurt analogue only, but not tofu analogue demonstrating that texture analysis can help articulate quality differences.

Grittiness is one of the texture attributes that is known to impact the mouthfeel of food products (e.g. chocolate and yogurt) that can be objectively measured. Hagura et al. (2011) evaluated the grittiness of dairy yogurts by measuring the flow behavior because grittiness is closely related to the particle sizes and concentration of the product. The grittiness of model yogurt with added coffee extraction residues included 5 concentrations from 0% to 2%. A flow characteristic evaluation device evaluated each sample using a flow characteristics evaluation device to calculate the flow velocity (±1 ml/min). The combination of fluctuation frequency (1/s) and the difference in maximum and minimum flow velocity reported a linear relationship to the particle distribution. Therefore, the flow characteristics can help determine the grittiness of yogurts. Although this study was on dairy yogurts, similar method should also be carried out for plant-based yogurt systems, as grittiness and graininess are often a negative quality attribute associated with plant proteins in some applications.

2.2.3 Water and Oil Holding Capacity

The water and oil holding capacities (WHC and OHC) of plant-based products measure how much water and oil, respectively, a food system holds which can provide insight to the mouthfeel (i.e., juiciness, perceived fattiness, richness (Hermansson 1986; Zayas 1997)). As a quality attribute, WHC and OHC indicate the ability to prevent water or oil, respectively, from being released from the three-dimensional protein structure (Zayas 1997). These are important physical properties to include in working with plant-based materials as protein-water and protein-lipid interactions will impact the overall function within a food application, including binding capacity, solubility, viscosity, swelling, emulsification, and gelation properties (Zayas 1997). The properties of the main plant proteins in products can greatly determine the final product's WHC and OHC. Moreover, they can impact the cook yield of the product, thus determine the total cost of the product. The most common methods to determine these parameters for the plant proteins, which are based on those proposed by Beuchat (1977) and Lin et al. (1974) involve dispersing a known mass (g/g) of protein in distilled water or vegetable oil followed by vigorous mixing. The solution is then centrifuged, and the excess water or oil is removed. The difference in the mass of the sample before and after centrifugation is calculated to determine how much water or oil the protein can hold (expressed as g H2O/g protein or g oil/g protein). The WHC and OHC of textured vegetable proteins that are commonly used in meat analogs for texture and baked doughs can also be measured in the same way.

Measuring the WHC and OHC in a plant-based product application as a whole, helps to predict/determine the overall product cook loss, shrinkage diameter and overall moisture content. Kamani et al. (2019) studied the impact of reducing the overall meat content in a chicken sausage through the use of partial and total replacement of meat with plant-based proteins (soy protein, gluten, chickpea flour) by evaluating the mechanical and physico-chemical properties and sensory characteristics of the products. The cook loss of the plant-based sausage was analyzed by measuring the weight of the sausages before and after cooking to determine their ability to hold water and oil after protein denaturation. Comparing to the traditional chicken sausage, plant-based sausage resulted in lower cooking loss, suggesting that more water and oil might be retained in the product. The author also indicated that other factors like cooking temperature and method, type of oil and also additives could impact the cook loss too. For example, Majzoobi et al. (2017) showed that the addition of K-carrageenan and konjac mannan significantly reduced the cook loss of meat-free sausages. Compared to the steaming method, deep frying method would result in a higher cook loss in sausages as moisture will get evaporated by cooking oil. However, with the addition of hydrocolloids like K-carrageenan and konjac mannan, fried soy-based sausages have the potential to retain juiciness with increased WHC of the product.

2.2.4 Emulsifying Properties

In many plant-based analogues, emulsions are being formed, for example yogurt, sausages, eggs, etc. Therefore, the characterization of the emulsifying properties of these plant-based products is important. There are many factors including the size, shape, flexibility, charge, hydrophobicity, and aggregation state of the plant protein ingredients that can impact their emulsifying properties. The screening of plant proteins on their emulsifying property during product development can be done by looking at their emulsion droplet size, ƶ potential, surface hydrophobicity, interfacial tension, and rheology measurements. The main emulsifying properties, emulsifying capacity and emulsifying stability are usually being characterized to evaluate their ability to form and stabilize an emulsion. The emulsifying capacity of plant proteins can be tested by measuring the mean particle diameter versus protein concentration under standardized homogenization conditions to determine the minimum protein concentration (C_{min}) required to form small droplets can then be established and the minimum droplet diameter that can be achieved (d_{min}) (Gumus et al. 2017).

In meat analogs, emulsion stability is especially important as it shows how well the plant-based product can retain moisture and fat during cooking. Kamani et al. (2019) characterized the emulsifying stability of plant-based sausages by measuring the total expressible fluid (TEF) after being heated at 80 °C for 60 min in a water bath. Surprisingly, no TEF was found for the plant-based sausages while traditional chicken sausage shows high TEF. This shows that plant proteins in the sausage have high emulsion stability, so the emulsion can hold the moisture and fat in the system. Not only in meat analogs, salad dressings emulsions with plant proteins as emulsifiers was found to be promising replacement for egg yolk (Ma et al. 2016). The emulsifying ability of plant proteins can affect the flow behavior, consistency coefficient, apparent viscosity and linear viscoelasticity of the salad dressing, therefore they are used as parameters to compare with egg yolk.

2.3 Aroma

Analytical aroma characterization is important in looking at the combinations of the volatile compounds to create the final aroma. It is important to study the volatile compounds that have a strong sensory property that contribute to the overall sensory perception of a product. This is especially for plant-based ingredients like soy and pulses, which can develop an innate beany off-flavor due to the presence of lipoxygenases and unsaturated fatty acids (Meriles et al. 2000).

A common method to identify the different volatile compounds present in food products is by using the aroma compound analysis. Youssef et al. (2020) performed the aroma compound analysis on a pea-based yogurt like which were fermented with different yeast strains. The volatile compounds were identified by gas chromatography (GC/MS) and generated a heat map of the different volatiles that helped to visualize of the many different compounds. Using this approach resulted in 87 volatile compounds, of which reported that off-flavor molecules including aldehydes, ketones, and furans were significantly reduced for the pea-based yogurt fermented with yeast and *Lactobacillus* (LAB) than the one with only LAB. The determination of the threshold for these compounds would be helpful in discerning whether this technique can best remove the off flavors through sensory tests.

Electronic nose (E-nose), a man-made device, mimics the mammalian olfactory system to identify the volatile compounds in a food product. This is a less expensive method compared to the approaches described earlier that are used in the industry as well. Wijaya et al. (2017) used this device to monitor the quality of beef over time. In their mobile e-nose system, the sensor array consists of gas, temperature, and humidity sensors. Many different gas sensors are applied to detect the complex odor combinations of the product. The sensor in the olfactory bulb then extracts and transmits a signal to the computer for analysis. By utilizing this technique, they classified the meat samples into 4 classes (excellent, good, acceptable, spoiled) according to the product's freshness. Gas sensors (MQ135, MQ136, MQ2, MQ3, MQ4, MQ5, MQ6, MQ7, MQ8, MQ9), temperature, and humidity (DHT22) were monitored over time to track performance degradation to help determine the shelf life of the product.

2.4 Challenges and Factors Impacting in the Instrumental Characterization in Plant-Based Systems

For each of the instrumental characterization methods mentioned above, there are many factors that may impact the overall outcome of the functional properties, including pH, ionic strength, and temperature. pH has great impact on many functional properties of plant protein ingredients as their solubility can be greatly affected. As many of the plant protein ingredients (soy, chickpea, faba bean, pea, and lentil proteins) have isoelectric points around pH 4–6, their protein solubility is the lowest within this range of lower than approximately 20%. This may impart quality challenges as reduced solubility will indirectly impact other major functional properties such as emulsification stability and gelling properties. This is an important consideration during product formulation as many food products are around this pH range (including: plant-based yogurt – 3.99–4.56 (Grasso et al. 2020), meat analogue – 4.6–5.8 (Stephan et al. 2018), plant-based milk – 6.40–7.47) (Mäkinen et al. 2015). Moreover, the ionic strength of the protein solution also has an impact on their functional property as a lower ionic strength environment can cause plant proteins to aggregate, lowering its solubility. The ionic strength environment depends on the type and concentration of the salt present in the solution. For example, sulfate and ammonium salts promotes ion-water interaction, which can cause plant proteins to aggregate (Lam et al. 2018). Furthermore, the use of flavors and/or seasonings to improve the overall taste of plant-based foods often includes salt, which imparts an ionic charge to the system which may impact the overall quality (texture and flavor). Additionally, thermal exposure to plant proteins during processing (such as steaming, frying, extruding, etc.) can result in a change to appearance, mouthfeel, and flavor. Although various plant proteins have different denaturation temperatures, they usually denature at a temperature above boiling point. The denaturation process will unravel the plant proteins and can significantly hinder their functional properties (Wu and Inglett 1974). Consequently, the pH, ionic strength and the temperature are factors that should be kept constant while doing plant protein ingredient comparisons. By using the quality metrics outlined above, the quality assessments of plant-based foods can quantify the functional attributes to better drive the product development and develop consistent quality plant-based products.

3 Sensory Evaluation

Sensory evaluation is a discipline within food science that systematically measures human responses to a variety of attributes based on physico-chemical properties using different dimensions of sensory perception (Issanchou and Nicklaus 2006). As mentioned above, there are many quality attributes that can be quantified to ensure overall product quality using instrumental methods, including mouthfeel characteristics, color, and aroma. However, sensory evaluation can also provide information on the same quality attributes to assess the sensitivity that humans perceive. It is recommended to use both methods to make sure that the quality measurements align with consumer's expectation. As plant-protein applications advance, the use of sensory evaluation can help to build a library of the primary sensory attributes within different plant-based ingredients (and functions) which can help to provide an indicator of overall consumer acceptance. However, in order to maximize the value of sensory evaluation, it is important to discern the different methodologies as well as the benefits and limitations within each method.

Sensory evaluation has three main types of tests, including: discrimination, descriptive, and hedonic or acceptance tests. Discrimination tests are usually used to determine if consumers can differentiate between products. Of which, the most common test is the triangle test, where three samples are presented simultaneously using three coded sample identification (two samples are identical, one sample is different) and the panelist is tasked with choosing the sample they perceive as different (Lawless and Heymann 2010). Although discrimination tests can be done quickly, they are limited in determining only if products are different. For example, in the applications of plant-based materials, there may be value in fielding a discrimination test to determine if consumers can discern between a traditional food versus a plant-based analogue. However, the response will only determine if there is a difference and cannot indicate if the products were liked or if they were perceived as the same. Descriptive tests are sensory tests that articulate how products differ by quantifying different attributes through ranking scales using a small group (between 8 and 12) of trained panelists (Lawless and Heymann 2010). The intensity values of product attributes can be accurately quantified, which can guide the direction of the product development. With plant-based products, which exhibit unique color tones, off-flavors and tastes, quantifying their unique attribute characteristics can help determine what development approaches to more effectively achieve the product target. However, as with discrimination tests, descriptive analysis should not have panelists report liking of individual characteristics or overall acceptance of the sampled products. Lastly, hedonic or acceptance tests are a sensory test that measures consumers' liking of individual attributes or overall acceptance of products using a hedonic scale. The 9-point hedonic scale is the most common scale used for this sensory method; yet there are several others that are used. It is best practice that acceptability (hedonic) studies are fielded with at least 60–150 untrained consumers meeting the target demographic. While there are several different types of sensory tests, it is important to remember that each type of test is positioned to address a specific scientific question. It is important to remember that conclusions regarding liking, acceptance, preference or palatability should not be gained from discrimination and/or descriptive studies, but rather from untrained consumers in a hedonic study.

Selecting a sensory method is important in understanding the sensory attributes of your product. For plant-based products, there are two frequent questions asked by product developers and researchers. One question is how the new plant product compares to its traditional animal-based product. A second question is how the addition of an ingredient(s) or processing change improves the plant-based product compared to another formulation of the plant-based product. Selection of the control for these studies is important in helping to answer the research question. The control can be the targeted animal-derived product that is aiming to be mimicked (such as an all-beef burger or bovine milk), a commercial plant-based product already in the market that has high popularity. When making conclusions based on sensory data, it is always important to consider what product was selected as the control and whether it was appropriate. In some cases, it is important to address more than one question of the plant-based product. For example, in some cases, studies compare sensory attributes between a traditional animal product and a new plant-based product and conclude that some of the attributes of plant-based product had comparable or better performance than the resembling animal product. Yet upon examining the hedonic results, it can often be found that the test sample was not well liked even with better performance in some sensory characteristics (Kamani et al. 2019). Therefore, as with any sensory test, not just for plant-based products, it is important to carefully select the method and the control sample(s), as it is essential for making accurate conclusions about the test product. In some cases, multiple methods may be used to help address different research questions.

The recruitment of consumers to participate in sensory studies is also important to consider. The consumers in the sensory study should match important characteristics (e.g., age, gender, socio-economic status, diet type, etc.) of the target audience. For example, analogue products are typically targeting consumers seeking diet shifts to improve health or sustainability by reducing intake of animal products, but not wanting to compromise on taste, flavor, or mouthfeel. Alternatively, the target audience may be vegans and vegetarians but are likely to have much more prior experience with plant-based alternatives, which can impact their expectations for products (Hoek et al. 2011). Therefore, it is important to consider prior experience of the consumers, such as asking if they have tried plant-based products before or have been consuming plant-based products as part of their diet for at least 1 year. Prior research has shown that acceptance of plant-based products can vary based on dietary consumption (Graça et al. 2015). Other general considerations include if the consumer has food allergies or dietary restrictions, age group, and if they are the primary grocery shopper.

Many sensory attributes can be measured using standard sensory techniques using both discrimination and descriptive tests (Lawless and Heymann 2010). Key sensory attributes for plant protein food applications are still being explored and should be standardized so that future studies can follow to develop new plant-based foods. As many plant-based protein products mimic animal-based foods, key attribute terms are important to include to help with characterization. The sub-sections below discuss some of the key attributes of analogs (e.g., mouthfeel, taste, flavor, color, aroma and visual appearance).

3.1 Appearance

Visual appearance including color is important as consumers often initially judge a food by what it looks like. The visual cues can impact the expectation for a product's taste and flavor; therefore, it is important to create a product that has good appearance to attract consumers. Visual appearance is also challenging for products trying to mimic animal-based products, as consumers may expect analogs to have similar features. Monitoring the quality of appearance during purchasing, after opening, and during and even after cooking is important. As an example, meat products are expected to change color after being cooked from reddish pink to brown. The overall appearance of plant-based analogues should most closely match the traditional animal-based products to set positive expectations.

Different plant-based materials might undergo distinct chemical reactions which may introduce unexpected quality changes over time therefore shelf-life studies should be considered. For example, plant-based meat analogues often are challenged with discoloration over long storage due to being exposed to light or oxygen for a long time (Duque-Estrada et al. 2020). Plant-based yogurt usually has a gray or brown hue that may not be accepted as the original color of plant-based ingredients could be considered a defect by consumers. Therefore, the change of color in plant-based is needed to truly mimic traditional animal products.

As an approach to maintain product appearance quality, Gomez et al. (2019) used different sauces for meat analogues. A hedonic test was performed by 73 consumers who rated three visual parameters of both meat analogue and a beef equivalent that were treated with teriyaki and beer marinade. No significant difference in hedonic scores was detected between the samples, suggesting that the meat analogues were equally accepted as the beef samples in terms of visual appearance. On the other hand, the instrumental color analysis shows significant differences in the color between meat analogues samples and beef samples for both marinades. Therefore, sensory experiments can help to guide which quality attributes are most important with consumers, even though significant difference was found by measuring the colorimetric values.

Other ingredients can also affect the color and appearance of meat analogues. Faujan et al. (2018) developed meatless nuggets with different ratios of chickpea flour to texturized vegetable protein. The hedonic scores of color of the meat analogue increased liking with chickpea flour concentration. This is due to the presence of carotenoids naturally in chickpea contributing to the yellow color, which was more preferred with panelists. Although the hedonic scores for color increase with increased ratio of added chickpea flour, the overall acceptance scores for the meat analogues decreases. This shows that color might not be the main factor that impacts the main overall liking as the texture attributes has a lower score at high ratio of chickpea flour. Therefore, it is important to analyze multiple sensory attributes for overall consumer acceptance.

Visual surface texture such as surface pattern, roughness, glossiness, etc. can also have significant impact on food perception,expectation, and acceptance. Sensory evaluation methods can help assess the correlation of surface texture attributes and likings of food products. As sensory measures of visual properties are not time dependent, many studies have used pictures or images instead of actual food. This approach helps to reduce cost and decrease variability between samples. For example, Ngapo and Dransfield (2006) used computer programs to adjust fatness levels in meat images and showed them to consumers for sensory evaluation to understand the influence of fatness preference. Similarly, meat analogs might be able to use such techniques to understand the influence of visual fatness on consumer acceptance. By doing so, it might enhance consumer's expectation on the overall quality of the product.

When designing such sensory evaluation studies to quantify the influence of visual attributes, it is important to keep the lighting of the place for evaluation the same because difference in lighting can change consumer perception of color and the visual texture. Problems with inadequate lighting may give a product with darker color and harder for the consumer to evaluate the glossiness of a product. For refrigerated samples, they should be taken out from the refrigerator right before sensory evaluation to prevent any condensation on the food product, which may affect consumer perception on the appearance of the product. An improved visual surface texture of plant-based meats may improve consumer expectations and lead to greater adoption for plant-based products.

3.2 Mouthfeel Characteristics

Mouthfeel characteristics relate to a variety of different textural attributes within foods and will vary across product categories. For example, in meat analog, for each meat products like a burger patty and an emulsified hotdog product, the main texture attributes to focus can be vastly different, especially when the cooking method is different. Lin et al. (2002) conducted a descriptive test on different soy-based meat analog high moisture content with the terms tough, mushy, moist, layered, cohesive, springy, and chewy. They reported that the moisture content of the meat analog has profound impact on the scoring of all the texture attributes, where higher moisture resulted in higher score in mushy and moist and lower score in tough, layered cohesive, springy, and chewy. However, no hedonic test was done in this study on the meat analogs with different moisture content, therefore the association of the texture attributes to different meat analogs are only known, but not liking.

For yogurt analogs, different texture terms are being used to be evaluated the products. Greis et al. (2020) compared the mouthfeel properties of five plant-based yogurt-like products to two dairy yogurts with temporal dominance of sensations (TDS), a rapid descriptive method. The texture attribute terms evaluated were thick, thin, creamy, watery, sticky, and foamy. Upon that, a 7-point hedonic scale was also done on the overall mouthfeel liking by 87 consumers. The study reported that the attribute terms tested are related to both plant-based yogurt-like products and dairy yogurts. Surprisingly, these yogurt analogs are found to have similar and equal liking with their overall mouthfeel to dairy yogurts. The plant-based yogurt and dairy yogurts can be similar and equally liked by their mouthfeel profile. Since this study was conducted with untrained panelists, the utilization of both descriptive test and hedonic test helped to determine a correlation among textural attributes. Specifically, the "thickness" and "creaminess" of plant-based yogurt-like products were found to increase the mouthfeel liking while thinness and wateriness decreases the liking. Therefore, multiple sensory tests can help evaluate the plant-based products more entirely.

3.3 Taste, Aroma and Flavor

Taste refers to the perception of sweet, sour, bitter, salty, and umami. Aroma traditionally refers to the smell of the food prior to chewing (ortho-nasal olfaction). During chewing, odor compounds are released and are perceived through retronasal olfaction. The perception of odor compounds in the mouth are combined with taste and texture perception to impress upon consumers the overall flavor. Therefore, flavor is the combination of taste and aroma result. It is important to understand the difference between taste, aroma, and flavor for designing questions to understand the correct sense response you want of the product. Especially for plant protein products, it is important to understand these three sensory qualities as they can be much different from that of traditional meat products.

The biggest challenge for the taste of plant-based products is that plant proteins like soy and pulses have naturally have bitter and astringent tastes due to saponins and isoflavones, which can negatively impact the consumer acceptance (Damodaran and Arora 2013). Moreover, plant-proteins ingredients also give off a beany and grassy odor generated by the autoxidation of polyunsaturated fatty acids which contribute to the formation of off odors (Asgar et al. 2010). This eventually can contribute to the overall off-flavor of plant-based products. Therefore, questions regarding specific taste qualities that are expected from plant-based products may be included in sensory tests. To overcome this problem, again, various seasonings and flavorings are usually added to mask the undesirable taste and the beany, grassy or green aroma of pulses. The addition of meat flavorings into the meat analogues are common to mimic the traditional meat products as plant-based ingredients do not innately have that meaty taste. It is also important to evaluate the aftertaste of plant-based products as it also determines if the flavorings would really help increase the acceptability of taste of the product. The taste and aroma are usually being evaluated at the same time as flavor for a product, as they are closely related.

To overcome the beany flavor of soy protein, Katayama and Wilson (2008) added vegetable-based “chicken” and “shrimp” flavors to commercial textured soy meat analogues. Trained panelists tasted the meat analogues with different concentrations of flavorings and rated the intensity of 0–150 scale of the sensory attributes. The attributes regarding taste/flavor include beany, oily, saltiness, chicken-flavor/ fishy flavor and shrimp flavor. A consumer preference test was done in addition with a 9-point hedonic scale with 125 consumers. The sensory evaluation showed that the addition of chicken flavor increased the saltiness perception of the consumers, while the beany flavor decreased with the increase in flavoring concentration. However, the hedonic tests only tested on soy meat analogs with high concentration chicken flavor, therefore it is not known whether the increase in flavoring concentration increases the liking of this plant-based meat analog.

For yogurt-like beverages, different fermentation starters have been assessed to improve the overall flavor and taste of the product. Luana et al. (2014) selected different lactic acid bacteria (LAB) and enzymes for the formation of oat-based yogurt like beverage. For this study, 10 untrained consumers evaluated the intensity of the flavor and taste attributes of yogurt-like products on a scale of 0–10, including acidic, cereal, and sweet. Additional analysis also included flavor attributes including toasted flavor, earthy, cereal, savory, sweet and. The oat-based yogurt made without starter had a significantly higher scores on attributes such as earthy and cereal, showing that the fermentation process helps reduce some of the not as pleasing artificial and earthy tastes. On the other hand, oat-based yogurt fermented *with Lactobacillus plantarum* LP09c showed increased intensity in overall flavor, sour taste and after taste. While this work provides preliminary data that indicates the use of LAB can improve the quality of plant yogurts, future students should include a larger participant pool to confirm these findings.

Volatile compounds such as aldehydes, phenols, esters, etc.can impact the overall aroma and flavor of foods and therefore can be an early quality attribute of plant-based products (Kosowska et al. 2017). Furthermore, the beany and/or greeny notes associated with plant-based proteins can be off-putting for omnivore consumers, therefore the aroma of plant-based foods should be explored. For example, soy-based milk, the effect of using hot water for the blanching and grinding process on the beany and non-beany flavor was evaluated (Lv et al. 2011). It was reported in past studies that hot water can help eliminate the beany flavors as it can inactivate lipoxygenase from soy (Lv et al. 2011). However, this study also evaluated the effect on non-beany odor using trained panelists that rated the beany and non-beany odor of traditional soymilk and hot water blanched soymilk at different timepoints (0, 2, 4, 6, 8, 10 min) on a 15-point intensity scale. The non-beany odors include oxidized, total off-flavors, cooked beans, fruity, and sweet. Although a longer blanching and grinding in hot water time reduced the beany flavors, the ratings for total aroma also decreased. The positive aroma profile including the sweet and fruity aroma also decreases with the blanching and grinding time. Therefore, to balance out the aroma, optimized conditions blanching and grinding time should be chosen to maximize quality.

3.4 Challenges and Factors Impacting Sensory Design of Plant-Based Systems

As outlined above, there are many challenges in developing plant-based products that have desirable sensory attributes. Plant-based ingredients do not readily deliver visual, smell, taste, texture, and flavor attributes that mimic characteristics of conventional animal products. Therefore, assessing these sensory characteristics is essential to product success. Sensory science is a scientific discipline which can be used to help answer questions related to product performance and quality, and consumer acceptance. However, just as with any experiment, great consideration is needed in designing a sensory study. Here, we briefly note a few challenges often observed in studies conducting sensory analysis for plant-based products and some important factors that should be considered when planning a sensory study. There are three main types of sensory methods (discrimination, quantitative, and hedonic), with each methodology based on following specific protocols and designed to answer particular product questions. One common mistake is mixing methodological approaches. For example, for trained panels (quantitative), members of the panel are trained. They, therefore should not be asked to report on acceptability or liking as their training can result in experimental bias. Knowing your specific scientific question regarding the product is key in selecting and constructing the sensory study. Another critical factor is the selection and recruitment of consumers, which is important for a few reasons. First, are there enough participants to allow for uncovering statistical significance in your study? Too few participants would not provide enough power to find differences, which could wrongly conclude that there were no differences between products. A third factor is the inclusion of a control product based on the research question. The conclusions drawn from the study are firmly based on the control product. Another common mistake when conducting a hedonic study is ensuring the control product is well-liked. If an unsatisfactory control product is included, the results may show the test product outperformed the control product. This could potentially be misleading and difficult to interpret how the test product would perform in the marketplace. In summary, a well-designed sensory study is paramount to developing plant-based products, as it is critical to making decisions in determining whether a product meets consumer expectations and delivers desirable attributes.

4 Sensory and Other Quality Attributes of Various Plant Protein Foods

In the above paragraphs, we have described the many quality attributes that are usually characterized for plant-based products in both instrumental methods and sensory methods. It is important to learn the important quality attributes for different food applications to design experiments that can target specific food applications. Moreover, some quality attributes described above can be more important to specific plant-based applications and we will be discussing what they are in distinct categories of plant-based products below.

4.1 Meat Analogues

Different plant proteins have been explored in the application as meat analogs. In building plant-based meat analogs many different attributes must be accounted for to mimic traditional meat products including flavor, color, texture, juiciness as animal proteins are vastly different from that of plant proteins. Within the product category of meat analogues, applications include emulsified material (such as a linked product), whole muscle (such as a filet), and minced proteins (such as ground beef) – each presenting their own respective quality considerations.

Whole muscle meat analogs usually require layered structures to represent the fibrous morphology of real meat products. This is a challenging problem as plant-based protein has a huge difference in their protein structure compared to meat products. In the plant protein industry, the utilization of extrusion is commonly found to create layered structure by using plant proteins, which is usually called texture vegetable proteins (TVPs). It is very common to use soy protein powder as the ingredient to make TVPs, however, new plant protein materials like oat and pea protein are explored as novel ingredients (Kaleda et al. 2020). During the extrusion process, the different parameters like screw speed, temperature, moisture, can affect the turn out of the texture of the TVPs. By using texture profile analysis and scanning electron microscopy, the structure of the TVP can be analyzed and the springiness and hardness can be determined for comparison with real meat products. With sensory evaluation, the consumer desired texture attributes can be known to be set up for instrumental analysis standards.

Emulsified meat analog applications such as deli meats and/or linked sausages, the use of physicochemical characterization can help optimize product formulations that can later be confirmed with sensory methods. However, due to the protein globular structure of plant-based proteins, the way that the animal proteins and plant proteins gel is very different. Often it is challenging to produce the true mimicking properties of animal proteins. Stephan et al. (2018) compered various vegetable proteins (soy, pea, sunflower proteins) and two different mycelia of *Pleurotus sapidus* in a vegan boiled sausage analog system to traditional 100% meat based German and Russian sausages as a control. While many of the attributes including the water activity, pH value, cooking loss are similar to that of traditional German sausages, the texture profile of vegan sausages resulted in a significantly lower cohesiveness and more elasticity compared to both traditional sausage controls. This suggests that mimicking the texture of sausage analogs is more challenging than other attributes. Sensory evaluation using a 9-point hedonic scale further confirmed these findings as the texture results of the German sausage ranked higher than any of the vegan sausage analogs over time (time = immediately and 4 weeks after production). Comparing to all the proteins added in the vegan boiled sausage analog system, the vegetarian sausage analog (egg white based) had the closest liking on texture to German sausage, however, would not be considered as plant-based product.

Therefore, the softer texture of plant-based meat analogues can deter consumers. However, with the help of sensory evaluation on the addition of different ingredients in newly made plant-based products, it is possible to increase consumers acceptance of product texture. A common approach to enhance the gel strength of meat analogs is by adding hydrocolloids. The addition of konjac mannan and K-carrageenan was found to significantly increased the general acceptability of plant-based sausages considering texture, color, smell and appearance (Majzoobi et al. 2017). However, they only used seven-point hedonic scale on overall acceptability and did not rate the sensory attributes individually, therefore it remains unknown how the overall acceptability is correlated with just texture. If one's focus is on texture, additional descriptive tests would result in better sensory evaluation of the usage of hydrocolloids.

The color of red meat changes with myoglobin level, therefore seen as purple when freshly cut, red when raw and brown when cooked. The difference in color of meat and plant proteins is challenging for mimicking as plant proteins are not naturally red in color. The changes in cooking condition also add extra hurdle especially to whole muscle products when the change in color is most observable. However, for other emulsified products, which can range in color by mixing with other ingredients, the expectation for real meat color might be lower. Savadkoohi et al. (2014) explored the addition of tomato pomace in plant-based sausage as color agent. By measuring the lightness, yellowness and redness of the plant-based sausages using the L*a*b* values, plant-based sausages were found to have higher lightness, redness and yellowness with added tomato pomace, showing the potential of tomato pomace in adding that redness to sausage analog to mimic red meat. However, the acceptability of color intensity decreases with the increase in addition of tomato pomace. Surprisingly, it was found that the yellowness of the traditional beef frankfurter and beef ham are different, suggesting the importance of specifically choosing the type of meat product to be the control for comparison in these studies.

Juiciness is also an important attribute expected in meat products, for example in burger patties and sausages for consumers to evaluate the meat product's quality. This sensory attribute is usually being compared to the water holding capacity of plant-based sausages because the more water it can hold, the lesser chance of losing water during cooking. The addition of tomato pomace in sausages was found from the sensory evaluation that the acceptability of juiciness of the meat-free sausages was lower than that without tomato pomace. In many cases, the odor and flavor of meat are expected for meat analogs to truly mimic real meat products, however, this depends on the daily diet of consumer. A vegetarian consumer looking for plant-based meat analog might not be expecting a meat flavored product, but an acceptable flavor for the usual plant-based analogs.

4.2 Meat Hybrids/Extenders

While fully plant-based meat analogues can significantly improve health and sustainability, the quality attributes thus far are accepted by a smaller grouo of consumers. However, the use of blending plant-based proteins and meat ingredients has helped to provide parity and improved quality while helping to reduce meat consumption (or extend). It has been found that the addition of plant proteins in as extenders in meat products are able to significantly lower the cooking loss and thus reduce the cost of product. Plant protein also was used as fat replacers in meat products to reduce the amount of fat in meat products. However, the sensory attributes including juiciness, texture, flavor, and appearance should be considered in determining the amount of extender to be added. Whole cowpea flour replacement 5% of ground beef in frankfurter type sausages was found to have increased liking the appearance, juiciness, texture, flavor, and aftertaste compared to no replacement (Akwetey et al. 2012). However, starting from the addition of 10%, all the sensory attributes received lower liking compared to control, showing 5% is the optimum replacement amount in beef frankfurter sausage.

4.3 Yogurt Analogues

With the popularity of dairy products in western countries, plant-based milks are also utilized to produce yogurts, cheese, and ice cream. Again, there are many quality attributes in the various dairy products that should be focused, especially many of them are emulsion-based. The main yogurt quality attributes include smooth, creamy gel-like structure that holds a mild sweet aroma and dairy flavor. The use of plant-based proteins has been used for yogurt systems, yet current products are challenged with achieving a product that is more similar to the traditional dairy-based quality due to the off-flavors, gritty texture, grey-blue coloring, and beany aftertaste.

One of the biggest limitations for plant-based yogurt is their off-flavor compounds, which are mainly associated with the presence of aldehydes, ketones, furans, and alcohols. To reduce these off-flavors, the selection of starter culture is important as the fermentation process can be used to reduce these off-flavors (Youssef et al. 2020). Youssef et al. (2020) tested a starter culture of lactic acid bacteria (LAB) in a 4% pea protein solution with different yeasts (*Kluyveromyces lactis*, *Kluyveromyces marxianu*s, or *Torulaspora delbrueckii*), which is a yogurt-like product. By performing descriptive analysis with trained panelists, 13 sensory descriptors of the yogurt like product for example sour, tangy, sparkling, vegetal, leguminous plant etc. are rated for the intensity to learn the impact of different LAB and yeast on the product's sensory perception. For the yeast added samples, there is a significant reduction in the average intensity of green flavor/vegetal and leguminous plant sensory attributes which contributes to the off-flavor. The gas chromatography/mass spectrometry analysis also supported this fact by analyzing the volatile compound profile. The degradation of many off-flavor compounds including aldehyde, ketone, and furan compounds was found for fermented products with LAB or LAB with yeasts.

A white color for appearance in plant-based yogurt is also expected to mimic the cow milk's yogurts. When soymilk yogurt was compared to cow milk's yogurt, the hedonic scale rating of the color had significant lower ratings although the overall acceptability has no significant difference than cow milk's yogurt (Farinde et al. 2009). This is again due to the innate yellowish color of plant proteins; therefore, coloring agent might be needed. However, for products with flavorings like strawberry milk and chocolate milk, the need of change of plant-based milk to white color is lowered as the main color has been replaced by the pink or brown color, which can be easily mimicked by adding coloring agents.

The mouthfeel characteristics such as the thickness and creaminess as mentioned above are also main concerns for plant-based yogurts as the coagulation of animal proteins differs from plant proteins. Therefore, a lot of commercial plant-based yogurts were found to be added with different thickeners. To limit the use of hydrocolloids, Demirkesen et al. (2018) evaluated the use of microfluidization on hazelnut-based yogurt like product instead of additional thickeners to improve thickness. The texture and rheology attributes of the fermented hazelnut-based products were analyzed by using texture analyzer and rheometer. The complex modulus values from the rheology analysis showed that fermented hazelnut-based products have similar overall resistance to deformation to that of conventional cow milk yogurt. The elastic and viscous modulus revealed that the adjustment of water content in the yogurt mix before fermentation can determine the final texture and rheological properties. Therefore, a favorable mouthfeel characteristic of plant-based yogurt can be produced to match cow milk yogurt.

Consequently, plant-based yogurt like hazelnut-based yogurt can be made without addition of hydrocolloids using microfluidization.

4.4 Plant-Based Cheese

Cheese analogues are defined as products which are intended to partly or fully substitute or imitate cheese and in which milk fat, milk protein or both are partially or replaced with non-milk-based alternatives (Fox et al. 2017). Cheese analogues have been a common product as they are less expensive to produce and have a longer shelf life than traditional animal-based cheese products. However, there is limited past published work specific to plant-based cheese analogues and plant-based ingredients are usually used with dairy ingredients in the production of cheese analogs.

One of the current limitations of cheese analogues is flavor as the unique fermented flavors for each cheese are hard to mimic. The additional dairy flavoring is also often interrupted by the green, beany and off-odors of plant-based ingredients. To tackle this problem, methods have been explored to improve the flavor to closely relate to the real cheese product. Ahmad et al. (2008) reviewed how to improve the flavor of soy-based cheese analog showed that where plant-based cheese analog has an extra challenge where plant proteins like soy innately has beany flavor. One recommendation made by the author is to use the addition of flavor-enhancing LAB nonstarter cultures like *Lactococcus lactis* ssp. lactis, *Lactobacillus helveticus, Lactobacillus casei* and *Lactococcus lactis* ssp. This method can increase flavor profile in cheddar cheese and lower the intensity of bitterness, therefore they suggested this method has potential to improve soy cheese flavor. These cultures can help produce flavor compounds, as soy curds contain the amino acids needed for the LAB cultures, providing desirable flavor characteristics. Therefore, the selection of culture for making plant-based cheese analogs and choice of plant proteins with the right mix of amino acids is an important factor.

Mimicking the textural properties of hard dairy cheeses is a challenge due to the coagulation dynamics of plant proteins as it is hard to create a gel to reach similar hardness as dairy cheeses. Therefore, many plant ingredients are used in cheese products like cheese spreads. Li et al. (2013) reported the soy cheese spreads that was made by combined glucono-lactone coagulation and lactic acid bacteria fermentation methods, resulted a more stable and less fractured structural system and achieved higher scores of sensory acceptances among different processed soy cheese spreads. Moreover, enzyme hydrolysis process can degrade large soy protein molecules into smaller particles, which results in a smoother cheese spread. However, more research on strengthening texture of plant-based cheese analogs should be done because it is hard to form hard cheeses with plant-based materials.

4.5 Non-vegan/Hybrid Cheese

As said above, the majority of cheese analogs are hybrid cheese where some even contain dairy materials but in small amounts. To mimic dairy based cheese, there are many quality attributes used to characterize the physicochemical properties for application (unheated and heated) including, flavor, texture (shreddability, sliceability, spreadability), shelf life, cooking behavior (browning, crispiness, flowability, meltability, oiling off, structurability) (Fox et al. 2017; Masotti et al. 1990).

Texture of cheese analogues are important for there are expectations on its functional applications for example, in the application of pizza, cheese analogs are expected to be shreddable and melt rapidly on the pizza (Bachmann 2001). However, there are many factors that can influence the texture of cheese analogues. For example, the moisture in the protein network can impact the elasticity of cheese analogues. Cheese analogs with lower moisture content was found to have the highest storage modulus (G' and G"), therefore cheese analogs with higher moisture content are more elastic (Pereira et al. 2001). The fat content and addition of pectin gel both can impact the texture of cheese analogs (Liu et al. 2008).

Low fat cheese analogs compared to full fat cheese analogs had lower hardness, gumminess, chewiness and adhesiveness. However, with the addition of pectin gel in low fat cheese analogs can result in overall reduction in fat and higher liking scores among cheese analogs in sensory evaluation (Liu et al. 2008). The addition of pectin gel in cheese analogs also decreases their melt enthalpy, therefore increasing their meltability. The amount of citric acid and sodium chloride can also impact the cohesivenesss, springiness and firmness of cheese analogues (Stampanoni and Noble 1991). By using sensory evaluation, the effect of citric acid and sodium chloride on the perception of texture attributes of cheese analogs are found. With higher citric acid and sodium chloride level of cheese analogs, the firmness of cheese analogs increases, while springiness decreases. By increasing citric acid alone, the decrease in cohesiveness and adhesiveness is being observed. However, the cohesiveness and adhesiveness of cheese analogs is affected more by the fat content than the citric acid and sodium. The addition of an enzyme- modified soybean beverage in production of a non-vegan cheese like product was found to improve its texture. By utilizing the texture profile analysis, it was found that the hardness values of all cheese-like products decreased significantly over the ripening period. Although the non-hydrolysis soybean beverage cheese-like product had much lower hardness than that of the cheese-like product made from cow milk only, the hardness of high hydrolysis soybean beverage cheese-like product was found to have no significant difference than the cheese-like product made from cow milk only. The high hydrolysis soybean beverage cheese-like product has also higher storage modulus values and more compact structure than that of non-hydrolysis soybean beverage cheese-like products.

4.6 Fluid Plant-Based Milk

Novel plant-based milk substitutes, such as rice, oat and pea based- milk have been gaining popularity as soy and almond milks have concerns with being declared allergens within the US market. While these plant-based milk substitutes often aim to mimic the unique creaminess of dairy milk attributed by animal fat-based emulsions, many aspects on the emulsion properties of the milk substitute are measured including the surface hydrophobicity, particle size distribution and rheological behavior. The utilization of the high-pressure homogenization can help increase the protein solubility and the surface hydrophobicity of lentil-based milk substitute (Jeske et al. 2019). This leads to the particle size of the lentil-based emulsion to be reduced by more than 100-fold, creating a more stable emulsion.

Again, the grassy or beany note of plant-based materials must be masked as a milk substitute, however, individuals who have been consuming such drinks might be used to these flavor notes and can be a unique feature that some consumers become to accept and like. There are many cases that products that are seen as off-flavors may become the signature of the product, for example the sulfur note in Hershey chocolate.

Appearance, especially color, again can place a key factor in acceptance due to the distinct white color of milk. However, other factors also play a role, for example taste, flavor, and mouthfeel. When using the 9-point hedonic scale comparing 5 commercial plant-based milks, including milks made of soy, almond, oat, rice, and hemp, the color of hemp-based milk received the highest rating, as it most resembled dairy milk's white color. However, the overall acceptability score of hemp-based milk is the lowest which was attributed to its low flavor score.

4.7 Plant-Based Ice Cream

One of the key quality attributes associated with ice cream products is the smooth, silky texture attributed to the emulsified fat. For traditional dairy-based ice cream, the fat source comes from cream, milk and condensed milk which contributes to the creaminess of the product. With plant-based ice cream, the most common fats use coconut oil, peanut oil, cashew oil and palm oil, however, legumes, nuts, grains, and seed oils have also been used. The non-fat solids of dairy ice cream like non-fat dry milk are usually replaced by plant-based flour, concentrates and isolates. Additionally, plant-based ice creams usually need to consider plant-based emulsifiers as traditional dairy ice cream commonly uses egg yolk. To create the desired mouthfeel characteristics for plant-based ice cream, different attributes must be tested including overrun, melting time and rheological characteristics including viscosity, flow behavior index, consistency index. Sensory attributes that should be taken in consider are mostly the same as other dairy analogs said above, however, as there are a lot of flavors for ice cream product for example coffee, chocolate, strawberry, etc., the color of ice cream may not be a big concern.

Some of the hurdles for making plant-based ice cream to reach the desired mouthfeel characteristics like dairy ice cream is to create a stable emulsion and control of the water activity. When replacing dairy milk with plant-based milk (soy milk and coconut milk) in making ice cream, the melting rate of the ice cream decreases (Aboulfazli et al. 2014). Soymilk-based ice cream was found to have a much lower melting rate than that of coconut milk and dairy milk-based ice cream due to soymilk proteins high water retention, preventing free water movements, and therefore resulting in higher viscosity. The 100% replacement of dairy milk by plant-based milk in making ice cream had a significantly lower body and texture rating, which can be due to the less stable plant-based emulsion which allow separation of water and oil in the emulsion to create an icier ice cream. The increase in free water in ice cream happens especially in the case of using coconut milk as coconut proteins are less soluble in water. Therefore, it is also important to add stabilizers like locust bean gum and inulin to help bind water molecules and stabilize the texture of plant-based ice cream (Góral et al. 2018).

4.8 Plant-Based Egg Replacers

Eggs are a great protein source, and it is an established functional ingredient that acts as an effective emulsifier in ice cream, salad dressings and other foods. It provides thermally reversible gel structures (omelets, cakes, and foams). The mimicking properties of plant-based egg replacements must help to deliver these functions. Plant-based materials are found to act as good emulsifiers in food systems and can mimic the foaming ability of egg white. However, some of the plant-based egg replacers may add a hydrocolloid to add strength to the gelling property of the food product.

In baked good applications, it is important to measure the texture characteristic of the product especially foamability because egg replacers play a significant role for the fluffy texture in cakes and holds its shape. As said above, hydrocolloids are usually paired with plant proteins as egg replacers for example soy and wheat-based egg replacer can be paired with algin, carrageenan or sodium alginate (Ratnayake et al. 2012). However, using a 100% replacement of plant-based eggs replacers resulted in cake products that had problems with off flavor, uneasiness of removal from pan and/or moist texture independently. The bake loss of all products made with plant-based egg replacers was higher than that of liquid and whole eggs. However, the cohesiveness of yellow cakes made by egg replacers is significantly less than that of real eggs where the moistness was higher than that of real eggs. Therefore, the yellow cakes produced with egg replacers have much stickier texture. Due to the current textural and flavor limitations with existing plant-based egg replacers, research continues to identify other alternatives for egg replacers.

Aquafaba, the wastewater from canned chickpea, was found to be a potential egg replacer because of its foaming and emulsifying property (Mustafa et al. 2018). The foamability of the aquafaba was comparable to that of egg white, however in the application of cake product, the result cake volume of aquafaba replacement was slightly lower than that of egg white. Moreover, by texture analysis, the cake made with aquafaba was found to be less springy and less cohesive than that made with egg white. Therefore, additives may need to be added to improve the texture attributes of the cakes made with aquafaba replacement. Aquafaba was also tested in the application of mayonnaise as a good emulsifier and an egg yolk replacer (Raikos et al. 2020). With the measurement microstructure analysis, a fine emulsion was able to be formed with aquafaba replacement while able to remain stable for 21 days refrigerated. The ratio of aquafaba to oil can impact the texture of the mayonnaise, therefore it is important to have consistent ratio for quality control.

4.9 Seafood Analogs

As consumer awareness and interest in more sustainable foods increase, seafood analogs are starting to emerge. The most common crab analog in the market is surimi, however its main component is fish, which is still in the seafood category. Starting from the appearance, seafood usually has a white color especially fish, which makes it harder to mimic because plant-based ingredients usually have a more yellowish or greenish color. However, with some seafood products that are deep fried, additional flour coating on the surface may help to hide such appearance. For flavor, seafood analogs are expected to have a 'sea' flavor that is hard to mimic and that mix of flavorings might be needed to produce the right flavor quality for the product or risk off-flavors of 'old fish.' As the muscle fiber alignment for seafood is different from other meat analogs, the way to resemble a seafood muscle will be different from meat analog. Moreover, within the seafood category, fish, crab, and shrimp all have different texture, therefore the expectation of how it shreds apart would be different. Like other plant-based muscle analogs, texture analysis is usually conducted to check the tensile strength of the product to reach a targeted texture. Most often, seafoods are more homogenized gel compared to the fibrous texture found in whole muscle meat analogs. Therefore plant-based seafood analogues commonly use hydrocolloids such as konjac flour, alginate, and carrageenan in creating such analogs to optimize the texture. Patented seafood analog had suggested using pea protein with konjac and fenugreek to produce meatless fish stick, tuna, and salmon (Wang 2020). Although there have been only a few publications specific to seafood analogues, with increasing interest in plant-based products, more research on plant-based seafood analog is expected.

4.10 Traditional Plant-Based Foods

Historically established vegetarian products are usually the center of the plate as protein source, which includes products like tofu, seitan and tempeh. Although they can still be seen in the market, more and more new plant-based alternatives are introduced. These traditional plant-based foods do not tend to mimic animal products, therefore should not be called analogs.

While new plant-based alternatives are trying to mimic the flavor and texture of animal protein, the traditional plant-based products should be evaluated with different sensory questions. Among these traditional plant-based foods, each has their protein structure, therefore, they have different quality characteristics to control. For example, tofu by coagulation of soy proteins, results in a soft semi-solid gel structure while tempeh was formed into a soy cake surrounded by mycelium by fermentation. On the other hand, seitan has a firm, fibrous structure that is attributed to a very tight gluten matrix. The different formulated structures result in a variety of different perceived textural qualities. Moreover, these foods have unique tastes attributed to the ingredients and processes (which may include fermentation). Therefore, each of the traditional plant-based food should be assessed individually. In some consumer demographics, certain attributes may be more accepting. For example, the beany flavor of soy products is widely accepted in Asian countries. Yet novel approaches have been made to further improve the quality of traditional vegetarian protein foods. For example, Yang et al. removed lipoxygenases from soybeans to produce tofu for better flavor (Yang et al. 2015). By using descriptive analysis, off-flavors designated to soybeans including fresh pea aroma, grassy rancid aroma are rated to measure effect of using lipoxygenase lacking soybeans in the tofu production. Tempeh has also been modified by adding *Saccharomyces cerevisiae* to the fermentation process to reduce the beany aroma from soybean, however, this method reportedly only works in fried tempeh applications (not in raw tempeh). This might be due to volatile compounds being generated during the frying process from the *Saccharomyces* (Kustyawati et al. 2017). Additional studies further support that the overall acceptability of deep-fried tempeh scored significantly higher in all aspects including color, texture, taste, odor, and mouthfeel than stewed tempeh (Refaat et al. 2018).

5 Conclusion

As technology advances for the processing of plant-based materials into novel applications, it is imperative that instrumental and sensorial methods are integrated to help maximize quality attributes that are expected by consumers. Leveraging physical characterization and sensory evaluation methods can help to guide the critical quality components throughout the development life cycle to optimize quality efficiently. Through physicochemical characterization, many prototypes can be investigated efficiently to assess key quality attributes, including appearance, color, mouthfeel, taste, flavor, water and oil holding capacity, and emulsifying properties. Sensory experiments then determine consumer segments and overall consumer acceptability. Please note, however, that it is imperative sensory experiments make careful consideration into the experimental design as the consumer segment within the plant-based category is widely segmented (i.e., full vegan diets, flexitarians and full meat-based diet).

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Chapter 12
Amino Acid Profile and Bioavailability of Plant-Based Protein-Rich Products

Alan Javier Hernández-Álvarez, Matthew G. Nosworthy, and Martin Mondor

1 Introduction

Amino acids are the building blocks of polypeptides and proteins which play many critical roles in human body. Amino acids are classified according to the side chain group type, core functional groups' location, polarity or pH level, but for nutritional purposes, amino acids are arranged in essential, non-essential and conditionally essential amino acids (Bhutta and Sadiq 2013). During gastrointestinal digestion (GID), proteins are hydrolyzed to small peptides and amino acids so that these can be absorbed. GID involves a coordinated series of events that includes proteolytic enzymes interaction with proteins to form smaller molecules that can be absorbed and delivered into the bloodstream. Gastric and pancreatic enzymes, like pepsin, trypsin, chymotrypsin, elastase and carboxypeptidases A and B, are the main responsible for food protein breakdown (Fig. 12.1) (Bhutta and Sadiq 2013). Nine amino acids are required for human growth and maintenance (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) which cannot be synthetized by the body, thus they must be obtained from a wide variety

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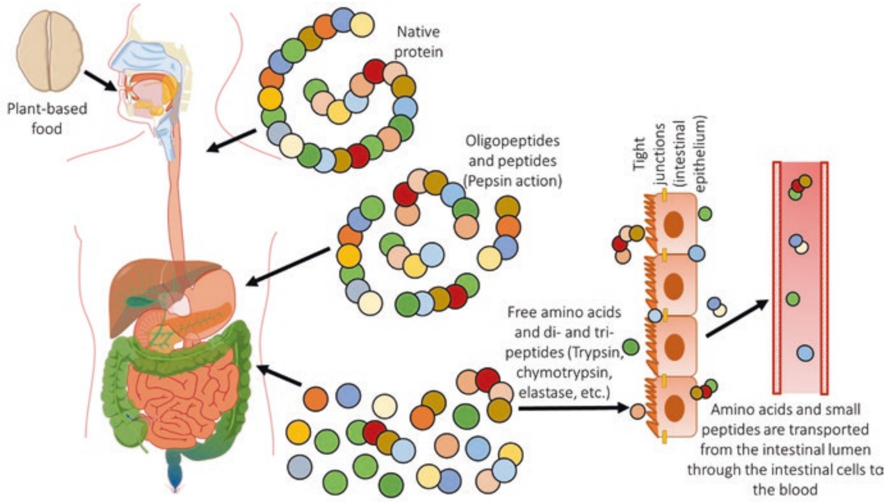

Fig. 12.1 Protein digestion and absorption

of food sources (Bhutta and Sadiq 2013). On the other hand, non-essential amino acids (alanine, arginine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine) are synthetized by most of the human body cells during all stages of life, even if they are not acquired from diet (Puigserver 2018). Meanwhile, conditionally essential amino acids (arginine, cysteine, histidine and tyrosine) are synthetized by the human body in adults, but they are needed when there is an illness, stress conditions, and during child growth (FAO/WHO/UNU 1985; USDA 2019). When dietary proteins are broken down by gastrointestinal digestion, free amino acids and small peptides are produced. Proteins provide approximately 14–18% of total food energy intake, about 65% of which are of animal origin (meat, dairy and eggs) (USDA 2019). However, grains and cereals are important protein supplies, since they comprise an average of 16–20% of dietary protein intake around the world (RDA 1989).

Legumes, such as soybean, pea, chickpea, among others, contain about 17–25% protein, with a high predominance of globulins (35–72%) and albumins (30–50%). Globulins have important quantities of arginine, asparagine, aspartic acid and lysine, while albumins are rich in cysteine, lysine and methionine. But overall, pulses' proteins have limiting amounts of methionine, cysteine and tryptophan (Singh 2017). Cereals contain about 6–20% protein (Goldberg 2003), but the protein fractions vary between crops. In wheat, gliadins and glutenins are the major protein fractions, while in rice it is oryzenin (glutelin), maize it is zein (prolamin), barley proteins are predominantly hordeins (prolamin) and glutelins, and oats are globulins and prolamins (avenin) (Kulp and Ponte 2000). In cereals, globulins have higher amount of essential amino acids than prolamins (40–80%), since prolamins mainly consist of proline and glutamine oligopeptides (30–70%) (Shewry and Halford 2002; Mäkinen et al. 2017). Glutelins (>45%) consist of hydrophobic amino acids (leucine, phenylalanine, proline, valine and tyrosine) (Ewart 1967), while albumins (*N*-terminal peptides) are rich in cysteine (>10%) (Mitra et al. 1979).

In most cereals, lysine and threonine (EAA) are considered as *limiting amino acids*, as well as tryptophan in maize, while in pulses methionine and cysteine are included in this category. Thus, while protein intake as a blend of different protein food sources (animal and plant) is recommended, a diet based on a combination of cereals and legumes results in a good protein quality diet, by balancing the deficiency of some amino acids in these protein sources. This is known as *supplementary effect* of protein sources (Hayward and Hafner 1941; Puigserver 2018). However, farming technologies may also help to increase the content of certain amino acids of nutritional interest. For example, a genetically enhanced barley variety showed an increase of 55% in lysine, present mainly in albumin + globulin protein fractions, compared to the wild type NP 113 barley variety (Joshi et al. 1988).

In this book chapter, we will review the amino acid composition and bioavailability of different plant-based proteins and of their derived food products, the assessment of protein content claims, the health benefits of amino acids, as well as factors affecting amino acids bioavailability and how to measure it.

2 Amino Acid Composition of Different Plant-Based Proteins and of Their Derived Food Products

The protein and essential amino acid content of certain unprocessed and processed plant protein material and plant protein processed foods are presented in Table 12.1, while the non-essential amino acids are presented in Table 12.2. The products discussed range from high protein pasta and spinach (Filip and Vidirh 2015), dry and cooked pulses (Nosworthy et al. 2017), soy ingredients and chips, wheat flour bread, and sausage meatless (United Stated Department of Agriculture. FoodData Central n.d.), broccoli (Kmiecik et al. 2010), plant-based protein isolates (Carrasco-Castilla et al. 2012; Sánchez-Velázquez et al. 2021; Sánchez-Vioque et al. 1999) and a commercially available food – Huel (2020). The study involving high protein pasta and high protein spinach accomplished this increased protein content by including pea protein isolate into the formulation (Filip and Vidrih 2015). Although there is a reduction in protein content on an as-is basis after cooking, this pasta still retains a higher protein content than that of cooked pulses as determined by Nosworthy et al. (2017), and much higher than that of broccoli alone (Kmiecik et al. 2010). The protein present in the commercially available product Huel is derived from pea protein, oats, brown rice protein, flaxseed, and medium chain triglyceride (MCT) powder (Huel 2020). Although there are five different protein containing ingredients, it is the combination of pea and rice protein that serve as the primary protein sources and, due to their complimentary amino acid profile, result in the relatively high essential amino acid content of the product.

Table 12.1 Protein content and essential amino acid composition of plant protein sources (g/100 g)

	Protein content	Trp	Met	Thr	Val	Iso	Leu	Phe	Lys	His	Arg
	Plant protein material – unprocessed										
High Protein Spinach[a]											
Dry	39.60	0.32	0.36	1.30	4.90	1.70	3.10	2.10	2.50	0.90	3.10
Red Kidney Beans[b]											
Dry	23.94	0.22	0.24	1.05	0.96	0.79	1.80	1.24	1.61	0.66	1.15
Navy Beans[b]											
Dry	24.52	0.23	0.30	1.10	1.14	0.94	1.94	1.40	1.70	0.67	1.28
Whole Green Lentils[b]											
Dry	26.27	0.21	0.21	1.11	1.15	1.01	2.12	1.38	2.13	0.70	2.25
Split Red Lentils[b]											
Dry	29.51	0.26	0.22	1.23	1.36	1.18	2.48	1.63	2.21	0.80	2.40
Split Yellow Peas[b]											
Dry	25.26	0.20	0.26	0.96	1.10	0.98	1.84	1.19	1.82	0.61	1.93
Split Green Peas[b]											
Dry	26.24	0.26	0.19	1.01	1.04	0.87	1.96	1.31	1.85	0.65	1.89
Black Beans[b]											
Dry	23.95	0.25	0.25	1.26	1.17	1.00	2.12	1.43	1.81	0.73	1.41
Chick Peas[b]											
Dry	21.91	0.15	0.30	0.89	1.06	1.00	1.85	1.44	1.62	0.64	2.09
Pinto Beans[b]											
Dry	22.68	0.19	0.27	1.06	1.04	0.90	1.90	1.27	1.66	0.67	1.22
Soy Beans[c]											
Dry	12.95	0.16	0.16	0.52	0.58	0.57	0.93	0.59	0.78	0.35	1.04
Broccoli[d]											
Raw	2.85	nd	0.07	0.13	0.16	0.12	0.22	0.12	0.22	0.09	0.18
	Plant protein material – processed										
High Protein Spinach[a]											
Cooked	17.20	0.15	0.17	0.73	0.90	0.78	1.40	1.00	1.10	0.42	1.40
Red Kidney Beans[b]											
Cooked	8.27	0.08	0.08	0.36	0.33	0.27	0.62	0.43	0.56	0.23	0.40
Navy Beans[b]											
Cooked	8.76	0.08	0.11	0.39	0.41	0.33	0.69	0.50	0.61	0.24	0.46
Whole Green Lentils[b]											
Cooked	6.72	0.05	0.05	0.28	0.29	0.26	0.54	0.35	0.54	0.18	0.58
Split Red Lentils[b]											
Cooked	7.30	0.06	0.05	0.30	0.34	0.29	0.61	0.40	0.55	0.20	0.59
Split Yellow Peas[b]											

(continued)

Table 12.1 (continued)

	Protein content	Trp	Met	Thr	Val	Iso	Leu	Phe	Lys	His	Arg
Cooked	6.81	0.05	0.07	0.26	0.30	0.26	0.50	0.32	0.49	0.16	0.52
Split Green Peas[b]											
Cooked	7.39	0.07	0.05	0.28	0.29	0.25	0.55	0.37	0.52	0.18	0.53
Black Beans[b]											
Cooked	8.39	0.09	0.09	0.44	0.41	0.35	0.74	0.50	0.63	0.26	0.50
Chick Peas[b]											
Cooked	7.57	0.05	0.10	0.31	0.37	0.35	0.64	0.50	0.56	0.22	0.72
Pinto Beans[b]											
Cooked	7.85	0.07	0.09	0.37	0.36	0.31	0.66	0.44	0.57	0.23	0.42
Black Bean protein isolate[e]											
	88	0.2	0.8	4.2	5.7	5.5	9.9	6.8	7.4	3.5	6.0
Chick Pea protein isolate[f]											
	88.1	nd	1.6	4.3	6.0	6.3	10.7	8.5	7.4	3.3	11.8
Soy protein flour (defatted)[c]											
	51.10	0.62	0.62	1.97	2.31	2.31	4.11	2.86	3.06	1.27	3.93
Soy protein isolate[c]											
	88.32	1.12	1.13	3.14	4.10	4.25	6.78	4.59	5.33	2.30	6.67
Oat protein flour[g]											
	15.85	1.50	0.64	3.31	3.43	nd	6.80	5.22	3.40	1.84	6.29
Oat protein isolate[g]											
	87.24	0.69	0.99	3.35	4.14	nd	7.93	6.54	3.53	2.08	7.35
Oat protein isolate[g]											
Cooked	87.24	0.55	2.45	2.83	5.64	nd	8.53	6.58	3.40	2.58	7.14
Broccoli[d]											
Cooked	2.51	nd	0.06	0.10	0.15	0.11	0.19	0.10	0.20	0.08	0.15
	Plant protein processed foods										
High Protein Pasta[a]											
Dry	36.40	0.32	0.37	1.20	1.60	1.40	2.60	1.80	2.10	0.78	2.60
Cooked	15.60	0.15	0.18	0.57	0.81	0.72	1.30	0.90	1.10	0.39	1.30
Soy chips[c]											
	26.5	0.38	0.38	1.13	1.34	1.26	2.14	1.37	1.68	0.70	2.07
Wheat flour bread[c]	11.98	0.14	0.21	0.32	0.50	0.44	0.83	0.59	0.23	0.25	0.42
Sausage meatless[c]											
	20.28	0.28	0.25	0.79	1.03	0.97	1.59	1.06	1.26	0.52	1.52
Huel Vanilla Protein Powder[h]	30.00	0.36	0.48	1.07	1.51	1.17	2.31	1.56	1.66	0.98	2.30

[a]Filip and Vidirh (2015), [b]Nosworthy et al. (2017), [c]United Stated Department of Agriculture. FoodData Central (n.d.), [d]Kmiecik et al. (2010), [e]Carrasco-Castilla et al. (2012), [f]Sánchez-Vioque et al. (1999), [g]Sánchez-Velázquez et al. (2021), [h]Huel (2020)

Table 12.2 Non essential and conditionally essential amino acid composition of plant protein sources (g/100 g)

	Cys	Asn	Ser	Gln	Pro	Gly	Ala	Tyr
	Plant protein material – unprocessed							
High Protein Spinach[a]								
Dry	0.35	3.70	1.90	7.80	2.40	1.50	1.50	1.10
Red Kidney Beans[b]								
Dry	0.18	2.85	1.56	3.62	1.00	0.98	1.06	0.65
Navy Beans[b]								
Dry	0.24	2.89	1.56	3.43	1.02	1.01	1.08	0.70
Whole Green Lentils[b]								
Dry	0.26	3.37	1.50	4.84	1.25	1.24	1.27	0.85
Split Red Lentils[b]								
Dry	0.22	3.71	1.80	5.18	1.35	1.27	1.37	0.92
Split Yellow Peas[b]								
Dry	0.31	2.86	1.25	4.08	1.04	1.08	1.09	0.73
Split Green Peas[b]								
Dry	0.20	3.13	1.53	4.46	1.17	1.12	1.18	0.69
Black Beans[b]								
Dry	0.21	3.23	1.78	3.90	1.11	1.12	1.21	0.78
Chick Peas[b]								
Dry	0.29	2.89	1.29	4.01	1.03	0.98	1.05	0.62
Pinto Beans[b]								
Dry	0.21	2.84	1.54	3.51	0.98	1.00	1.07	0.70
Soy Beans[c]								
Dry	0.12	1.51	0.72	2.43	0.61	0.54	0.58	0.46
Broccoli[d]								
Raw	0.05	0.31	0.15	0.5	0.17	0.14	0.15	0.08
	Plant protein material – processed							
High Protein Spinach[a]								
Cooked	0.17	1.69	0.90	3.60	1.10	0.67	0.70	0.50
Red Kidney Beans[b]								
Cooked	0.06	0.98	0.54	1.25	0.35	0.34	0.37	0.22
Navy Beans[b]								
Cooked	0.08	1.03	0.56	1.23	0.37	0.36	0.39	0.25
Whole Green Lentils[b]								
Cooked	0.07	0.86	0.38	1.24	0.32	0.32	0.33	0.22
Split Red Lentils[b]								
Cooked	0.05	0.92	0.44	1.28	0.33	0.32	0.34	0.23
Split Yellow Peas[b]								
Cooked	0.08	0.77	0.34	1.10	0.28	0.29	0.29	0.20
Split Green Peas[b]								
Cooked	0.06	0.88	0.43	1.26	0.33	0.32	0.33	0.19
Black Beans[b]								

(continued)

Table 12.2 (continued)

	Cys	Asn	Ser	Gln	Pro	Gly	Ala	Tyr
Cooked	0.07	1.13	0.62	1.37	0.39	0.39	0.43	0.27
Chick Peas[b]								
Cooked	0.10	1.00	0.45	1.39	0.36	0.34	0.36	0.21
Pinto Beans[b]								
Cooked	0.07	0.98	0.53	1.21	0.34	0.35	0.37	0.24
Black Bean protein isolate[e]								
	0.3	11.3	7.1	17.1	2.0	4.3	4.1	4.1
Chick Pea protein isolate[f]								
	1.2	13.7	7.1	19.1	nd	4.7	5.3	3.8
Soy protein flour (defatted)[c]								
	1.01	4.98	2.70	9.08	2.84	1.79	2.86	1.74
Soy protein isolate[c]								
	1.05	10.20	4.59	17.45	4.96	3.60	3.59	3.22
Oat protein flour[g]								
	1.70	9.18	5.01	23.52	nd	4.61	4.76	3.50
Oat protein isolate[g]								
	1.20	9.41	5.01	27.97	nd	3.87	4.80	3.75
Oat protein isolate[g]								
Cooked	2.37	6.99	3.38	23.53	nd	4.30	4.15	3.52
Broccoli[d]								
Cooked	0.05	0.25	0.11	0.5	0.18	0.13	0.13	0.06
	Plant protein processed foods							
High Protein Pasta[a]								
Dry	0.35	3.10	1.70	6.90	2.20	1.20	1.30	0.95
Cooked	0.17	1.60	0.82	3.30	1.00	0.61	0.64	0.51
Soy chips[c]								
	0.43	3.21	1.50	5.06	1.50	1.21	1.26	1.03
Wheat flour bread[c]								
	0.27	0.48	0.58	4.20	1.41	0.41	0.37	0.33
Sausage meatless[c]								
	0.31	2.31	1.09	4.22	1.12	0.82	0.85	0.69
Huel Vanilla Protein Powder[h]								
	0.45	3.05	1.49	5.32	1.3	1.29	1.36	1.14

[a]Filip and Vidirh (2015), [b]Nosworthy et al. (2017), [c]United Stated Department of Agriculture. FoodData Central (n.d.), [d]Kmiecik et al. (2010), [e]Carrasco-Castilla et al. (2012), [f]Sánchez-Vioque et al. (1999), [g]Sánchez-Velázquez et al. (2021). Supplementary data, [h]Huel (2020)

Recently, Gorrissen et al. (2018) compared the amino acid composition of a large selection of plant-based protein sources (oat, lupin, wheat, hemp, microalgae, soy, brown rice, pea, corn, potato) with animal-based proteins (milk, whey, caseinate, casein, egg). The WHO/FAO/UNU recommend, for an adult, a protein intake of 0.66 g/kg body weight/day. Based on that protein intake, the essential amino acid contents of the plant-based proteins from oat (21%), lupin (21%), wheat (22%), hemp (23%) and microalgae (23%) are below the WHO/FAO/UNU amino acid requirements, while those of soy (27%), brown rice (28%), pea (30%), corn (32%) and potato (37%) meet the requirements. All animal-based proteins meet the requirements: milk (39%), whey (43%), caseinate (38%), casein (34%) and egg (32%). It was also observed that there is a large difference among plant-based proteins in terms of their amino acid profile with, for example, leucine contents ranging from 5.1% for hemp to 13.5% for corn protein. When compared to animal-based proteins, methionine and lysine are found in lower amounts in plant-based proteins (1.0% ± 0.3% and 3.6% ± 0.6% vs 2.5% ± 0.1% and 7.0% ± 0.6%, respectively). The authors concluded that amino acid profiles similar to those of animal-based proteins could be obtained by combining various plant-based protein isolates or blends of animal and plant-based proteins.

2.1 *Impact of Supplementation on the Amino Acid Profile of Food Products*

2.1.1 Bread

Supplementation of bread with plant protein ingredients to improve its protein content has been the topic of a number of studies recently (Crockett et al. 2011; El-Shafei et al. 1983; El-Sohaimy et al. 2019; Erben and Osella 2017; Mondor et al. 2014; Mubarak 2001; Serventi et al. 2018; Villeneuve and Mondor 2014; Villeneuve et al. 2015; Zhou et al. 2018), but only a few of them discussed the impact of supplementation on the amino acid profile of the resulting bread. El-Shafei et al. (1983) determined the profile of lysine and essential amino acids in corn flour and corn bread. The results indicated that corn flour contained higher amounts of threonine, leucine, phenylalanine, lysine, histidine and arginine, with respective values (g/100 g) of 0.428, 0.112, 0.246, 0.128, 0.233 and 0.175, compared to 0.368, 0.094, 0.153, 0.086, 0.117 and 0.088 in corn bread. Corn bread was richer in valine, methionine and isoleucine, with respective values (g/100 g) of 0.121, 0.443 and 0.675, compared to 0.077, 0.269, and 0.404 in corn flour. Mubarak (2001) substituted wheat flour bread with various ingredients derived from Sweet lupin (*Lupinus albus*) seed (flour, protein isolate 1, protein isolate 2, and protein concentrate). The protein contents of the various ingredients were the following: 34.9% for the lupin flour, 84.1% for lupin protein isolate 1; 86.2% for lupin protein isolate 2; and 38.8% for the lupin protein concentrate. Supplementation of the wheat flour bread increased the protein content of the resulting bread, except for the bread substituted with lupin flour, for which the increase was not significant (12.6% for wheat bread flour; 14.0% for bread substituted with lupin flour, 19.1% for bread substituted with lupin protein isolate 1; 19.3% for the bread substituted with lupin protein isolate 2; and 14.2% for bread substituted with lupin protein concentrate). The total amino acid contents (g amino acid/16 g nitrogen) of the substituted bread were also increased when compared to the control bread (34.67 for the wheat bread flour; 35.21 for bread substituted with lupin flour; 36.61 for bread substituted with lupin protein isolate 1; 38.52 for the bread substituted with lupin protein isolate 2; and 36.21 for bread substituted with lupin protein concentrate). In terms of quality, no detrimental effect was observed on bread sensory properties, and no significant difference was recorded in loaf volume. El-Sohaimy et al. (2019) studied the impact of supplementing wheat flour flat bread with quinoa flour on its nutritional quality. The levels of substitution were 5%, 10%, 15%, 20%, 25% and 30% with quinoa flour. The bread protein content was increased from 12.12% ± 0.63% in the control (100% wheat bread) to 15.85% ± 0.06% with 30% quinoa flour. As expected, the total amino acid content also increased with increasing levels of substitution (12.07 g/100 g for the control bread vs 13.78 g/100 g for the 30% quinoa flour bread). Results in terms of specific volume, appearance, crust and crumb texture, aroma-odor and colour were evaluated and found to be excellent. The authors concluded that quinoa flour is a promising ingredient for the supplementation of wheat flat bread.

2.1.2 Pasta

Many papers have reported the supplementation of pasta with plant protein ingredients to improve their protein contents (Alireza Sadeghi and Bhagya 2008; Baiano et al. 2011; Carini et al. 2012; de la Pena and Manthey 2014; Filip and Vidrih 2015; Gallegos-Infante et al. 2010; Giménez et al. 2016; Howard et al. 2011; Jayasena and Nasar-Abbas 2012; Laleg et al. 2016a, b, 2017, 2019; Madhumitha and Prabhasankar 2011; Martínez-Villaluenga et al. 2010; Mercier et al. 2016; Petitot et al. 2010; Sabanis et al. 2006; Shreenithee and Prabhasankar 2013; Sinha and Manthey 2008; Torres et al. 2007; Ugarcic-Hardi et al. 2003; Villeneuve et al. 2013; Zhao et al. 2005). However, only a few of them discussed the impact of supplementation on the amino acid profiles of the resulting pasta. In their work, Martinez-Villaluenga et al. (2010) studied the impact of supplementing pasta made from durum wheat semolina with 10% germinated pea flour on the amino acid profile of the pasta. The method described by Frias et al. (2005) was applied for the germination of the pigeon pea seeds (20 °C, 90% relative humidity) for 4 days in the dark. The essential amino acid content of the pasta was not affected by the substitution for most essential amino acids (Histidine; Valine; Methionine+Cysteine; Isoleucine; Phenylalanine+Tyrosine; Tryptophan). However, the contents of leucine, lysine and threonine were significantly higher for the supplemented pasta, with respective values (g/16 g N) of 7.47, 3.79 and 3.47, compared to 7.19, 2.39 and 2.81 for the control pasta. Filip and Vidrih (2015) studied the impact of supplementing durum wheat semolina with pea protein isolate at a level of 40% on the pasta's amino acid profile. Dry pasta had a protein content of 36.4 ± 1.8 g/100 g of DM, which is high compared to pasta made from 100% durum wheat semolina, which has a protein content of about 10%. After cooking, the protein decreased to 15.6 ± 1.1 g/100 g of DM. The total essential amino acids in the supplemented pasta was 12.1 ± 0.3 g/100 g of DM, while ordinary durum pasta contains about 5.3 g/100 g of DM. The two most deficient amino acids in wheat are lysine and threonine. Supplementation of durum wheat semolina with pea protein isolate significantly increased the lysine content from 0.37 to 2.07–2.50 g/100 g of DM and the threonine content from 0.47 to 1.17–1.30 g/100 g of DM. Sensory analysis data indicated that the supplementation of durum wheat semolina with 40% of pea protein isolate satisfied sensory and nutritional requirements, allowing further development and evaluation for possible marketing. Laleg et al. (2016a, 2019) studied the impact of substituting wheat pasta with 35% faba bean flour on the protein digestibility and the amino acid profile of the pasta. They also studied the effect of low-temperature (55 °C, LT) vs very-high-temperature (90 °C, VHT) drying on the protein network structure and digestibility. They observed that the total essential amino acids was higher for the substituted pasta than for the control pasta (334 vs 294 mg/g protein) (Laleg et al. 2016a). The amino acid profile of pasta supplemented with faba bean flour was found to be better than that of the control pasta, with a high lysine content even when dried at a very high temperature (Laleg et al. 2016a, 2019). Supplemented pasta also showed a higher protein digestibility.

2.1.3 Sausages

Another food product of interest that is regularly supplemented with plant protein ingredients is sausages (Abo Bakr 1987; Ahmad et al. 2010; Ahn et al. 1999; Lee et al. 2017; Marti-Quijal et al. 2019a; Mokni Ghribi et al. 2018; Ramezani et al. 2003; Thirumdas et al. 2018; Wambui et al. 2017). Abo Bakr (1987) determined the amino acid composition of three sausage meat products, including two products that were partially supplemented with 20% chickpeas or 20% faba beans. They found that the total amino acid contents (g/16 g nitrogen) were 44.91 for the 100% sausage meat product, 42.77 for the product substituted with 20% faba beans, and 42.37 for the product substituted with 20% chickpeas. All products showed high levels of the essential amino acids when compared with the FAO/WHO reference patterns. Thirumdas et al. (2018) studied the protein content and the amino acid profile of fermented Spanish “chorizo” sausages supplemented with beans, lentils and broad beans, compared to sausages with soy protein. Protein content was significantly higher in the sausages with soy protein (35.62%) and broad beans (34.66%) compared to the samples enriched with protein from beans (31.81%) and lentils (30.56%). In terms of their amino acid profile, no significant difference was observed among the various sausages. The authors concluded that protein extracted from beans, lentils and broad beans can be used to enrich “chorizo” as an alternative to soy protein. Marti-Quijal et al. (2019a) evaluated the impact of adding vegetable protein sources (beans, peas and lentils) to the protein content and the amino acid profiles of pork sausages. Pork sausages with added soy protein were used as the control. The protein contents (%) of the sausages were 15.40 ± 0.18 for the control, 14.68 ± 0.26 for the sausages supplemented with peas, and 14.90 ± 0.23 and 14.80 ± 0.37 for the sausages supplemented with lentils and broad beans, respectively. When compared to the control, the sausages supplemented with peas and broad beans showed a significantly lower protein content. No significant difference was observed among the different sausage products in terms of total amino acid content and in terms of essential amino acid content. Considering texture traits (chewiness, gumminess and hardness), physicochemical parameters (pH and colour) and amino acid profiles across treatments, proteins from legumes provided profiles close to that of soy.

2.1.4 Other Food Products

Burgers: Marti-Quijal et al. (2019b) prepared turkey burgers supplemented at a level of 1% with soy, pea, lentil or broad bean. The protein content of the burgers was around 15%. The total amino acid contents expressed in g/100 g were 10.66 ± 0.81, 8.74 ± 1.37, 9.26 ± 1.66 and 12.53 ± 1.56 for the burgers substituted with soy, pea, lentil and broad bean, respectively. Only the burger substituted with broad bean had a total amino acid content significantly higher than the content of the other burgers. The taste was found to be similar among the different burgers. The burgers made with pea protein presented the highest values for pH and lightness, whereas those prepared with broad bean showed the highest redness.

Bars, cookies and muffins: A few studies on the enrichment of bars, cookies or muffins with plant protein ingredients can be found in the scientific literature (Amin et al. 2016; Bashir et al. 2015; Childs et al. 2007; James et al. 1989; Jarpa-Parra et al. 2017; Mohsen et al. 2009; Serrem et al. 2011; Shaabani et al. 2018; Shevkani and Singh 2014; Tang and Liu 2017; Watanabe et al. 2014). Serrem et al. (2011) studied the impact of various combinations of sorghum flour with defatted soy flour (100:0; 71.4:28.6; 50:50; 28.6:71.4) and various combinations of wheat flour with defatted soy flour (100:0; 71.4:28.6; 50:50; 28.6:71.4) on the nutritional value of cookies. Cookies made from 100% defatted soy flour were also prepared. Compared to the 100%-wheat-flour cookies, sorghum-soy and wheat-soy 50:50 ratio cookies had at least double the protein content, and the lysine content increased by between 500% and 700%. Composite cookies were rated as being as acceptable as the 100%-wheat cookies by school children over 4 days of evaluation. Watanabe et al. (2014) studied the impact of substituting wheat flour with quinoa flour, at levels of 7.5% and 15%, on the amino acid content of cookies. Quinoa substitution at a level of 15% resulted in an increase in the lysine and threonine contents (residues/1000 residues) when compared to the control, with respective values of 18 ± 1 (lysine control cookie) vs 24 ± 1 (lysine 15% quinoa cookie) and 30 ± 3 (threonine control cookie) vs 34 ± 1 (threonine quinoa cookie). Sensory evaluation indicated that the quinoa cookies were acceptable from an organoleptic point of view. Hence, the authors concluded that plant ingredients have considerable potential as protein-rich supplementary foods.

Drinks: Childs et al. (2007), Tan et al. (2018) and Bonke et al. (2020) have reported on the production of plant drinks. Tan et al. (2018) studied the amino acid profiles of three chocolate drink (50 g carbohydrate), each with 24 g of oat, pea or rice proteins added. Total amino acids (g/24 g protein) were 22.22 for the oat drink, 23.18 for the pea drink and 24.74 for the rice drink. However, the highest lysine content (g/24 g protein) was found in the pea drink, at 1.54, compared to only 0.80 and 0.66 for the rice drink and the oat drink, respectively. Bonke et al. (2020) tested different combinations of the following plant-based ingredients to prepare plant drinks with a balanced amino acid profile: whole-grain oat flour, pea (*Pisum sativum*) protein concentrate with 80% protein, and lentil (*Lens culinaris*) concentrate with 51% protein. A plant drink with 3.1% lentil concentrate, 2.0% pea protein isolate and 6.0% whole-grain oat flour had a total of 1664 mg/100 mL essential amino acids, while a plant drink with 4.2% lentil concentrate, 1.3% pea protein isolate and 6.0% whole-grain oat flour had a total of 1545 mg/100 mL essential amino acids. These were the two drinks with the highest total amino acids. Plant drinks with 6.3% lentil concentrate and 6.0% whole-grain oat flour had a total of only 789 mg/100 mL essential amino acids and were those with the lowest amino acid concentrations. An assessment of stability and sensory parameters was also conducted, and the authors concluded that there was an advantage of combining oat with pea.

3 Assessment of Protein Content Claims

Since the last decades, the scientific community has sought to establish rapid, easily, accurate and precise methods for assessing protein quality in digested foods for multiple purposes (Sarwar 1987). These methods must measure the basic parameters of protein quality being applicable to a wide range of foods, including protein digestibility, as well as bioavailability of essential and non-essential amino acids (Sarwar 1987).

The evaluation of protein quality, and subsequent assessment of content claim validity, is different depending on the jurisdiction being discussed. In North America, Health Canada requires the use of the Protein Efficiency Ratio (Health Canada 1981) while the United States Food and Drug Administration mandates the use of corrected protein level as % Daily Value through the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (21CFR101.9, USFDA). In Europe the basis for protein quality assessment is the amount that the protein content contributes to total energy present in the product (European Commission 2006), while in Australia it relies on the quantity of protein present in each serving (Food Standards Australia New Zealand 2015). There is also a more recent system for protein quality assessment based on the Digestible Indispensable Amino Acid Score (DIAAS) (FAO/WHO 2013), which is yet to be adopted by any jurisdiction for regulatory purposes. Additional information regarding these assessment methods are provided below.

3.1 Protein Rating System

In Canada the method for identifying whether a product meets the criteria for a protein content claim is the Protein Rating System (Government of Canada 2016). Prior to calculating the Protein Rating of a product, the Protein Efficiency Ratio (PER) must be determined. PER is a measurement of growth/weight gain per unit of protein consumed using a rodent feeding trial (Health Canada 1981). Briefly, young rats are fed with diets containing 10% protein by weight for 4 weeks, with diet consumption and weight gain being recorded. In addition to experimental samples, casein is also run in tandem with each experimental trial to identify any inter-trial variation and to act as a standardizing factor. After completing the trial, the PER of all samples is calculated by dividing the weight gain by the mass of protein consumed. An Adjusted PER is subsequently calculated by dividing the $PER_{Experimental}$ by the PER_{Casein} and multiplying by a standardized factor of 2.5, which is the average PER value of casein. It is this Adjusted PER that is used in the calculation of the Protein Rating. Protein rating is the product of multiplying the Adjusted PER and the quantity of protein in the Reasonable Daily Intake. If the resulting Rating is greater than 20, the food is considered to be a 'Good Source' of protein, with 'Excellent Source' of protein being granted if the Protein Rating is greater than 40.

The advantages that PER has over PDCAAS and DIAAS are twofold. PER is a much easier method to use because the only required measurements are protein consumption and weight gain. This protein quality measurement is also the only one that provides an indication of growth, which is essential for certain therapeutic foods and infant formulas. PER, however, is not without concerns. Standardization to casein for generation of Adjusted PER can impact the PER of the experimental protein due to inter-lab variation in casein measurement. This measurement also assumes that all energy is being devoted to growth and not maintenance of normal metabolic processes. Finally, PER mandates the use of a rodent assay. Since the amino acid requirement of rats is different from that of humans, concern has been raised as to whether the growth rates determined through this assay accurately reflects the growth rates of humans consuming the same protein.

3.2 Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The PDCAAS was introduced by the FAO/WHO in 1991 (FAO/WHO 1991) and has been used by the United States of America as their metric for protein quality since 1993 (21CFR101.9, USFDA). This method requires the quantification of fecal nitrogen digestibility in a rodent model corrected for endogenous protein loss, and the generation of an amino acid score (FAO/WHO 1991). The amino acid score is quantified by comparing the amino acid profile of the test protein with the reference pattern for children 2–5 years old outlined by the FAO/WHO in 1991. The lowest essential amino acid ratio value is considered the amino acid score, with the product of that value and the fecal nitrogen digestibility being PDCAAS. Protein content claims in the United States of America require the use of this PDCAAS value in further calculations. Initially, the corrected protein level in a food is generated by multiplying the PDCAAS and the protein content per reference amount customarily consumed (RACC). Subsequently, this corrected protein level is compared against a daily value (DV) of 50 g of protein to generate %DV. Should the %DV be greater than 10 the food is considered to be a 'Good Source' of protein, and if the %DV is greater than 20% the food is an 'Excellent Source' of protein (21CFR101.9, USFDA).

As with PER, there are advantages and disadvantages to PDCAAS as a metric for protein quality. Most notably, PDCAAS provides detailed information regarding the amino acid composition and digestibility of protein sources, compared to the growth measurement of PER. Concerns have been raised, however, by the FAO/WHO regarding the utility and validity of PDCAAS (FAO/WHO 2007). PDCAAS values are truncated to 1.00, meaning that no test protein can have a higher value than the reference protein, unlike PER where the final value can be above that of casein (2.5). Fecal protein digestibility is used in calculating PDCAAS, which is not an accurate representation of digestibility at the terminal ileum – the last point at which dietary amino acids are absorbed due to the activity of microflora in the colon. Specific amino acids, such as lysine and the sulfur amino acids, can be overestimated by not considering Maillard reactions and oxidation (Moughan 2005).

3.3 Digestible Indispensable Amino Acid Score (DIAAS)

In order to overcome the limitations of PDCAAS, DIAAS was proposed in 2013 (FAO/WHO 2013). There are similarities between PDCAAS and DIAAS, as both require the determination of amino acid composition and use of an *in vivo* assay to determine nutrient digestibility, corrected for endogenous loss. There are, however, multiple differences. While PDCAAS uses fecal protein digestibility as an indicator of nutrient absorption, DIAAS requires amino acid analysis of the digesta present at the terminal ileum. This means that rather than a reflection of protein digestibility, as in PDCAAS, DIAAS provides a measurement of individual amino acid digestibility. DIAAS is not a truncated measurement, so it is possible for a DIAAS value to be above 1.00 providing a more accurate indication of the protein quality. The amino acid reference patterns were also updated from the earlier 1991 document to better reflect the current understanding of human amino acid requirements. Overall DIAAS would provide a more accurate indication of the nutritive value of a protein, yet adopting DIAAS is not without complications.

In PDCAAS there is a requirement for three hydrolysis procedures to accurately determine amino acid composition, i.e., acid hydrolysis, oxidized acid hydrolysis (methionine and cysteine), and alkaline hydrolysis (tryptophan). This is doubled in

DIAAS as the analysis has to be done on both the protein ingredient and the ileal digesta. The cost of these analyses can be prohibitive for novel products, and accuracy is necessary for proper quality assessment. The ideal *in vivo* model for DIAAS is humans, otherwise, swine or rodent models for PDCAAS and PER are to be used. Ethical considerations of human trials aside, the cost of feeding trials for humans and swine far exceed that of rodents, although the accuracy of the data gathered would be more appropriate. A review published in 2017 describes in greater detail the factors to be considered regarding the adoption of DIAAS (Marinangeli and House 2017).

3.4 Protein Quality of Some Plant-Based Foods

PDCAAS: In commercial maize, PDCAAS values range between 30% and 50%, while quality protein maize (QPM) value is enhanced, which ranges from 54% to 72% due to a higher lysine content (Pachón et al. 2009). Sorghum has a more balanced amino acid profile, but low in protein digestibility and reduced bioavailability of limiting amino acids, with a PDCAAS of 20% (Duodu et al. 2003). The carbohydrate and protein contents not only affect the physicochemical properties of plant-based flours, but also the PDCAAS values. For example, *In vitro,* legume flours (chickpea, pea, soybean, lentils and faba beans) showed PDCAAS values in between 43.63% and 77.22% (16.7–38.7% protein; 1.3–46.5% starch), whereas in cereal flours (durum and CWRS wheats, hulless barley and oat) ranged from 44.56% to 66.96% (11.9–13.3% protein, and 52.9–60.1% starch) (Stone et al. 2019). Hamad and Fields (1979) compared the protein parameters of different plant protein sources. They reported that wheat and soybean PDCAAS values were 42% and 91%, respectively, while rice bran protein and casein showed a *true digestibility* (TD) of 94.8. This value was higher than rice endosperm protein, soy protein isolate and whey protein isolate (90.8, 91.7 and 92.8 respectively) (Han et al. 2015). However, to determine the quality of plant proteins, samples must undergo protein quality analysis (Zheng et al. 2019).

DIAAS: Despite the limiting amino acids present in cereals, for example lysine in rice, polished rice, oats, proso millet, foxtail millet and whole-wheat, these cereals have DIAAS values of 42, 37, 43, 7, 10 and 20, respectively. On the other hand, seeds deficient in sulphur amino acids, such as buckwheat and tartary buckwheat, have DIAAS values of 68 and 47, respectively (Joye 2019). Compared to animal proteins with typical DIAAS range of 107–114, cereals cannot be considered as complete protein sources (Hamad and Fields 1979; Joye 2019). However, processing of grains may or may not affect the final DIAAS values. For example, processing mung beans (*Vigna radiata*) as either dehulled-soaked, raw, unsoaked and soaked prior to boiling resulted in the DIAAS for sulphur amino acids being 16, 17, 18 and 19, respectively, showing no significant difference among treatments (Prachansuwan et al. 2019). However, a previous study on red and green lentils

(*Lens culinaris*) showed that baking decreased DIAAS values in comparison to boiling and/or extrusion (Nosworthy et al. 2018a). Extrusion enhanced the DIAAS of black (DIAAS 65) and red (DIAAS 60) kidney beans, respectively; while baking increased DIAAS in chickpeas (DIAAS 84) and faba beans (DIAAS 61). Cooking via boiling improved DIAAS values in navy (DIAAS 57) and pinto (DIAAS 70) beans (Nosworthy et al. 2018b, 2020).

3.5 Beyond Content Claims: Health Benefits of Amino Acids

The discussion of protein quality tends to be focused on the regulatory aspects of protein content claims and the physiochemical characteristics important for new product development. The biological activities of individual amino acids are worth considering. There has been much research done on bioactive peptides, particularly regarding reducing hypertension and cardiovascular disease (Pedroche et al. 2002; Garcia-Mora et al. 2015; Hong et al. 2008). This section will focus on discussing the non-nutritive biological activities of selected amino acids in plant-based proteins. These include arginine, which is high in certain plants such as hemp, and the essential amino acids which are limiting in certain plant-based proteins: methionine and leucine. It is noteworthy that although there is a significant body of literature discussing the health benefits of individual amino acids, no regulatory body currently allows for health claims based on a specific amino acid (Roberts 2016; Krasniqi et al. 2016; EFSA 2010).

3.5.1 Arginine

While not traditionally an essential amino acid, due to the capacity for the small intestine to synthesize adequate quantities in adults, arginine can be considered 'conditionally essential' in infants who do not have a fully developed small intestine, and individuals where the synthesis pathway is impeded (Wu 2009). Nitric oxide (NO), produced from arginine via NO synthase, is capable of interacting with many diverse tissues including skeletal muscle (Reviewed in Janero 2001; Botchlett et al. 2019). While most well known as a vasodilator, the function of NO in skeletal muscle is to act as a signalling molecule controlling cellular respiration, glucose uptake, and cellular differentiation (Stamler and Meissner 2001). There have also been indications that increasing arginine intake can lead to increased muscle mass (Campbell et al. 2006). Beyond skeletal muscle, arginine has been implicated in the reduction of coronary heart disease (Fiorito et al. 2008) obesity due to increased lipolysis (McKnight et al. 2010).

3.5.2 Methionine

The sulfur-containing amino acids, methionine and cysteine, are commonly limiting in plant-based proteins, such as those derived from pulse crops. There has been much research performed on the integral nature of methionine (MET) in one-carbon metabolism, it's ability to donate methyl groups to other biomolecules, and regulation of s-adenosylhomocystine/s-adenosylmethionine ratio which has been implicated in cardiovascular health (Ducker and Rabinowitz 2017). Unlike arginine, where an increased consumption can be beneficial, restriction of methionine intake can result in numerous health benefits. Restriction in dietary MET has been shown to prevent onset of diabetes in an obese rat model, potentially due to an increase in circulating fibroblast growth factor 21 (FGF21), which is a regulatory hormone (Castaño-Martinez et al. 2019). In that study, the authors also identified FGF21 levels in humans following a vegan diet, and when omnivores were placed on a vegan diet it increased circulating FGF21 in their plasma. MET restriction is also implicated in alterations in the intestinal microbiome that modulate health including 'leanness' and genetic methylation, with these alterations in 'leanness' and genetic methylation demonstrating sex-specific variation in mice (Wallis et al. 2020). There is also a growing body of work relating the restriction of MET in the diet to increased lifespan via the insulin/Insulin growth factor-1, a mammalian target of rapamycin (mTOR) signalling system (reviewed in Lee et al. 2016).

3.5.3 Leucine

Leucine is part of the triad of amino acids known as the branched chain amino acids, isoleucine, leucine, and valine. Leucine, in particular, has been investigated for its potential to increase muscle mass and exercise performance (Crow et al. 2006), as well as reducing the onset of sarcopenia in the elderly by increasing muscle protein synthesis (Casperson et al. 2012) if increased in the diet. A study in 2020 investigated leucine supplementation in the elderly undergoing bedrest or rehabilitation found that while leucine was able to prevent muscle loss it did not prevent the loss of muscle function (Arentson-Lantz et al. 2020). Leucine is also involved in hepatic lipid metabolism through mTOR, including a reduction in fatty acid transport (Bishop et al. 2020). The literature is less clear on the effect of leucine on obesity and diabetes. A study in mice fed a high fat diet in conjunction with increased dietary leucine showed reduced obesity and hyperglycemia, with the reduction in obesity being linked to increased resting energy expenditure (Zhang et al. 2007). Conversely, high concentration of BCAA in plasma is linked to increased insulin resistance (Lynch and Adams 2014). A study involving a diabetic mouse model determined that leucine restriction increased the proliferation of β-cells and modulation of the intestinal microbiota related to circulating blood glucose concentration (Wei et al. 2018). This investigation, however, also identified that leucine deficiency resulted in a reduction in muscle mass as well as having deleterious effects on hepatic steatosis.

4 Bioavailability of Amino Acids

Bioavailability of amino acids, sometimes referred as amino acid digestibility, expresses the proportion of the total amount of dietary amino acids that can be absorbed from the digestion of food protein sources (Sarwar 1987; Batterham 1992; Fuller and Tomé 2005; Levesque et al. 2010).

4.1 In Vitro vs In Vivo Measurement (Animal and Human Trials) of Amino Acids Bioavailability

Over the years, the amino acid bioavailability has been determined by several methods, such as the fecal balance method (Kuiken and Lyman 1948), measuring the disappearance of amino acids from the small intestine (ileal recovery) (Cho and Bayley 1972), or animal growth assays, such as PER (discussed above). But these methods have limited accuracy on a single sample and/or certain amino acids (Sarwar 1987; Batterham 1992).

Highly digestible proteins are recommended since these provide more amino acids for absorption during proteolysis, therefore, showing better nutritional value than those of low availability proteins (Singh 2017). However, *in vivo* experiments have demonstrated that endogenous and environmental conditions may influence the digestibility of plants proteins (Oser 1959; Wolfenson et al. 1981; Wolfenson 1986). For example, the *true digestibility of proteins* (TDP), evaluated in broiler female and male chickens, did not show differences between amino acids profile for intake of soybean meals in male chickens. But, in female chickens, an ambient room temperature of 32 °C decreased the TDP from 9% to 15% in comparison to a room temperature of 21 °C, specifically in alanine, aspartate, arginine, cysteine, glutamine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tyrosine and valine (Larbier et al. 1993). A chicken model for bioavailability of sulphur amino acids from soybean alkali-treated proteins showed a decrease of 71% in cysteine and 80% in histidine (Robbins and Ballew 1982). This could be due to the deficient digestibility of pulse proteins that limits their use in weaning food formulations. However, it is known that digestibility of pulse proteins is dependent on characteristic of granule starch, since digestibility of albumins and globulins from lentils and horsegram in the presence of starch has been linked to the opening of compact protein structures binding to the surface of starch granules and forming new bonds that facilitated the access of the proteolytic enzymes (Ghumman et al. 2016; Singh 2017). It is also well known that some bioactive compounds found in plant sources may influence the protein digestibility.

Amino acid *in vitro* and *in vivo* bioavailability experiments showed strong correlations in proteins from combined cereal grains ($r = 0.92$), but low correlations in soybean meal or corn gluten meal ($r = 0.29$) (Cave 1988). In humans, bioavailability of peptides, oligopeptides and amino acids is influenced by enzymatic degradation, hydrophobicity, molecular size/weight, and chemical stability (Xu et al. 2019). These factors affect directly their absorption capacity, that may follow passive (paracellular and passive transcellular diffusion) and/or active (transporter and transcytosis) routes. Some enzyme-resistant peptides, oligopeptides and amino acids can be transported into the bloodstream at concentrations in the micromolar range and remain intact for several minutes to hours to exert beneficial effects (Cave 1988; Xu et al. 2019).

To choose an *in vitro* or *in vivo* model for bioavailability of amino acids it is necessary to consider the pros and cons summarized in Table 12.3. *In vitro* bioavailability experiments are classified in three categories, chemical, enzymatic and microbiological. They can be performed individually or in combinations, according to research purposes and the experimental conditions required (Lewis and Bayley 1995). These methods are faster, cheaper and easier to conduct than the *in vivo* protocols, as well as avoiding ethical implications associated with animal experimentation. Also, *in vitro* assays can be performed following described procedures (i.e. digestive enzymes exposure followed by microbial bioavailability evaluations) and the conditions can be controlled by the experimental manipulators. However, sometimes the assayed parameters are not related to real physiological conditions. Moreover, chemicals, enzymes, and microbial population need to be carefully established, as currently data from these assays have low acceptance as a basis for diet formulation (Lewis and Bayley 1995; Metges 2000; Segura-Campos et al. 2011; Bhutta and Sadiq 2013; Neis et al. 2015; Brodkorb et al. 2019; Wang et al. 2020).

On the other hand, *in vivo* models are convenient under methodological circumstances, for example, animal facilities, depending on budget and time availability (Lewis and Bayley 1995). Chicken models are useful for the measurement of lysine/methionine bioavailability and indirect measurements of amino acids in plasma. Rodent assays could be used to estimate of ileal and fecal amino acid bioavailability close to humans and when various experiment repetitions (>3) are required. On the other hand, pig models allow to recover a higher amount of sample, which are closer metabolically to humans and are utilized to measure the capacity of a protein to provide specific limiting amino acids for promoting growth. Nevertheless, *in vivo* models require the compliance of strict bioethical procedures. Moreover, the differences between animal metabolism and amino acid requirement as well as external situations such as environmental conditions may influence the experimental parameters or the endogenous recycling rate of amino acids. These factors must be carefully taken into consideration during assessment (Kirk 1984; Batterham 1992; Larbier et al. 1993; Lewis and Bayley 1995; Fuller and Tomé 2005; Stein et al. 2007; Cortés-Cuevas et al. 2019).

To enhance the protein quality of cereals and pulses, it is necessary to formulate blends from different plant-based protein sources that complement the deficiencies of some amino acids and thus completes the essential amino acids requirements. The 'nitrogen in *vs* nitrogen out' (nitrogen recovery) of protein digestibility (PDCAAS) is a critical quality measurement for food protein sources. When comparing *in vitro* and *in vivo* methods for determining protein quality of plant sources,

Table 12.3 General considerations for using *in vitro* and/or *in vivo* models on amino acids bioavailability

Bioavailability model	Advantages	Disadvantages	References
In vitro			
Chemical	Rapid, cheaper, easier, low ethical implications. Can be designed as serial experiments. Can differentiate among samples on the same feedstuff. High correlation ($r = 0.96$) with chicken model measuring lysine and methionine bioavailability.	Is based on measure the ε-amino group of lysine (a limiting amino acid in some plant foods). Experiments on cereal grains show poor relationship *vs* biological estimates. Endogenous and microbial enzymes are not included.	Lewis and Bayley (1995), Fuller and Tomé (2005), Levesque et al. (2010)
Enzymatic	Rapid, cheaper, easier, low ethical implications. Individual enzymes can be added to the experiment in controlled concentrations. Can differentiate among samples on the same feedstuff.	A lack of consensus on the procedures available. The protein activity of mixes of enzymes (i.e. pancreatin) must be previously estimated. Various products of proteins or peptides consist in undigested macromolecules. Interactions among other biomolecules could give uncertain results. Gut microbial enzymes are rarely considered.	Lewis and Bayley (1995), Segura-Campos et al. (2011), Bhutta and Sadiq (2013), Brodkorb et al. (2019)
Microbiological	Rapid, cheaper, easier, low ethical implications. Specific microorganisms for some amino acids may be used. This method could be the continuation of a previous enzymatic or chemical test. This method provides information related to the breakdown of proteins from gut microbiota interactions.	There are numerous theoretical and practical concerns with microorganism management. Data generated from this method has not gained acceptance as a basis for diet formulation.	Lewis and Bayley (1995), Metges (2000), Neis et al. (2015), Wang et al. (2020)
In vivo			
Chickens	High correlation ($r = 0.96$) with *in vitro* lysine and methionine bioavailability. Indirect measurements of amino acid concentration in plasma are established.	Time consuming and expensive. Bioethical implications. Intake of amino acids are not easily quantified.	Larbier et al. (1993), Lewis and Bayley (1995), Cortés-Cuevas et al. (2019)

(continued)

Table 12.3 (continued)

Bioavailability model	Advantages	Disadvantages	References
Rodent	Amino acid estimation from ileal and fecal output are closer to human results than chickens. It allows repeat measurements that help to reduce variability.	Time consuming and expensive. Bioethical implications. Sulphur-amino acids metabolism and requirements are not same in humans. Exogenous conditions may affect the amino acid final results. Microbial fermentation in gut changes amino acid flux, leading to amino acid appearances or disappearances before incorporation to bloodstream. Small animals, insufficient sample may be obtained from one animal, so that digesta from 2 or more animals may need to be combined to provide sufficient sample.	Kirk (1984), Sarwar (1987), Lewis and Bayley (1995)
Pig	Indirect measurements of amino acid concentration in plasma are established There are six digestibility estimations to describe the protein digestibility: apparent, true and real for ileal and fecal measurements Measure the capacity of a protein to provide specific limiting amino acids and promote growth The net effect of all amino acids that can affect their bioavailability (digestion, absorption and utilization) Most metabolically similar to humans	Time consuming and expensive. Bioethical implications. Intake liming amino acids are not easily quantified. Microbial fermentation in gut changes amino acid flux, leading to amino acid appearances or disappearances before incorporation to bloodstream. Recycling endogenous amino acids need to be extracted from estimations. Sometimes the published yield data is for one amino acid only. It does not allow the repeated measurements that help to reduce variability. The sample obtained represents the digesta of only one short part of the feeding cycle and may, therefore, not be representative of 24 h flow.	Batterham (1992), Lewis and Bayley (1995), Fuller and Tomé (2005), Stein et al. (2007)

it is necessary to carry out several protein quality determinations to understand the relationship of these analytical techniques regarding the digestibility and bioavailability of amino acids from a wide variety of plant protein sources. In summary, more in depth studies are required to understand the effects of agri-food and food processing on protein and amino acid quality, as well as the need of establishing an international consensus about food digestion and protein quality assessment protocols, so that bioavailability and bioaccessibility values and/or tables can be proposed to be used as a worldwide reference for the evaluation of a wide variety of proteins on amino acids quality.

4.2 Bioavailability of Different Amino Acids in Plant Protein Foods

The true ileal digestibility (TID) of an amino acid is an indication of how well that amino acid is liberated from the protein during digestion and subsequently absorbed by the small intestine. While there are limited data from humans, most of the information on TID is derived from pig studies. Table 12.4 highlights the TID of amino acids from a variety of pulse classes, as well as milk and soy (modified from Fuller and Tomé 2005; Han et al. 2020). While the TID of most amino acids is high, the overall digestibility of the soy and milk proteins is higher than that of the pulse

Table 12.4 True ileal digestibility of different protein sources determined in pigs

	Kidney Bean[a]	Mung Bean[a]	Adzuki Bean[a]	Broad Bean[a]	Pea[a]	Chickpea[a]	Milk[b]	Soy[b]
His	57	68	89	85	75	73	99	95
Ile	80	83	89	82	88	85	98	97
Leu	89	91	94	96	93	90	99	96
Lys	84	86	90	83	91	88	99	97
Met	84	83	83	83	89	87	100	97
Cys	44	53	53	68	75	77	89	85
Phe	78	84	89	91	87	85	98	96
Tyr	59	77	85	89	83	76	99	97
Thr	75	77	87	89	83	79	95	91
Trp	78	82	77	84	87	87		
Val	80	82	89	91	87	83	98	96
Ala	70	73	87	91	84	80	96	96
Asp	88	90	94	95	93	93	98	97
Arg	84	88	93	91	96	96	98	98
Glu	86	88	92	95	92	91	98	100
Gly	47	55	84	88	77	76	90	90
Ser	82	83	88	92	88	85	97	97

[a]Han et al. (2020)
[b]Fuller and Tomé (2005)

proteins presented, most likely due to the presence of anti-nutritional factors in the pulse foods as well as overall differences in the food matrix. This is an important consideration as the digestibility of a protein is not necessarily indicative of the digestibility of its component amino acids.

In addition, grains and cereals are not only consumed as is but they are also processed into protein ingredients (flour, concentrate, isolate) that are being incorporated into food products. Processing will have a significant impact on the amino acid profile of the food products and on the bioavailability of those amino acids. For example, El-Shafei et al. (1983) determined the availability of lysine and essential amino acids in corn flour and corn bread. Lysine availability was determined by the growth response method on weaning rats using regression analysis of body weight gain or moisture gain against lysine consumed from corn flour and corn bread. The results indicated a positive correlation between weight and moisture gain and the amount of lysine consumed for both flour and bread. It was also observed that baking had a positive effect on lysine availability. Balance trials with rats were applied to determine the availability of essential amino acids. The results showed that the availability values for all amino acids except threonine were increased by baking. Giménez et al. (2016) supplemented corn (*Zea mays*) flour with 30% broad bean (*Vicia faba*) flour (CBB pasta) or 20% quinoa (*Chenopodium quinoa*) flour (CQ pasta) for the production of spaghetti. Pastas made from 100% corn flour (C pasta) were used as the control. Characterization of the pasta indicated that the net protein utilization was higher for the supplemented pasta than for the pasta made from 100% corn flour (34.81 ± 1.90 for C pasta vs 55.72 ± 2.11 for CBB pasta vs 58.65 ± 1.40 for CQ pasta). It was also the case for the protein digestibility-corrected amino acid score (37.62 for C pasta vs 49.90 for CBB pasta vs 51.02 for CQ pasta). The protein true digestibility was decreased by the substitution (90.93 ± 2.62 for C pasta vs 80.81 ± 2.13 for CBB pasta vs 78.06 ± 3.21 for CQ pasta). However, the supplementation of corn flour at those levels weakened the starch structure, negatively impacting some important sensorial characteristics of the pasta.

4.3 Impact of Antinutritional Factors in Plant Proteins on their Digestibility and on the Bioavailability of Amino Acids

Plants contain a number of bioactive compounds that can make their way into protein ingredients and food products upon processing. The most common bioactive compounds found in plant protein ingredients and plant-based protein food products are phytic acid, trypsin inhibitors and condensed tannins. These compounds play metabolic roles in animals or humans that frequently consume these foods. The effects of these compounds may be negative, positive or both (Campos-Vega et al. 2010). Among the different effects that these compounds may have, one of the most important is their impact on protein digestibility and on the bioavailability of amino acids (Gilani et al. 2012). Published data on the impact of these bioactive compounds on protein digestibility and on amino acid bioavailability are summarized in this section.

4.3.1 Phytic Acid

Phytic acid is a bioactive molecule found in plant seeds, where it serves as a storage form of phosphorous. Phytic acid accounts for about 80% of phosphorous found in plant seeds (Lolas and Markakis 1975). In terms of chemical structure, phytic acid is composed of six phosphate groups with two protons each. Of the 12 protons on phytic acid, six can dissociate at acidic pH, three at neutral pH, and the remaining three at basic pH (Woyengo et al. 2009). This abundance of negative charges confers to phytic acid its high binding potential. Phytic acid, with its net negative charge, can directly bind positively-charged molecules or indirectly bind negatively-charged molecules. In the latter case, a divalent cation bridge will allow the phytic acid to bind with negatively-charged molecules. In plant tissues, phytic acid is generally present as salts of monovalent and divalent cations (phytate). Plant proteins can carry a net negative charge or a net positive charge depending on the pH. Above the isoelectric point, plant proteins will have a net negative charge, while they will have a net positive charge below the isoelectric point. Thus, for pH above the isoelectric point of the proteins, which is around 4.5 for most plant proteins, phytic acid can bind with the proteins through divalent cation bridging, while for pH below the isoelectric point, phytic acid can directly bind to the proteins. Also, it is well known that aspartic acid, glutamic acid, lysine, arginine and histidine are amino acids that can be positively or negatively charged depending on the pH. For a pH superior to their pK, aspartic acid (pK = 3.9) and glutamic acid (pK = 4.2) are negatively charged. At a pH inferior to their pK, both amino acids will be uncharged. Lysine (pK = 10.5), arginine (pK = 12.5) and histidine (pK = 6.0) are positively charged for pH inferior to their pK, while they will be uncharged for pH superior to their pK. Thus, depending on the pH, the aforementioned amino acids may or may not interact with phytic acid. At low pH (for example in the stomach), phytic acid will directly interact with the positively-charged lysine, arginine and histidine (Gilani et al. 2012).

Serraino et al. (1985) studied the impact of phytic acid content on the *in vitro* protein digestibility (IVPD) and the relative rates of amino acid release of rapeseed flour. They compared the IVPD and the rates of amino acid release of raw rapeseed flour with those of rapeseed flours that were treated to reduce their phytic acid content by 51% and 89%. It was observed that the rapeseed flour with a 51% reduction in phytic acid had a higher rate of amino acid release than the control, but a rate similar to that of the rapeseed flour with a 89% reduction in phytic acid. The protein digestibility was not improved by the reduction in phytic acid. The same group carried out a study to determine the effect of phytic acid content on rapeseed protein digestibility and amino acid absorption using a rat model (Thompson and Serraino 1986). Weanling rats were fed with a diet containing 10% protein supplied by a high-phytate rapeseed flour (5.7%) or by a low-phytate rapeseed flour (2.4%). The results indicated that there was no significant difference between both diets in terms of protein digestibility and amino acid absorption. In their work, Chitra et al. (1995) studied the impact of phytic acid content on the protein digestibility of plant proteins from different grain legumes (chickpea, pigeon pea, urd bean, mung bean and soybean). Each seed was analysed for its phytic acid content and IVPD. Soybean was the seed with the highest phytic acid content (36.4 mg/g), followed by urd bean (13.7 mg/g), pigeon pea (12.7 mg/g), mung bean (12.0 mg/g) and chickpea (9.6 mg/g). *In vitro* protein digestibility of soybean ranged from 62.7% to 71.6%, while it varied from 55.7% to 63.3%, from 60.4% to 74.4%, from 67.2% to 72.2%, and from 65.3% to 79.4% for urd bean, pigeon pea, mung bean and chickpea, respectively. Statistical analysis indicated that there was a significant negative correlation between phytic acid content and IVPD. In general, an increase in phytic acid content resulted in a decrease in IVPD. In another work, Liu et al. (2018) studied the effects of supplementing phytic acid on the apparent digestibility and utilization of dietary amino acids in juvenile grass carp. Five diets with different levels of phytic acid were considered (0.2, 4.7, 9.5, 19.1 and 38.3 mg/g, coded as P0, P5, P10, P20 and P40, respectively). A feeding trial was conducted for 8 weeks, in which triplicate groups of fish (initial weight: 22.37 ± 0.16 g) were fed twice daily (08:00 and 16:00 h). The crude protein content in whole body significantly ($p < 0.05$) decreased in fish fed with the P20 and P40 diets. Supplemental phytic acid (>4.7 mg/g) significantly reduced the apparent digestibility coefficient of amino acids (Asp, Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Leu, Phe, Lys, Pro, His and Arg). The authors concluded that supplemental phytic acid decreased the apparent digestibility and utilization of amino acids and thus reduced the feed utilization of grass carp, suggesting that the level of total phytic acid should be below 4.7 mg/g in the grass carp diet. In another work, Woyengo et al. (2009) carried out a feeding trial with piglets to study the impact of supplementing phytic acid (as sodium phytate) at 0, 5, 10 or 20 mg/g on ileal mineral and amino acid digestibilities and ileal endogenous amino acid flow. The basal diet was a casein–maize starch-based diet formulated to meet National Research Council energy and amino acid requirements for piglets. The results indicated that phytic acid can reduce the apparent ileal digestibility of Na and Mg, partly by increasing endogenous losses of these minerals. However, phytic acid had a limited effect on the digestibility and endogenous losses of amino acids. Onyango et al. (2009) applied a 3 × 2 factorial design to study the impact of the form of phytic acid (free phytic acid or magnesium-potassium phytate) on endogenous losses of amino acids in 10-week-old male broilers. Chickens were intubated and were fed six dextrose-based combinations of phytic acid and phytase consisting of phytic acid form (no phytic acid, 1.0 g free phytic acid or 1.3 g magnesium–potassium phytate) and phytase (0 or 1000 units). Chickens fed with both phytic acid treatments showed increased endogenous loss of threonine (84 mg), proline (116 mg) and serine (75 mg) compared with the no-phytic acid treatment (69, 96 and 63 mg, respectively). All the aforementioned studies reported conflicting results regarding the impact of phytic acid on the digestibility of protein and amino acids. One possible explanation is that the impact of phytic acid on protein and amino acid digestibility could be a function of its concentration in the diet. This indicates that additional works are required to fully assess the impact of phytic acid on protein and amino acid digestibility.

4.3.2 Trypsin Inhibitors

Trypsin is an enzyme involved in the breakdown of proteins during digestion. Trypsin inhibitors are proteins that reduce the biological activity of trypsin. They compete with dietary proteins to bind with trypsin and therefore render it unavailable to bind with dietary proteins during the digestion process.

In their work, Grosjean et al. (2000) studied the impact of different levels of trypsin inhibitor activity on the ileal digestibility of protein and amino acids of feed peas in pigs. Thirteen pea samples with trypsin inhibitor activity ranging from 2.3 to 11.8 UTI mg/DM were mixed with a basal protein-feed diet containing equal portions of sucrose and maize starch. Each experimental diet had 170 g crude protein/kg. The results indicated that standardised ileal protein and amino acid digestibility decreased linearly with increasing levels of trypsin inhibitor activity, except for alanine. Wiseman et al. (2003) developed two pairs of near-isogenic lines of peas with high and low concentrations of trypsin inhibitors. The pea samples were named HA5 and LA5 and HB5 and LB5 and contained 8.73 ± 0.19, 1.45 ± 0.19, 7.40 ± 0.65 and 1.78 ± 0.15 trypsin inhibitor units per mg dry weight, respectively. The effect of feeding young broilers with diets containing the aforementioned pea samples on the apparent ileal amino acid digestibility was studied. The results indicated a significant difference in the coefficient of apparent ileal amino acid digestibility among the amino acids. However, for all amino acids reported in this work, the data clearly demonstrate that pea samples with low levels of trypsin inhibitor had a higher coefficient of apparent ileal amino acid digestibility than those with high levels of trypsin inhibitor. In another work, Clarke and Wiseman (2005) studied the effect of the level of trypsin inhibitor of soybean meals on the apparent ileal digestibility of amino acids in young broilers. Trypsin inhibitor values of soybean meals varied from 1.1 to 3.6 mg/g. No correlation was found between the levels of trypsin inhibitors and the coefficients of digestibility for individual amino acids. These results are in contradiction with those reported by the same group for peas (Wiseman et al. 2003), indicating that other factors may also affect the amino acid digestibility of soybean meals. Despite the potential negative impact of trypsin inhibitors on amino acid digestibility, it was also demonstrated that ordinary cooking, pressure cooking and microwave cooking effectively remove trypsin inhibitors in peas, eliminating their potential negative impact on digestibility (Habiba 2002). Laleg et al. (2016b) also demonstrated that trypsin inhibitory activity (mg/g of DM) was significantly reduced by cooking of pasta, reporting the following value for faba bean pasta, lentil pasta and black-gram pasta before and after cooking, respectively: 7.84 vs 2.48, 8.24 vs 1.52 and 11.26 vs 2.13. Similar results had been previously reported by Zhao et al. (2005) for spaghetti made from semolina containing 5% to 30% milled flours of green pea, yellow pea, chickpea and lentil.

4.3.3 Tannins

Tannins are polyphenolic compounds that are soluble in water and that can complex with proteins and precipitate them (Gilani et al. 2012). They can be classified into hydrolysable and condensed tannins. Condensed tannins are the most present in consumable food products.

Longstaff and McNab (1991) conducted a feeding trial in which 3-week-old chickens were fed with a diet substituted with 400 g hulls/kg diet from three varieties of beans (*Viciu fubu L.*), which was compared with a control diet without hulls. Each variety of beans had different levels of condensed tannins. The objective was to determine the effects of polysaccharides and tannins present in the hulls on the amino acid digestion. The results indicated that the diets substituted with hulls containing high levels of tannins (varieties Brunette and Minica) caused a large reduction in the digestion of amino acids compared with the control diet without hulls. Ortiz et al. (1993) fed chickens with diets based on 67.5% dehulled faba beans supplemented with different levels of freeze-dried tannin extract (0, 8, 16 and 24 g/kg diet). Diets supplemented with tannins significantly ($P < 0.01$) reduced protein digestibility from 88.8% to 80.8% compared to the control diet not containing tannins. The results indicated a high correlation between the digestibility values and the level of tannins in the diet. Amino acid digestibility showed a pattern similar to that of the crude protein, and the mean differences among treatments were in the range of 5.4–12.6%. In their work, Mariscal-Landín et al. (2004) studied the effect of tannins in sorghum on the coefficient of apparent ileal digestibility and on the coefficient of standardised ileal digestibility of amino acids. Four samples with different levels of tannins were considered (1.4, 4.6, 9.8 and 10.0 mg/g). The highest coefficient of apparent ileal digestibility was observed on the sorghum sample containing 1.4 mg of tannins/g, and the lowest was observed on the sorghum sample containing 4.6 mg of tannins/g. Digestibility was significantly different among the amino acids, with Leucine and glutamic acid being the most digestible in the four samples of sorghum, while the least digestible were found to be glycine, lysine, threonine and cysteine. As tannin levels increased, the proline coefficient of apparent ileal digestibility decreased ($P < 0.05$). The coefficient of standardised ileal digestibility of amino acids in the sorghum sample with 1.4 mg of tannins/g was higher than that of the sorghum sample with 4.6 mg of tannins/g, except for proline. Similarly, the coefficient of standardised ileal digestibility for isoleucine, lysine, threonine, valine, alanine and aspartic acid was similar among sorghums containing 1.4, 9.8 and 10.0 mg of tannins/g. The results did not show a clear detrimental effect of tannins on the coefficient of apparent ileal digestibility and on the coefficient of standardised ileal digestibility of amino acids. The authors suggested that this finding may indicate that both coefficients may be more influenced by the protein profile of the grain than by the tannin content. More recently, Reis de Souza et al. (2019) studied the impact of kafirin and tannin concentrations in sorghum on the ileal digestibility of amino acids in growing pigs. Two hybrids of sorghum were considered in that study. Sorghum 82G93 had a low tannin content (LT), while sorghum 81G67 had a high tannin content (HT). Each hybrid was available with either low or high levels of kafirins (LK and HK, respectively). A feeding trial was conducted in which pigs were fed four experimental diets that were formulated with sorghum as the sole source of crude protein and amino acids: LT-LK, LT-HK, HT-LK and HT-HK. The results indicated that the apparent ileal digestibility of glutamic acid and histidine were negatively correlated with the level of kafirins ($P < 0.05$), as was the apparent ileal digestibility of alanine, aspartic acid and valine ($P < 0.10$). Levels of tannins were also negatively correlated with the apparent ileal digestibility of lysine ($P < 0.001$), cysteine ($P < 0.01$), histidine ($P < 0.01$), methionine ($P < 0.01$), aspartic acid ($P < 0.05$), leucine ($P < 0.05$) and threonine ($P < 0.05$). Concerning standardised ileal digestibility values, those of alanine, glutamic acid, histidine and valine were negatively correlated with the level of kafirins ($P < 0.10$), while tannin level negatively affected the standardised ileal digestibility of lysine ($P < 0.001$), cysteine ($P < 0.01$), histidine ($P < 0.01$), aspartic acid ($P < 0.05$), leucine ($P < 0.05$), methionine ($P < 0.05$), serine ($P < 0.05$), threonine ($P < 0.05$) and valine ($P < 0.05$). The results of this study indicated that kafirins had a significant but minimal effect on the criteria studied. However, amino acid digestibility in growing pigs was reduced by the tannins present in sorghum. Most of the aforementioned studies indicated that tannin level is negatively correlated with amino acid digestibility. However, tannins can be partly eliminated by cooking (Habiba 2002).

5 Markets for Plant-Based Products

Over the past decade there has been continued consumer interest in foods that contain plant-based ingredients. In the United States alone, sales rose by 29% between 2017 and 2019 ($3.9b USD to $5.0b USD) (Good Food Institute 2020). In 2019 the majority of sales were plant-based dairy products, $3.4b USD, with the fastest growing area being plant-based eggs. In Canada, the sale of plant-based protein products rose by 7% in 2017 totalling over $1.5b CAD in sales, with 40% of Canadians including more plant-based foods into their regular diets (Agri-food Innovation Council 2019). To further demonstrate the consumer interest in plant-based products, in 2020 a Canadian website, Vegansupply.ca, listed over 500 vegan products ranging from cereals and pastas to simulated cheeses, such as parmesan and cheddar, to meat-like products of jerky, chicken, bacon, and burger patties. Similar trends have occurred in Europe with over $5.8b USD being spent on plant-based protein items in 2018 and an expectation for that to rise to over $9.4b USD by 2027 (Research and Markets 2020).

6 Conclusion and Future Perspectives

During the last couple of years, consumers have been shifting from an animal protein diet to a flexitarian or plant-based diet. This shift in consumers diet and the growing need for sustainable food systems, along with a rising demand for plant-based livestock feed, pet food and aquaculture feed, make plant proteins an economically viable alternative to animal protein. However, plant proteins and their derived food products differ in terms of their essential amino acid contents and protein quality. In addition, processing and composition may also have a significant impact on the amino acid profile of the proteins contained in food products, as well as on the bioavailability of those amino acids. This makes the evaluation of plant proteins' fate, and of their derived products (peptides and amino acids), in human gastrointestinal tract (e.g., digestion and bioavailability) of utmost importance. Several methods (*in vitro* and *in vivo*) are available to determine the bioaccessibility and bioavailability of proteins and of their derived products, with each method having their own advantages and disadvantages. These *in vitro* and *in vivo* assessment methods should be used with care, as intra- and inter- laboratory comparison can be complex, thus making it difficult for comparison purposes. The method(s) selected for analytical purposes should be justified depending on the aim of the study. More studies are needed to understand the relationship of these analytical techniques regarding the digestibility and bioavailability of amino acids from a wide variety of plant protein sources. In-depth understanding of proteins from plant sources will be tailored for specific applications in innovative products development, following the current trend of "plant-based foods" in the food industry.

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Chapter 13
Bioactive Components of Plant Protein Foods in the Prevention and Management of Non-communicable Diseases

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1 Introduction

Plant protein foods perform vital functions in human nutrition as they are important not only in meeting the protein adequacy in everyday diet but also provide other essential nutrients. The demand for plant protein-based products is growing substantially because of their perceived potential health benefits and increased consumer's fears about the unfavorable health effects of eating animal protein foods due to environmental and ethical issues in animal production. The nutritional quality of plant proteins with respect to some essential amino acids may however be inferior compared to animal proteins. Many public health initiatives are currently promoting the use of plant protein-based diets to improve the public health nutrition. It has been shown that a healthy eating approach and higher education level and not the socioeconomic conditions were found to be associated with more plant-protein consumption. The data on sociodemographic factors indicate that plant-based diets may be a convenient and economical way to improve the diet quality in all income groups. Future research is however required to evaluate the quality of plant proteins with respect to supply of essential amino acids and overall health

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benefits (Aggarwal and Drewnowski 2019). Appropriate strategies should therefore be developed to meet the essential amino acid requirements through improved consumption of combination of plant protein foods (Hertzler et al. 2020). Plant protein foods consist of a variety of bioactive compounds with diverse chemical configurations and traits, which have been reported to beneficially regulate the metabolic processes and offer health benefits. They can modify the antioxidant potential, inhibit, or induce the enzyme actions, alter the gene expression and receptor activities as well as nutrient bioavailability (Carbonell-Capella et al. 2014; Bordoni et al. 2019). Use of plant protein foods have been shown to influence energy intake, reduce pro-inflammatory state, oxidative stress, and metabolic disorders, with different intracellular signaling pathways and posses many other therapeutic benefits (Siriwardhana et al. 2013; Moreno-Valdespino et al. 2020).

Epidemiological studies have suggested that higher consumption of food bioactive compounds (BACs) with antioxidant activities, such as vitamins, phenolic compounds, flavonoids, carotenoids, can have positive health outcomes and may lower the risk of many chronic diseases like diabetes, cancer, heart diseases, stroke, alzheimer disease, cataracts, and age-related neurodegenerative disorders (Siriwardhana et al. 2013; Fraga et al. 2019; Moreno-Valdespino et al. 2020). The plausible health effects of bioactive compounds can vary depending upon the digestive processes in the body, which determine their bioavailability and bio-accessibility. However, only limited information is available about their bioavailability in humans (Carbonell-Capella et al. 2014). Plant-based diets contain thousands of phytochemicals, which may act as anti-nutrient particularly in individuals with some physiological disabilities. However, some of these phytochemicals may act as antioxidants, detoxifying and immunity-potentiating agents, and may act as neuropharmacological mediators with different functional abilities. For example, Vitamins E, C and provitamin A carotenoids, phenolic compounds, flavonoids and isothiocyanates possess antioxidant potential and reduce the risk of NCDs (Rao 2003; Del Bo et al. 2019). They are termed as BACs as they may boost the body's antioxidant defense mechanisms, reduce inflammation, and risk of lifestyle related chronic diseases. Studying the low molecular weight compounds presents in body biofluids has shown the potential protective role of BACs in the prevention and/or management of non-communicable diseases (Rangel-Huerta and Gil 2016). Acknowledging the role of these bioactives in health promotion is therefore important to exploit their potential health benefits (Petroski and Minich 2020). Several experimental studies have proved that food BACs have a positive biological impact on human health and protect against NCDs and neurodegenerative disorders (NDDs). The presence of secondary metabolites in plant protein foods such as polyphenols, glucosinolates, carotenoids, terpenoids, alkaloids, saponins, vitamins, and fibers have been shown to have antioxidant, antiatherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodilator properties. Polyphenols are one of the most copious bioactive compounds that can be used in the development of effective preventive agents against NCDs. However, the bioavailability and bio-accessibility of BACs is a big challenge for their potential industrial and environmental applications (Câmara et al. 2021).

Food bioactive compounds are very heterogeneous class of compounds with different chemical shapes, configurations, and attributes. They are asymmetrically distributed in nature with variable amounts in foods. They have amenable site of action, quench the reactive oxygen species, induce, and inhibit the gene expression and possess specificity in biological actions (Carbonell-Capella et al. 2014; Porrini and Riso 2008; Correia et al. 2012). The bioavailability of each bioactive compound may differ greatly because of several interfering (Manach et al. 2005; Parada and Aguilera 2007; Correia et al. 2012). Similarly, the concentrations of active metabolites in the target tissues may vary considerably depending on food source or chemical interactions among the phytochemicals and biomolecules (Manach et al. 2005; Carbonell-Capella et al. 2014). Because of their variable diverse individual responsiveness, these bioactive compounds should be explored further. An unusual global increase in the morbidity and mortality rates of NCDs has set forth potentially serious socioeconomic consequences. It has been estimated that almost 41 million global deaths, which is equivalent to 71% of all global deaths, occur due to NCDs. Almost 77% of all NCDs deaths occur in low- and middle-income countries, which can be prevented with appropriate improvement in dietary and lifestyle strategies (WHO 2021). Obesity is linked to an elevated risk of several chronic diseases, including type 2 diabetes, coronary heart disease, stroke, asthma, chronic respiratory diseases, and several types of cancers. Foods of natural origin play a major role in the prevention and management of NCDs; therefore, nutrition interventional strategies are essential to be implemented at all stages of life. It has been suggested that improving the dietary and lifestyle patterns, physical activities, and cessation of smoking, may be effective strategies in the prevention and management of NCDs (Budreviciute et al. 2020). Pharmacological interventions are not only expensive but have also failed to decrease the mortality in the existing population, and therefore, there is a necessity to develop inexpensive measures to control the rise in CVDs and diabetes and their associated secondary complications. The food bioactives like resistant starch, cyclo (His-Pro), a food-derived cyclic dipeptide; and plant polyphenols could be cheap alternatives in the prevention and management of NCDs (Prasad et al. 2015). The empirical and bioinformatics studies have primarily provided in vitro data and only limited clinical relevance to justify the incorporation of bioactive peptides and hydrolysates in the production of nutraceuticals and functional food supplements for health promotion. Well-designed randomized control trials and clinical studies are highly warranted to understand the true effects of polyphenol-bioactive peptides interactions on digestion, absorption, metabolism and biological functions as well as to find cutting-edge evidence to support the health claims about nutraceuticals and functional foods containing BACs (Li-Chan 2015; Pérez-Gregorio et al. 2020).

2 Major Bioactive Compounds in Plant Protein Foods

Legumes and pulses are globally recognized as the main sources of dietary proteins as they contain many BACs including phytochemicals, enzyme inhibitors, phenolic compounds, phytohemagglutinins (lectins), phytoestrogens, saponins, lignans, unsaturated fatty acids and antioxidants compounds such as organic acids, tocopherols, carotenoids, oligosaccharides, dietary fiber, zinc, selenium, and play many important physiological roles in the prevention and management of NCDs (Ciudad-Mulero et al. 2020). The quantities of BACs depend on the type and variety of plant protein foods, the processing and chemical nature of phytochemical. For example, the lutein content among pulses can vary from 4.6 to 818.9 μg/100 g whereas the phenols from 15.0 to 284.3 mg/100 g (Margier et al. 2018). The colour of the seed coat indicates the presence of antioxidant phytochemicals e.g., the black colour of beans shows the existence of high amounts of anthocyanins, polyphenols, and flavonoids (Chávez-Mendoza and Sánchez 2017). Lentils are found in different colours (yellow, orange, red, green, brown, or black), and contain variable amounts of flavan-3-ols, proanthocyanidins and some flavonols that are dependent on the cultivar, composition of seed coats and cotyledons (Ganesan and Xu 2017). The color, smell, taste, and other agronomic traits of crop plants are regulated by the composition of secondary metabolites. Genes have been shown to affect the composition and amounts of secondary metabolites and facilitate the plant adaptations to climate changes, and promote beneficial interactions with biotic factors (Ku et al. 2020). Alcázar-Valle et al. (2020) evaluated 18 accessions of *Phaseolus* spp. and reported significant differences in the amounts of phenolic compounds in various bean species with a strong positive correlation between antioxidant activity and the contents of flavonoids, anthocyanins, and lectins (Alcázar-Valle et al. 2020).

The legume phenolics can interact with digestive enzymes and have been shown to lower the apolipoprotein B secretion from HepG2 cells, which in turn reduce the oxidation of low-density lipoprotein (LDL)-cholesterol (Amarowicz 2016; Martinez-Gonzalez et al. 2017; Hui-Fang et al. 2018). The daidzein and genistein present in soybean and chickpeas possess the antioxidant anti-inflammatory potential and can favourably influence in the prevention of several chronic diseases including CVDs and certain types of cancer. However, the effect of isoflavones in inhibiting digestive enzymes need further clarification (Chakrabarti et al. 2014, 2018; Nediani and Giovannelli 2020). The data from *in vivo* studies endorses the role of legume polyphenols, particularly the isoflavones in the prevention and management of diabetes and obesity by reducing oxidative stress and inflammation. However, how soybean isoflavones impact the sensitivity of leptin, demand further studies (de Camargo et al. 2019). Peas (*Pisum sativum* L.) contain many bioactive phenolic compounds like gallic acid, epigallocatechin, naringenin, and apigenin, which have been shown to possess chemopreventive potential in cancer treatment (Stanisavljević et al. 2016). Anthocyanins from black soybeans displayed anti-inflammatory activities in rat model (Sohn et al. 2014). In another study, the phenolic acids, flavonoids, and anthocyanins from navy and black beans were shown to suppress the mRNA expression of colonic inflammatory cytokines such as IL-6, IL-9, IFN-g, and IL-17A in a mouse model of acute colitis (Zhang et al. 2014). The ethanol extract of red beans containing catechin-7-β-d-glucopyranoside efficiently inhibited the nitric oxide (NO) production in both *in vitro* and *in vivo* experimental models (Park et al. 2011).

The lectins, trypsin inhibitors and amylase present in legumes can selectively bind with trypsin, chymotrypsin, disaccharidases, and alpha amylases to form glycoconjugates and may inhibit their activities. Although the prolonged ingestion of residual levels is unlikely to pose any health risk, an increased intake may impair the protein digestibility (Lajolo and Genovese 2002). On the other hand, these antinutritional compounds may have beneficial properties when consumed at low levels. Lectins are believed to activate innate defense mechanisms, reduce certain types of cancer, and manage obesity. Similarly, the protease inhibitors have been shown to reduce the incidence of certain types of cancer and showed potent anti-inflammatory actions. Although, processing can improve the nutritional quality and palatability of legumes, it may negatively impact the amount of antioxidant phytochemicals, which has however been still considered sufficient to produce beneficial health effects (Ciudad-Mulero et al. 2020). The processing methods can affect the amount of inositol phosphates, galactosides, protease inhibitors and phenolics and may define the potential health benefits of the processed pulse-based products (Pedrosa et al. 2021).

Plant bioactive peptides are believed to be as health beneficial ingredients in the formulation of functional foods. Bioactive peptides (BAPs) have exhibited several benefits in biochemical assays, cell culture, and animal studies. The skills to translate these findings into their commercial use however stays impeded because of paucity of data on the correlation between the *in vitro* findings with *in vivo* results. Ingestion of these BAPs inhibit the digestive enzymes during their transit in the gastrointestinal (GI) tract and cross the intestinal epithelial barriers and eventually reach the target organs in an active form to exert their health-promoting actions. Extensive research studies are therefore needed to understand the *in vivo* physiological effects of these food BAPs with respect to their gastrointestinal stability and transport in the body (Amigo and Hernández-Ledesma 2020). BAPs obtained through the enzymatic hydrolysis of food proteins possess several health-promoting properties against several disease conditions. Protein hydrolysates contain these BAPs and therefore can serve as functional foods (Dhaval et al. 2016). The impact of BAPs depends on their absorption and bioavailability at target tissues. The antihypertensive, anticancer, anticalmodulin, hypocholesterolemic, and multifunctional properties of food protein-derived peptides depend on their structure-function parameters. Future investigations on BAPs should therefore be directed towards elucidation of their *in vivo* molecular mechanisms of action in maintaining homeostasis during aberrant health conditions in human subjects at safe dose levels (Udenigwe and Aluko 2012). Cryptides are the functional peptides (usually having 2–20 amino acids linked by amide bond in a specific sequence), which are encoded within the primary protein sequence. These peptides can also be produced during *in vitro* processing of food proteins with acid, alkali, heat, and enzymatic hydrolysis. In the gastrointestinal tract, they are produced during the digestion process. The most studied cryptides are from serum albumin, immunoglobulins, hemoglobin, and saliva and milk proteins. The BAPs derived from food proteins act as antioxidative, anti-inflammatory, immunomodulatory, antihypertensive, hypocholesterolemic, mineral binding, anti-obesity, antimicrobial, and opiate-like agents (Chakrabarti et al. 2014) and can help in reducing and regulating the onset of prolonged degenerative diseases like cancer, cardiovascular diseases, hypertension, diabetes, inflammation, microbial infections etc. (Iavarone et al. 2018; Priya 2019). The BAPs are more bioavailable, less allergenic and therefore are in great demand (Priya 2019).

The resistant starch, oligosaccharides, and fiber in pulses possess prebiotic functions as they escape digestion and are fermented by colonic bacteria. Increased dietary fiber intake in Canadians resulted in reduced healthcare costs associated with the management of constipation (Abdullah et al. 2015). Lignans, the diphenolic compounds, are present in a wide variety of plants. Though a large variety of lignans exist in nature, only a few of them are converted into enterolignans, enterodiol, and enterolactone by the intestine's bacterial population that are readily absorbed by the human body (Senizza et al. 2020). Flaxseed is an excellent source of lignans. The other sources include grains, legumes, vegetables etc. An inverse association has been reported between lignan levels and the risk of breast, endometrial, ovarian, and thyroid cancers in women (Arts and Hollman 2005). However, McCann and colleagues reported that an inverse association between lignans and breast cancer was only observed in premenopausal US women but not postmenopausal US women (McCann et al. 2002). Enterolactone and enterodiol were shown to decrease in human colon cancer cells in a dose- and time-dependent manner (Qu et al. 2005). There is strong evidence from the *in-vitro* human cell culture studies that enterolactone (EL) have the anticancer and antimetastatic mechanisms in several types of cancers (Mali et al. 2019). There is ample scientific data to suggest that flaxseed lignans have multiple targets and modes of action in the chemoprevention of cancer (De Silva and Alcorn 2019).

Studying the synergistic and antagonistic effects of phytochemicals in food matrix is of great challenge, particularly in understanding their role in the prevention and management of NCDs and cardiometabolic risk (Bouchenak and Lamri-Senhadji 2013). It has been suggested that pulses have the potential to inhibit the pathways of inflammation, DNA damage, cell proliferation, and metastasis. In vitro studies demonstrate that extracts from pulses containing phenolic compounds, proteins and short chain fatty acids induce apoptosis in cancer cells and prevent cancer development. However only a few *in vivo* studies support this hypothesis, and further in vivo studies and translational approaches in humans are required to confirm these findings (Rao et al. 2018). Data from various epidemiological studies reveal that plant phenolics exert antioxidant, anti-inflammatory, antibacterial, anti-analgesic, anti-allergic, and anti-alzheimer effects and show protective effects against chronic inflammation and inhibit Parkinson and Alzheimer diseases (Shahidi and Yeo 2018). Epidemiological studies and clinical nutrition trials suggest that the consumption of plant-based foods have beneficial effects in improving fatty liver disease (FLD). Studies in mice showed that mung bean protein isolate was effective in reducing the hepatic lipid accumulation and can be used to prevent the development of non-alcoholic fatty liver disease (NAFLD) (Watanabe et al. 2017). BACs like resveratrol, anthocyanin, curcumin, and tea polyphenols, could alleviate FLD by ameliorating hepatic steatosis, oxidative stress, inflammation, gut dysbiosis, and apoptosis, and regulate the autophagy and enhance the enzymes of ethanol metabolism. Although plant-based foods are well tolerated, the safe levels of effective doses of their bioactive compounds need to be established in future studies (Sim et al. 2020).

The plant bioactive compounds such as anthocyanins, catechins, β-glucan and n-3 long chain polyunsaturated fatty acids are being studied as functional foods for their possible use in controlling obesity, diabetes, and various biomarkers of metabolic syndrome (Moreno-Valdespino et al. 2020). There is however inconclusive evidence whether the bioactive molecules are efficient in weight management and prevention of metabolic syndrome. This variability may be because of few controlled intervention trials, inconsistencies in study design related to duration, amount and delivery of bioactive supplementation in food products. It has therefore been suggested that well-designed intervention trials should be conducted to substantiate their potential in the treatment of obesity, metabolic syndrome, and related disorders (Bordoni et al. 2019). The kaempferol and quercetin have been shown to reduce the risk of cardiovascular diseases whereas genistein inhibits the growth of breast and prostate cancer cells, and cyanidin 3-glucoside and ferulic acid exhibit antioxidant properties (Chávez-Mendoza and Sánchez 2017). It has been suggested that various bean species have a high biological potential in the prevention of cancer, cardiovascular diseases, and obesity, among others and therefore their conservation, production, and extended human utilization be encouraged and promoted (Alcázar-Valle et al. 2020). Although the bioactivity of individual plant phenolic compounds has now been well characterized, more efforts are needed to understand the synergistic and antagonistic actions of various combinations of phenolic compounds to develop state-of-the-art food products, nutraceuticals and dietary supplements for the prevention and management of different chronic and degenerative diseases (Santana-Gálvez et al. 2019, 2020). It has been suggested that the quantity of phenolic compounds in horticultural crops can be increased through the application of postharvest abiotic stresses. Simultaneously appropriate non-thermal food processing technologies should be developed to retain the antioxidant phenolic compounds and to obtain shelf-stable food products (Jacobo-Velázquez and Cisneros-Zevallos 2020; Jacobo-Velázquez et al. 2020).

3 Mechanism of Action of Plant Protein Bioactive Molecules

Plant bioactive molecules produce their health promoting effects through the metabolites produced by the gut microflora rather than the food components *per se*. Their main impact is on improving the intestinal functionality as they increase the gut microbial diversity, promote healthy bacterial populations, improve endothelial and cognitive functions, reduce bone loss and many other potential benefits on human health and development. The usual daily dietary intake of these complex phenolic food components may exceed 100 mg, which are transformed into simple phenolic metabolites in the colon by gut microbiome (Hartono et al. 2015). Non-digestible starch polysaccharides (dietary fiber) like inulin, raffinose, stachyose act as prebiotics and selectively promote the proliferation of health-promoting bacterial populations in the colon (Slavin 2013; Hou et al. 2019; Praznik et al. 2021). Pulse seed extracts containing soluble fiber have also been shown to improve gastrointestinal motility, intestinal functionality and morphology, and mineral absorption (Singh et al. 2017b; Awika et al. 2018). The soluble extracts can positively affect the intestinal health by increasing mucus production, the number and thickness of goblet cells, villus surface area, and crypt depth and may act, directly or indirectly to increase the mineral solubility and bioavailability. As a result of fiber fermentation and production of short chain fatty acids (SCFA) the intestinal pH is reduced that inhibits the growth of pathogenic bacteria. The SCFA also increase the propagation of epithelial cells thereby increasing the absorptive surface area, contributing to higher assimilation of nutrients (Reed et al. 2018; Tako 2020).

Plant polyphenols have also been proposed as neurotropic chemopreventive agents because of their antioxidant potential and controlling properties in the maintenance of metabolic homeostasis. They can however trigger some toxic effects and therefore should not be recommended indiscriminately without considering their possible risks. Though several phenolic acids and other phytochemicals have been shown to affect the expression and activity of enzymes involved in the production of inflammatory mediators leading to the development of gut disorders including colon cancer. However, it is still ambiguous, which compound is more beneficial for the healthy gut microbiome (Miranda et al. 2018). Low phytate peas (*Pisum sativum* L.)-based diets have been shown to improve the iron status, gut microbiome, and brush border membrane functionality *in vivo* (*Gallus gallus*) (Warkentin et al. 2020). Overall, the dietary plant-origin BACs positively affect the intestinal functionality and growth of gut microbiome (Tako 2020). The immune response is believed to be one of the most complex mechanisms to protect the host against the invading pathogens and toxins. It involves a strong cooperation among various body's cell types and the host-microbe symbiosis to defend the body against any potentially obnoxious agents (Brambilla et al. 2008; Childs et al. 2019; Zheng et al. 2020). Protein deficiency at an early life stage may cause deleterious consequences on immune system and can impede the production of immunoglobulins (Wei et al. 2019). Dietary proteins and other nutrients such as vitamins C, D, E, zinc, selenium, and omega-3 fatty acids play important role in the maturation of the immune system and have been shown to have beneficial effects against infectious and chronic diseases (Shakoor et al. 2021; Smith et al. 2018; Maggini et al. 2018). Lentil proteins provide not only dietary amino acids but are also a source of BAPs that provide many health benefits. The nutritional quality of lentil protein can be enhanced through food processing applications. The antioxidant and antihypertensive properties of lentil peptides have been linked to the primary structure of their C-terminal heptapeptide. Angiotensin converting enzyme (ACE) inhibition relies on the formation of hydrogen bonds between C-terminal residues of lentil peptides and residues of the ACE catalytic site (García-Mora et al. 2017). Genetic strategies in breeding programs to introduce favourable genes are currently being applied to improve the nutritional quality, amino acid composition, and processing fractions of lentil (Khazaei et al. 2019).

4 Plant Protein Foods in Weight Management and Obesity

The health promoting effects of plant protein foods on appetite, body weight, metabolic and glycemic responses, cardiovascular and muscle health are well documented. Although data shows the health-promoting outcomes of plant protein foods, the evidence is still inadequate to formulate precise dietary references for daily meal plans to replace animal proteins with environment friendly plant protein alternatives. More evidence-based studies are required to formulate and validate the health claims for plant protein components (Lonnie et al. 2020). Pulses contribute to improve satiety, reduce food intake, obesity risk and manage diabetes. Regular consumption of pulses in amounts >5 cups per week compared to starchy foods, can help to manage hyperglycemia and hyperlipidemia in diabetic patients (Ramdath et al. 2016). It has been suggested that legumes including the Australian sweet lupins, as a part of traditional diet, can be beneficial to health (Kouris-Blazos and Belski 2016). Pulses, alone or as a part of low-GI or high-fiber diets improved the markers of long-term glycaemic control in humans. Low-glycaemic index diets containing pulses have been shown to increase satiety, control food intake, and prevent the development of coronary heart disease in diabetic and healthy subjects (Rizkalla et al. 2002). Obesity raises the risk of complications from immune-related diseases. Dietary intake patterns are the most important modifiable risk factor in the progression of obesity and related diseases. More data is therefore required to determine the effects of dietary pulses on sustainable long-term weight-loss (Li et al. 2017; Kim et al. 2016). The results of a 28 days randomized controlled trial with overweight and hypercholesterolemic subjects showed that subjects who consumed muffins containing pea flour (equivalent of one-half cup of pulses per day) compared to those who ate control muffins made with white wheat flour showed reduced insulin resistance. Whole pea flour has also been reported to reduce android adiposity in women (Marinangeli and Jones 2010; Marinangeli et al. 2017).

Along with the presence of many phytochemicals, pulses contain dietary fiber, have low glycemic index value, and possess antioxidant and anti-carcinogenic properties. Pulse consumption can therefore not only improve serum lipid profile but can also favourably affect several CVD risks factors like platelet activity, and inflammation, particularly in ageing population. However, well-designed long-term randomized controlled trials are required to validate the immediate effects of pulses on these diseases (Mudryj et al. 2014). Peas (*Pisum sativum* L.), specifically the green and yellow cotyledon dry peas have many health benefits, as its fiber and cell wall constituents contribute to gastrointestinal functions. Amylose content of pulses reduces the digestibility of starch, lowers glycaemic index, whereas the galactose oligosaccharides exert prebiotic effects. Data from a 16-week randomized clinical trial found that plant protein foods not only improved the body composition but also showed a decrease in body weight and insulin resistance. Lower intake of leucine was associated with lower body fat mass whereas the histidine was found to trigger improvement in insulin resistance (Kahleova et al. 2018). Plant protein foods enhance gut microbiome environment, reduce low-grade inflammation, and therefore help to improve immune function and prevent disease severity (Kamboj and Nanda 2018). Use of beans as part of dietary strategy can help to control obesity, prevent cardiovascular and colon protective effects and can improve immune-related disease risk (Mullins and Arjmandi 2021).

5 Plant Protein Foods in Reducing the Risk of Diabetes

Randomized controlled studies indicate that dietary intake of pulses, either alone and/or in combination with high-fiber low GI diet, improve the markers of glycaemic control and reduce hyperglycemia in subjects with and without type 2 diabetes (T2D) (Thomas and Elliott 2010; Gaesser et al. 2019; Ramdath et al. 2016). Hemoglobin A1c (HbA1c) is considered as a diagnostic tool to determine the optimal cut off points for detecting the prediabetes and diabetes conditions. A decrease in HbA1C of >0.3% is proposed as a clinically meaningful threshold (Sievenpiper et al. 2009; Nam et al. 2018). Inflammation is the root cause in the pathogenesis of T2D and its secondary complications. The plant BACs improve insulin resistance and suppress the inflammatory signaling pathways. The BACs act as a therapeutic tool in chronic inflammatory diseases. Polyphenols influence various cellular and molecular pathway and act as metabolic modulators. Plant bioactive peptides from soy, oat, pulses (chickpea, beans, peas, and lentils), canola, wheat, flaxseed, and hemp seed can exert health beneficial properties. The bioactive peptides are encrypted in the native protein sequences and can modulate the biological functions of the body through intracellular signaling pathways. No substantial evidence is however available about their mechanism of action, gastrointestinal bioavailability and potential health claims and therefore did not find clinical commercial usage (Chakrabarti et al. 2018). The Canadian Diabetes Association has recommended plant-based diets in medical nutrition therapy for persons with T2D (Rinaldi et al. 2016).

The data from several epidemiological and randomized controlled trails indicate that nutritional and bioactive components of pulses have the potential cardiovascular health benefits and hence dietary approaches be developed to consume pulses for reducing the risk of CVDs in people living with and without diabetes (Lukus et al. 2020). Epidemiological data shows a steady inverse association between the intake of pulses and the risk of chronic diseases suggesting the possible synergistic effects of phytochemicals in producing beneficial health effects (Rebello et al. 2014). Replacing energy-dense foods with legumes has been shown to produce beneficial effects in the prevention and management of cardiometabolic diseases. Wennberg et al. (2015) observed that higher intake of pulses was associated with a reduced the risk of abnormal glucose metabolism in Mauritian men and women with impaired fasting blood glucose. It has been suggested that instead of aiming at dietary restriction, advisement to increase the intake of pulses would help to reduce the risk of metabolic syndrome (Wennberg et al. 2015). A study examined the effects of consumption of pulses in 44 overweight or obese subjects for 8 weeks, on the risk factors for metabolic syndrome. The group that consumed 5 cups of pulses per week as part of an 8-week ad libitum diet as compared to a reduced energy (500 kcal/day reduction) diet, not only had reduced energy intake but also improved its metabolic markers, including waist circumference, blood pressure, HbA1C, and insulin resistance. The group that consumed pulses also experienced some additional health benefits such as an increase in fasting HDL-cholesterol and C-peptide, a measure of insulin production (Mollard et al. 2012).

It has been suggested that the use of appropriate fiber rich items in the development of new food products will help to improve the health (Veronese et al. 2018). It was observed that substituting half a serving/day of eggs, bread, rice, or baked potato in the Mediterranean diet with legumes lowered the risk of diabetes incidence in Spanish population, particularly in high-risk older cardiac patients (Becerra-Tomás et al. 2018). Chickpea (*Cicer arietinum* L.) is a good source riboflavin, niacin, (thiamin, folate, and the vitamin A precursor β-carotene). Chickpea in combination with other pulses and cereals, can have beneficial effects in the prevention and management of CVDs and other human chronic diseases (Jukanti et al. 2012). In addition to improving the nutrient profiles of meals chickpeas help to delay gastric emptying and slowing of carbohydrate absorption. Chickpeas and chickpea-based food products like hummus contain many bioactive compounds such as phytic acid, sterols, tannins, carotenoids, and other polyphenols that may help in body weight management and prevent the onset of chronic diseases (Wallace et al. 2016).

6 Plant Protein Foods in Hypertension and Cardiovascular Diseases

Cardiovascular diseases are the leading cause of global deaths and are associated with dyslipidemia, hypertension, T2DM, chronic inflammation, and obesity. Dietary intake patterns can potentially alter the initiation and progression of CVDs. Dietary intake of fruits, vegetables and whole foods containing higher quantities of bioactive compounds have been shown to be more cardioprotective because of their antioxidant, anti-inflammatory and antithrombotic effects that interrupt and slow down the development of CVDs (Badimon et al. 2019). Consumption of legumes has been shown to have protective effects against the biomarkers of cardiovascular disease risk and lowered blood pressure in hypertensives (Souza et al. 2015). The pea proteins yield bioactive peptides that showed antioxidant and ACE inhibitor activities and its polyphenolics can exert hypocholesterolemic and anticarcinogenic activity (Dahl et al. 2012). Plant-based protein supplements contain many bioactive peptides that possess ACE-1 inhibitory activity and reduce blood pressure. It has been suggested that specific bioactivities of these peptides must be further tested in high quality randomized controlled clinical trials to formulate precise functional supplements (Roy et al. 2010; Giromini et al. 2017; Jayalath et al. 2014). The individual response to bioactive compounds is diverse as some individuals may benefit more than others and it has been not much explored. Understanding the individual variability with respect to their impact on cardiometabolic outcomes is crucial not only to systematically measure the concentration and nature of circulating metabolites but also to provide evidence-based dietary recommendations (Milenkovic et al. 2017). Pulses have also been shown to have additional beneficial health outcomes on HIV together with reduced risk of chronic diseases (Mudryj et al. 2014). Data from experimental, epidemiological, and clinical studies indicate that bioactive components in foods such as flavonoids, lycopene, resveratrol, omega-3 fatty acids, have prophylactic and therapeutic cardioprotective effects and should be supplemented in all population groups who have a high prevalence of CVDs (Shukla et al. 2010). Data from interventional studies has revealed the role of nutrition in cardioprotection as reduction in dietary fat helped to lower serum cholesterol. The whole diet approach in the Mediterranean-style diet, that emphasizes the use of vegetables, fruit, fish, whole grains, and olive oil has proved to reduce the cardiovascular events better than low-fat diets as indicated in statin trials (Dalen and Devries 2014).

There is compelling evidence that food derived components, food groups, and healthy diets markedly affect dyslipidemia and lower CVDs risk. Dietary patterns that include Mediterranean and Nordic style diets, dietary approaches to stop hypertension (DASH), Portfolio and vegetarian type dietary patterns, are therefore recommended in clinical practice (Trautwein and McKay 2020). The cholesterol lowering benefits of plant protein food sources such as soybean, pulses, and nuts, are well documented (Li et al. 2017). However, data from prospective cohort studies and randomized controlled trials suggest low- to moderate-quality evidence that consumption of pulses have limited beneficial effect on reducing the cardiometabolic risk factors. Additional high-quality large prospective cohort studies and randomized clinical trials must therefore be conducted to determine the advantages of legumes and pulses consumption in the prevention and management of overweight, obesity, diabetes, and CVD (Viguiliouk et al. 2017, 2019).

It is anticipated that a well-defined, plant-based diet can be used as a dynamic therapeutic strategy in clinical practice to alleviate cardiovascular risk factors and lessen the need of pharmacological drug load (Najjar et al. 2018). Data from the pre-clinical and clinical studies shows that plant derived BACs have cardioprotective properties and may prevent coronary artery diseases (Sharifi-Rad et al. 2020). Soybean products contain many BACs, which have been associated in glycemic control, cholesterol reduction, and prevention of bone loss, cardiovascular disease and cancer (Isanga and Zhang 2008). Phytoestrogens, present in soybean, have favorable effects on CVDs risk factors but their effects on cancer are variable and likely complex. Although sufficient evidence is available to suggest that consuming plant protein food sources rich in bioactive compounds has many health beneficial effects, additional data is required to make recommendations for their use in daily meal plan (Kris-Etherton et al. 2002). The soy protein antigens show anti-cancer, anti-human immunodeficiency virus (anti-HIV), anti-microbial infection, prevent mucosal atrophy and improve the drug efficiency. They have also been shown to boost nutrient absorption and thereby reduce the risk of type 2 diabetes and obesity (Xiao 2008). Gomes et al. (2020) evaluated the influence of protein hydrolysates of common beans (*Phaseolus vulgaris* L.) on lipid metabolism and endothelial function in male adult BALB/c mice (*Mus musculus*, class Rodentia) fed an atherogenic diet with and without protein hydrolysate for 9 weeks. They observed that the rats fed on atherogenic diet with protein hydrolysate (700 mg/kg/day) had reduced feed intake, weight gain, lipid profile, tumor necrosis factor-α, angiotensin II (94% and 79%, respectively) and increased endothelial nitric oxide synthase (62%) activity as compared to other groups (normal and only atherogenic diet). They concluded that protein hydrolysate from common beans possess hypocholesterolemic activity, prevent inflammation and dysfunction of vascular endothelium, decrease oxidative stress leading to reduced atherogenic risk (Gomes et al. 2020). Cysteine, glutathione, glutamate, and arginine have been shown to prevent changes that cause hypertension (Vasdev and Stuckless 2010; Zhubi-Bakija et al. 2020). It has been suggested that consuming about 25 g soy protein daily may reduce the risk of heart disease. The food product containing 6.25 g of soy protein per serving can be labelled as part of a diet low in saturated fat and cholesterol and if the food product contributes 25 g soy protein/d, it may be labelled as heart healthy protein. The phytoestrogens isoflavones and other bioactive substances present in soybean and plant-based diets have however some safety concerns (Rizzo and Baroni 2018).

7 Plant Protein Foods in the Prevention and Management of Cancer and Other Diseases

Several plant phytochemicals such as artemisinin, lupeol, curcumin, and quercetin, brazilin, catechin, ursolic acid, β-sitosterol, and myricetin are currently being evaluated for their efficacy as potential cytotoxic agents (Mazumder et al. 2020). The bioactive food components (BFCs) including folate, polyphenols, selenium, retinoids, fatty acids, isothiocyanates and allyl compounds are involved in DNA methylation and histone modifications and possess anticancer potential. Diet-epigenome interactions start occurring in utero and therefore early-life nutrition is of great significance. Dietary influences on DNA methylation and histone modifications focus on epigenetic impacts on candidate genes. The influence of early life nutrition in particular the impact of BFCs on cancer risk programming should therefore be further examined for cancer prevention (Ong et al. 2011). Data from some *in-vitro* and *in-vivo* studies shows that saponins and phytosterols act as chemopreventive agents and may reduce the risk of certain cancers. Saponins are argued to have hypocholesterolemic, immune-stimulatory and anti-carcinogenic properties. Phytosterols are structurally related to cholesterol and compete with cholesterol for their absorption. This differential absorption of sterols is the main mechanisms by which they offer their anti-carcinogenic properties (Rao and Koratkar 1997; Jiang et al. 2019).

Phytic acid, lectins, tannins, saponins, amylase inhibitors and protease inhibitors have been shown to reduce the availability of nutrients and can cause growth inhibition in infants and children. However, at low levels they have been shown to reduce blood glucose, plasma cholesterol and triglycerides levels and improve insulin responses to starchy foods (Samtiya et al. 2021). In addition to this, phytates, tannins, saponins, protease inhibitors, goitrogens and oxalates have been related to reduce cancer risk (Chikara et al. 2018; De Mejía and Prisecaru 2005; Singh et al. 2017a). Phytic acid is a strong chelating agent that can form complexes with protein and minerals (e.g., Ca, Fe, Zn and Mg) making them unavailable for absorption. Low level of phytic acid in diets have however some health beneficial effects and thus can be considered as a bioactive molecule (Ojo 2021). Phytic acid has also been shown to possess anticancer properties in the colon and mammary glands in rodent models and in various tumor cell lines *in vitro*. In view of these beneficial effects, it may be termed as bioactive component (Rickard and Thompson 1997) suggesting that anti-nutrients might not always be harmful. The balance between the beneficial and hazardous effects of plant anti-nutrients however depends on their concentration, chemical structure, time of exposure and interaction with other dietary components. Although anti-nutritional factors are undesirable, their positive impacts on health has led to name them as bioactive substances (Gemede and Ratta 2014). The plant BACs like flavonoids, phenolic acids, alkaloids, saponins, polysaccharides have also been shown to exhibit hypouricemic functions by inhibiting the uric acid production and improving renal uric acid elimination thereby help to manage hyperuricemia (Jiang et al. 2020). The addition of pulses in traditional wheat-based food products will add protein and dietary fiber. Low-phytate pea varieties appeared to provide greater Fe^{2+} bioavailability and moderately improved Fe^{2+} status and significantly improved gut microbiota and the functionality of duodenal brush border membrane suggesting the efficacy and safety of crop biofortification approach (Warkentin et al. 2020).

Pulses are among the most extensively consumed global foods since many decades. Mung bean (*Vigna radiata* L.) has a long history of use as traditional medicine in Asian countries. It has been known to ameliorate hyperglycemia, hyperlipidemia, hypertension, cancer, and carry hepatoprotective and immunomodulatory activities (Hou et al. 2019; Watanabe et al. 2017; Dahiya et al. 2015). Vitexin and isovitexin are the major polyphenols and peptides in mung bean that possess higher bioactivity (Hou et al. 2019). Chickpeas, green peas, and kidney beans are the main legumes consumed in Australia. It has been suggested that to promote their consumption innovative culinary techniques highlighting their nutritional significance and promising health attributes must be employed (Figueira et al. 2019). Lentils (*Lens culinaris* L.) contain many bioactive and functional compounds that possess high total antioxidant capacity. The fiber and phytochemicals of lentils help to improve hyperglycemia, lipid and lipoprotein metabolism and body weight management in diabetic patients (Aslani et al. 2015). Some plant-based protein sources (e.g., soy legume products, sea vegetables, and brassica vegetables) can contain high levels of purines that may increase uric acid levels and trigger gouty arthritis. Additional studies are therefore required to establish the safe intake of plant protein foods, particularly in hyperuricemic individuals (Jakše et al. 2019).

8 Plant Protein Food Sources as Part of a Typical Everyday Diet

The global interest in the use of plant-based diets is increasing because of their health benefits in the prevention and management of chronic diseases (Agnoli et al. 2017; Campbell 2019; Medawar et al. 2019). The global increase in the intake of saturated and trans-fats, refined sugars, salt, processed meat, dairy products, and highly processed industrial food products have increased the risk of NCDs. In addition to this, the improper policy planning and imbalanced nutrition has increased the risk of malnutrition and NCDs in developing countries. It is important to identify cost-effective solutions to promote healthy dietary and lifestyle policies in combating the emergence of NCDs. Dietary globalization has increased the variety and nutrient adequacy in traditional monotonous diets, yet it has also introduced the Westernized foods leading to high intake of saturated fats, refined and processed grains that need caution. It is a big global challenge as the multinational fast-food companies are vehemently exploring new world markets to promote their products. It is proposed that well-planned dietary policy interventions and persistent efforts are required to beneficially modify the adverse trends in the consumption of energy-dense Westernized foods. However, the plant protein-based diets may have variable effects on CVDs risk factors because of nonprotein compounds. Increased consumption of protein-rich foods can result in lower intakes of other nutrients, which may simultaneously influence the gut microbiota. More conclusive evidence is needed to establish whether the plant or animal protein food sources are more beneficial (Richter et al. 2015). It has been suggested that the dietary patterns, which provide more unprocessed plant protein food sources rich in bioactive components have been shown to be more beneficial to health compared to energy-dense typical American diet in plummeting the risk factors and mitigating the pathological impact of CVDs, diabetes, cancer and neurodegenerative disorders (Richter et al. 2015; Samtiya et al. 2021).

Dietary intake patterns such as Mediterranean-Style dietary patterns, Dietary Approaches to Stop Hypertension (DASH) or a combination of Mediterranean-DASH Intervention that promote healthier foods like intake of fruits, vegetables, whole grains, legumes, seeds, and nuts with a simultaneous decrease in highly processed animal-based foods containing high amounts of saturated fats, sugars and processed meats and foods, positively influence human health and help to avoid the risk of NCDs and neurodegenerative disorders (Cena and Calder 2020). The impact of unhealthy lifestyle behaviours on health outcomes can vary considerably among individuals and must be explored further. There is sufficient evidence to suggest that lifestyle-associated oxidative and inflammatory responses are the primary drivers for the cell and tissue damages which in turn can lead to the promotion of NCDs. Monitoring the biomarkers of oxidative and inflammatory activities can be an efficient way in the prevention of NCDs and development of effective treatment strategies (Seyedsadjadi and Grant 2021). It has been shown that traditional plant-based foods of Native Americans deliver substantial amounts of bioactive compounds with distinct health outcomes and must be explored further to address the NCDs challenges not only in indigenous population but also in other communities globally (Sarkar et al. 2020).

9 Conclusion and Recommendations

Noncommunicable diseases (NCDs) such as obesity, diabetes mellitus, cardiovascular diseases, and cancers are increasing sharply worldwide and imposing an unusual burden on the socioeconomic status of people, especially in developing countries. These diseases are driven by several factors including rapid unplanned urbanization, aging population, improper policy planning, imbalanced nutrition, unhealthy lifestyle and dietary patterns. It is important to find low-cost consumer friendly solutions to promote healthy dietary and lifestyle policies in preventing and managing the NCDs. Plant bioactive products have proved to be competitive candidates for alternative therapy in several disorders. Plant protein foods comprise a variety of BACs with diverse chemical structures and traits, which have been reported to modulate the metabolic processes and offer many health benefits. They have the potential to modify the antioxidant potential, inhibit or induce the enzyme actions, impact gene expression and receptor activities as well as nutrient bioavailability. Besides, they have the potential to influence energy intake, reduce pro-inflammatory state, oxidative stress, and metabolic disorders, with different intracellular signaling pathways and possess many other therapeutic benefits. The consumption of legumes has been shown to have protective effects against the biomarkers of numerous chronic or non-communicable diseases because of the presence of a diverse range of polyphenols. Some polyphenols such as quercetin, curcumin and isoflavones have undergone clinical trials. Well-designed randomized control trials and long-term interventional dietary approaches are required to characterize and comprehend the underlying mechanisms of plant bioactives in the prevention and management of NCDs.

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Chapter 14
Antinutritional Factors and Biological Constraints in the Utilization of Plant Protein Foods

Amanat Ali, Sankar Devarajan, Annamalai Manickavasagan, and Athar Ata

1 Introduction

Proteins are the principal supplier of nitrogen for growth, repair, and maintenance of tissues and vital physiological functions. The quality of proteins implies to its amino acid composition, digestibility, and their bioavailability and varies with protein structure, processing, and the presence of certain antinutritional factors (ANFs) that may limit the digestion (Sá et al. 2020). The quality and digestibility of plant protein foods is considered lower as compared to animal protein foods. This is because of the presence of numerous non-nutrients that affect the bioavailability of protein and certain minerals, particularly of calcium, iron, and zinc. Besides plant protein foods have limited supply of some essential amino acids such as lysine, methionine, threonine, and tryptophan, and do not provide vitamin B12 (Rogerson 2017; Agnoli et al. 2017). Plants produce an array of noxious compounds to safeguard themselves against different types of predators like herbivores, insects, pathogens, and microbes as well as to fight against the adverse environmental conditions. These secondary metabolites are produced by normal metabolism of plants through different mechanisms and impact their colour smell, and taste (Dang and Van Damme 2015; Ku et al. 2020). Both the genetic and environmental factors have

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been reported to affect these chemical compounds in plant foods, particularly in soybean under different agroclimatic conditions (Maria John et al. 2017). A wide variety of toxic proteins and gene derived polypeptides have also been discovered in different plant protein foods (Csaky and Fekete 2004; Dang and Van Damme 2015). These chemical compounds have relatively no or little nutritional significance and have been shown to impact the optimum use of food nutrients, and may have either beneficial or harmful effects on body functions under certain conditions (Williamson 2017). The compounds, which impact the optimum use of food nutrients and reduce their digestion, absorption and metabolic utilization and may generate adverse health effects, are termed as antinutritional factors (ANFs) or antinutrients. Some of these antinutrients are however basically useful for the growth and survival of crops. ANFs play important active roles in determining the use of plant protein foods for humans. Depending on their structure and molecular weight, these secondary metabolites in plant protein foods limit the utilization of their nutrients and may trigger variable adverse physiological effects in humans (Samtiya et al. 2020; Matsuura and Fett-Neto 2017).

The individual responses to these dietary ANFs as toxic compounds may be acute, sub-acute or chronic and can vary widely depending upon several factors like the nature of toxicant, dose, timing, synergistic and antagonistic action of other ingredients etc. The metabolism of these chemical compounds can however be modified by several extrinsic and intrinsic factors that impact the normal functioning of the organism. Changes in the toxicity of these food components can be due to changes in their metabolism, as most of the events that lead to overt toxicity involve activation, and/or detoxification of the original food component. These compounds may however also exert some beneficial health effects when consumed at low concentrations. The level or concentration of these antinutrients in plant protein food sources can vary with respect to plant species, cultivar, post-harvest treatments as well as the use of processing methods (Akande et al. 2010). The major antinutrients and toxic compounds present in plant foods are; protease inhibitors, α-amylase inhibitors, α-galactosides, anti-minerals, phytates, oxalates, tannins, polyphenols, different types of alkaloids, lectins, phytohaemagglutinins (PAH), gossypol, saponins, cyanogenic glycosides, compounds causing favism, lathyrogens, goitrogens, phytoestrogens, lipoxygenases, anti-vitamin factors, uricogenic nucleobases in yeast protein products as well as the toxic proteins and food allergens (Khokhar and Apenten 2003; Gilani et al. 2012; Gupta 1987; Kiranmayi 2014). Details about the presence of various ANFs in different plant protein food sources are listed in Table 14.1. The ANFs are of major concern in human nutrition as they may inhibit the enzyme activity and form complexes with nutrients to reduce the digestibility and nutrient bioavailability of plant protein foods. However, the adverse effects of ANFs and toxic metabolites have not been appropriately addressed globally. Some of these ANFs in plant protein foods can be toxic (e.g., some lectins, cyanogenic glycosides, non-protein amino acids (NPAAs)), some make them unpalatable and add bitter taste (e.g., tannins or NPAAs), whereas the others may have adverse effects leading to reduced consumer's growth and fitness through nutrient complexation (e.g., phytates), metabolic inhibition (e.g., NPAAs, cyanogenic glycosides,

Table 14.1 Antinutritional factors in plant protein food sources

Plant protein foods	Antinutritional factors	References
Legumes (grains and seeds in general)	Protease inhibitors, amylase inhibitors, lectins (phytohemagglutinins), lathyrogens, cyanogenic glycosides, alkaloids, chlorogenic acid, non-protein amino acids [NPAAs], saponins, tannins, phytic acid, oxalates, gossypol, goitrogens, isoflavones, compounds causing favism, phytoestrogens, lipoxygenase, allergens, factors affecting digestibility	Akande et al. (2010), Bennetau-Pelissero (2019), Bohn et al. (2008), Gilani (2012), Gupta (1987), Samtiya et al. (2020)
Wild legumes of South India	Total free phenolics (0.41–5.96%), tannins (0.04–0.60%), L-DOPA (1.34–8.37%), trypsin inhibitor activity (13.48–65.43 TIU/mg protein).	Vadivel and Janardhanan (2005)
Pulses (in general)	Protease inhibitors (trypsin and chymotrypsin inhibitors), amylase inhibitors lectins, phytic acid, polyphenols, tannins, lathyrogens, saponins, flatulence factors, oligosaccharides (raffinose, stachyose, verbascose and ciceritol), antihistamines and allergens	Singh (2017), Sharma et al. (2013), Parca et al. (2018), Jain et al. (2009)
Faba beans and seeds (*Vicia faba* L.)	Trypsin inhibitors, lectins pyrimidine glycosides vicine and convicine (v-c), pro-anthocyanidins, phytic acid, phytates, tannins,	Mattila et al. (2018), Cantoral et al. (1995), Khazaei et al. (2019)
Chickpea (*Cicer arietinum* L)	Trypsin inhibitors, lectins-hemagglutinin activity, α-galactosides, tannins, saponins; phytic acid, stachyose, raffinose	El-Adawy (2002), Frias et al. (2000), Olika et al. (2019)
Black gram (*Vigna mungo L. Hepper*)	Antinutritional factors (phenolic compounds, tannins, saponins, phytic acid, trypsin inhibitors)	Suneja et al. (2011)
Horse gram & moth beans	Trypsin inhibitors, hemagglutinin activities and polyphenols	Kadam and Salunkhe (1985)
Common bean cultivars (*Phaseolus vulgaris*, L.), white and red beans	Trypsin inhibitors, lectins-phytohemagglutinins, phytic acid, alpha-amylase, and glucosidase inhibitors, Arcelins (lectin-related proteins)	Bollini et al. (1999), Barrett and Udani (2011), Batista et al. (2010), Carbonaro et al. (2000), Dang and Van Damme (2015)
Dolichos lablab beans (*Lablab purpureus*) of Kenya	Tannins, phytates and trypsin inhibitory activity	Kilonzi et al. (2019)
Rice bean (*Vigna umbellata*)	Trypsin inhibitor activity and phytic acid, polyphenolic compounds and saponins	Kaur and Kapoor (1992), Katoch (2013)

(continued)

Table 14.1 (continued)

Plant protein foods	Antinutritional factors	References
Cowpea (*Vigna unguiculata* L.) and Tribal pulse	Trypsin inhibitors, phytic acid, tannins, hydrogen cyanide, oligosaccharides L-Dopa (L-3, 4-dihydroxyphenylalanine)	Kalpanadevi and Mohan (2013), Khalid and Elhardallou (2016), Khattab and Arntfield (2009), De-Paula et al. (2018), Price et al. (1980), Jayathilake et al. (2018)
Soybeans (*Glycine max*)	Trypsin inhibitors, lectins, agglutinin-lectins, phytic acid, tannins, saponins and isoflavones, allergenic proteins, glycinin, beta-conglycinin	Anderson and Wolf (1995), Bajpai et al. (2005), Cantoral et al. (1995), Wang et al. (2014)
Grass pea (*Lathyrus sativus* L.)	Antinutritional factors, neuroexcitatory amino acid, beta-N-oxalyl-l-alpha, beta-diaminopropionic acid (beta-ODAP), endopeptidase protease inhibitors.	Xu et al. (2019), Miranda et al. (2019)
Field Pea (*Pisum sativum*)	Phytates, tannins, trypsin inhibitors and lectins	Vidal-Valverde et al. (2003), Cantoral et al. (1995)
Plant proteins (in general)	enzyme inhibitor, lectins, and allergens, phytic acid,	Jie et al. (2001)
Tepary bean proteins	Trypsin inhibitor activity (TIA), hemagglutinating activity, and phytic acid.	Idouraine et al. (1992)
Jatropha kernel Protein isolates	Phytate, tannin and saponin and phorbol esters and cyanogenic glucosides reduced to minimum	Devappa and Swamylingappa (2008)
Fababean protein isolates	Hemagglutinins, phenols, phytic acid, vicine, convicine, trypsin inhibitors and α-chymotrypsin inhibitors	Arntfield et al. (1985)
Mung bean protein isolates	tannins, phytic acid and trypsin inhibitor	El-Adawy (2000)
Linseed protein fractions	Trypsin inhibitors	Anaya et al. (2015)
Mustard and rape seed proteins	Glucosinolates, gossypol	Gilani et al. (2012)

isoflavones, alkaloids) and interference with digestion and absorption of nutrients such as protease inhibitors, α-amylase inhibitors, lectins etc. (Gemede and Ratta 2014; De Angelis et al. 2021; Parca et al. 2018). For example, the trypsin inhibitors and phytates reduce the digestibility of proteins and bioavailability of minerals, respectively. The amount of naturally occurring ANFs in plant protein foods regulates their use for human consumption (Mattila et al. 2018). Depending upon their toxic impacts, some of these compounds have received considerable attention whereas the others have not been well studied. This chapter features about the presence of these ANFs in plant protein food sources, their mechanism of action, use of processing methods to reduce or eliminate them and lists their adverse health effects in humans.

2 Antinutritional Factors and Their Impact on Human Health

2.1 Protease Inhibitors

Plants produce protease inhibitors (PIs) not only to protect them against the attack of herbivores, insects, fungus, and bacteria but also to prevent the proteolytic degradation of their storage proteins in seeds. PIs are widely present in a variety of plant protein foods and are important to assist the innate defense mechanism in plants to maintain the physiological homeostasis. During the propagation and sprouting of seeds, the ANFs like enzyme inhibitors support the early plant growth (Savelkoul et al. 1992). PIs inhibit the digestive enzymes of the pests and insects that attack the seeds leading them to starvation or produce hemolysis in their gastrointestinal tract causing their death (Hellinger and Gruber 2019; Anderson and Wolf 1995; Aviles-Gaxiola et al. 2018). PIs are a heterogenous group of proteins with molecular weights ranging from >15 kDa to <15 kDa (Birk 1996). About 104 plant families with diverse properties have been identified to express a range of PIs, including polyphenols, terpenes, flavones, saponins, alkaloids, tannins, amino acids, di- and tripeptides, and derivatives thereof as well as plant peptide- or protein-based PIs (Polya 2003; Gomes et al. 2011; Hellinger and Gruber 2019). PIs exert their negative impact on the nutritional quality of proteins by initiating pancreatic hypertrophy that results in growth inhibition. PIs play crucial role in the regulation of endogenous proteases and may display the antifeedant, antifungal, antitumor and cytokine inducing activities. Some may have useful therapeutic impacts and may help to lower blood cholesterol or prevent cancer (Srikanth and Chen 2016).

Soybeans contain many antinutritional compounds, which have different biological functions such as hormonal, immunological, bacteriological, and digestive impacts (Gemede and Ratta 2014; Mangena 2020). Liener (1996) estimated that consuming raw soybeans can inhibit about 40% of growth mainly because of the presence of trypsin inhibitors (TIs) like Kunitz inhibitor and Bowman-Birk inhibitor. The agglutinins present in soybean inhibit growth, whereas undenatured protein result in poor digestibility (Liener 1996). Soybean contains the highest amount of TIs that can trigger considerable reductions in protein and amino acid digestibility (Gilani et al. 2012). The data from rat studies showed that the impact of ANFs on protein quality and digestibility was more noticeable in elderly as compared to young (Gilani et al. 2012). The presence of ANFs in plant protein foods not only adversely affect the nutrients bioavailability but also reduce the protein digestibility and may cause growth inhibition in infants and children (Gemede and Ratta 2014). It has therefore been suggested that while designing the daily meal plans for elderly people one must consider the impact of ANFs on protein digestibility. PIs isolated from the *Dolichos biflorus* (horse gram) were stable over a wide range of pH (2.0–12.0) at 100 °C for 20 min and inhibited the action of trypsin and chymotrypsin (Kuhar et al. 2014). TIs are one of the most relevant and widely studied ANFs (Aviles-Gaxiola et al. 2018). Idouraine et al. (1992) reported that ethanol extracts of tepary bean protein showed variable trypsin inhibitor activity (TIA) ranging from 1·9 TIU/mg sample for 2-ME to 161·01 TIU/mg sample. The highest TIA was observed in sodium phosphate buffer (SPB) whereas the lowest was in ethanol fraction. No hemagglutinating activity (HA) was recognised in sodium dodecyl sulfate (SDS) fraction (Idouraine et al. 1992). The TIs contents of soybean and common beans ranged from 43–84 to 21–25 TIU/mg of sample, respectively. The TIs content of different Lathyrus cultivars, ranged from 19–30 TIU/mg sample whereas the chickpea and pea contained 15–19 and 6–15 TIU/mg sample. Lentils and faba bean had low values in most cultivars ranging from 3–8 to 5–10 TIU/mg of sample, respectively. The amount of TIs varied with legume species and variety (Guillamon et al. 2008). Plant PIs are a diverse family of polypeptides, which play important role in body's homeostasis. Regulation of proteases function is therefore essential to avoid pathogenesis. As the PIs target human proteases, they are now the targets for new drug discoveries against cancer, inflammation, and neurodegenerative diseases (Hellinger and Gruber 2019).

2.2 Lectins

Lectins are widely distributed in plant protein foods. Lectins are thought to be implicated in plant's defense mechanism against predators through building a symbiotic relationship between legumes and N-fixing bacteria (Liener 1997). They are non-catalytic carbohydrate-binding proteins of non-immune origin and many of them are not easily denatured by heat and may resist proteolytic digestion. Depending on the genetic and environmental factors, high amounts of lectins get accumulated in the seeds of legumes. In legumes, lectins structurally constitute a diverse class of glycoproteins, which may represent up to 10% of the protein. They have been extensively studied to develop biochemical tools for the isolation and characterisation of glycoprotein. Although their biological role remains mysterious, they may act as toxic allergens and hemagglutinins prompting intestinal disorders (De Mejía and Prisecaru 2005; He et al. 2018). Recent proteomic methodologies have made it possible to develop and validate sensitive and specific assays for detecting trace amounts of harmful lectins in foods (Nasi et al. 2009). Lectins from the common bean *Phaseolus vulgaris* act as phytohemagglutinin and can lead to gastroenteritis, nausea, and diarrhoea. The consumption of improperly cooked legumes can result in non-pathogenic food-borne poisoning (Lam and Ng 2011; Kenmochi et al. 2015). Lectins promote changes in bacterial microflora by gradually interfering with nutrient metabolism as result of binding with glycoprotein receptors on the epithelial cell lining of intestinal mucosa. Lectins can enter the circulatory system and can produce hormonal and immunomodulating effects on the intestinal transport system. They can promote degeneration of internal organs and tissues and can inhibit growth by systematically interfering with nutrient absorption and metabolism (Vasconcelos and Oliveira 2004). Lectins have been shown to produce selective cytotoxicity against cancer cells and may also have the antimicrobial and insecticidal activities. Some of the plant lectins have been shown to inhibit the exocytosis and repair mechanism of plasma membrane of gut epithelial cells (Miyake et al. 2007). Lectins from the white kidney beans (*Phaseolus vulgaris*) were presumed to be the causative agent for the acute intestinal symptoms in Japanese population (Ogawa and Date 2014).

Consumption of high amounts of uncooked or partially cooked kidney beans containing high quantities of lectins, saponins, phytates, and PIs has been shown to cause food poisoning (Bender and Reaidi 1982; Vasconcelos and Oliveira 2004; Dolan et al. 2010). Kidney beans (*Phaseolus vulgaris* L.) may also trigger allergenicity via its allergenic proteins (Kumar et al. 2013). Lectins such as phytohemagglutinin (PHA) from red kidney beans (*P. vulgaris*), can lead to acute gastroenteritis because of loss of epithelial resistance. Lectins have been shown to disturb the hormonal homeostasis and can promote pancreatic hypertrophy in a dose-dependent manner (Vasconcelos and Oliveira 2004). Lectins can resist digestion, persist through the gut passage, and may enter the circulation retaining full biological activity (De Mejía and Prisecaru 2005). In addition to this, some legume lectins can inhibit the activities of various intestinal and brush border enzymes such as sucrase, maltase, aminopeptidase, and dipeptidyl peptidase. Although most of the foods are cooked before eating, some lectins may appear to be quite stable and do not get denatured by heat escaping proteolytic digestion and therefore can impact the functions of digestive system (He et al. 2018). Consumption of high amounts of raw or improperly cooked kidney beans have been reported to cause many incidents of food poisoning (Rodhouse et al. 1990). Although certain lectins may be harmful to health, the overall dietary effects of lectins are minimal (Panacer and Whorwell 2019). In general, the dietary intake of lectins is low and may not have any measurable negative impact on nutritional performance (He et al. 2018). Small amounts of lectins may however positively impact the biological system exhibiting the mitogenic, antitumor, immunomodulating, antifungal, antibacterial, antiviral, and insecticidal activities. Lectins-related amylase inhibitors obtained from bean extracts have shown their utility as “weight-blockers” in nutritional formulations for obesity treatment. Lectins as bioactive proteins provide great opportunities to develop pharmaceutical and nutritional products for their practical applications in the context of disease prevention and safety issues (Roy et al. 2010; Muramoto 2017; Liener 1997; He et al. 2018).

Weder et al. (1997) observed that the ANFs in Anasazi bean were significantly less as compared to traditional pinto bean (*Phaseolus vulgaris* L.). They categorised lectins of Anasazi beans as nontoxic whereas of pinto beans as toxic. However, no significant differences were observed in the TIA of both types of beans (Weder et al. 1997). Similar differences were reported among various grass pea accessions (*Lathyrus sativus* L.) with respect to ANFs including the neuroexcitatory amino acid beta-N-oxalyl-l-alpha, beta-diaminopropionic acid (beta-ODAP) and PIs (Xu et al. 2017). It has been suggested that grass pea lines containing low ANFs be developed through breeding programs (Xu et al. 2019). The amount of ANFs (trypsin inhibitors, phytates, tannins) in all desi cultivars of chickpea were significantly higher compared to their Kabuli counterparts. The tannin contents ranged from

0.07% to 0.22% whereas the amounts of trypsin inhibitors were from 9 to 31 mg/g in both the cultivars of chickpea. *In-vitro* starch digestibility of kabuli chickpea was higher than desi chickpea cultivars (Sharma et al. 2013). Suneja et al. (2011) also reported significant variability in the amounts of ANFs (phenolic compounds, tannins, saponins, phytic acid, trypsin inhibitors) in different cultivars of black gram (*Vigna mungo* L. Hepper). They suggested that cultivars with low contents of ANFs should be developed through improved breeding programs (Suneja et al. 2011).

2.3 Phytic Acid or Phytate

Phytate or phytic acid (PA) is naturally present in cereal grains, legumes, and many other plant foods. Phytic acid normally occurs as calcium, potassium, and/or magnesium salt and accumulates in seeds during the developmental stages and its concentration reaches maximum at maturity. During the germination the seed, the phytate is hydrolysed by endogenous phytases to release phosphate, inositol, and micronutrients to meet the needs of growing seedling (De-Paula et al. 2018). Because of its six phosphate groups, phytate can act as a strong chelating agent and forms complexes with proteins at physiological pH (Selle et al. 2012) and can inhibit the action of digestive enzymes such as alpha-amylase, lipase, or proteinase (Kumar et al. 2020). Such inhibition of enzymes can not only lower the digestibility of proteins but may also reduce the bioavailability of other nutrients (Bohn et al. 2008; Burgos and Armada 2019) (204). Sharma and colleagues reported that the protein digestibility of millet grains was negatively correlated with the amount of phytic acid (Sharma and Gujral 2019). Phytic acid can strongly bind to metallic cations (Ca, Fe, K, Mg, Mn and Zn) forming complexes, which make these metal ions biologically unavailable. In the presence of calcium, phytate can chelate with zinc and iron making them unavailable for absorption (Gibson et al. 2018; Singh 2017). Phytic acid and its derivatives are thought to be involved in RNA export, DNA repair, signalling pathways, endocytosis, and cellular vesicular trafficking. Biochemical data indicates the scope and application of isolated phytases in food industry (Bohn et al. 2008). De-Paula and colleagues tested forty-three genotypes of cowpea beans and reported significant differences in their phytic acid, phosphorus, and zinc contents. They observed that the cultivar L042 had the lowest phytic acid content (9.630 ± 1.725 mg/g) and considered it the best based on the bioavailability of minerals. They suggested that it should replace the currently consumed varieties of cowpea beans (De-Paula et al. 2018). Khattab and Arntfield (2009) reported that kidney beans contained the highest amounts of antinutrients whereas only small quantities of tannins and phytic acid as well as the lowest TIA were recorded in Canadian cowpea (Khattab and Arntfield 2009). Idouraine et al. (1992) reported that phytic acid was high in tepary flour (TF) (4·61 mg/g) (Idouraine et al. 1992). The dietary intake of phytate varies among different populations and largely depends on their dietary intake patterns. The people consuming whole grains and plant-based diets can have higher intakes that may exceed >2 g/day (Amirabdollahian and Ash

2010). As both the environmental and genetics factors influence the oligosaccharides, phytate, saponin and lectin contents of *P. vulgaris*. Oxalic acid present in foods not only reduces the bioavailability of certain minerals but may also lead to the formation of urinary tract stones causing a disease known as urolithiasis (Petroski and Minich 2020). The appropriate varieties of dry beans with lower concentrations of ANFs such as a-galactosides, inositol phosphates, saponins and lectins should be selected for human consumption and large-scale cultivation (Muzquiz et al. 1999).

2.4 Alkaloids

Alkaloids constitute a critical group of naturally occurring plant secondary metabolite that primarily contain nitrogen in their structures. Based on their structure, alkaloids can be classified as indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, xanthine alkaloids, tropanes, terpenoids and steroids. They are present in many economically important plant protein foods and constitute an important component of everyday human diet (Schramm et al. 2019). The major alkaloids in coffee and cacao seeds, and in tea leaves are caffeine, theobromine, and theophylline whereas in tomatoes tomatine and in potatoes solanine. Plant extracts containing alkaloids like morphine, quinine and colchicine have been commonly used since centuries to treat and cure people from pains and illnesses (Jie et al. 2001). The toxic effects of these alkaloids depend on their dose, exposure time, and individual characteristics. Depending on their use in pharmacological context, they can have both harmful as well as beneficial effects (Matsuura and Fett-Neto 2017; Adamski et al. 2020). It has been suggested that genetically engineered breeding programs should be developed to enhance the production of legume varieties, containing favorable secondary metabolites with health beneficial effects, for facilitating their adaptations to climate changes (Ku et al. 2020).

2.5 Phenolics, Tannins, Saponins, Glucosinolates, Goitrogens, Phytoestrogens, Gossypol, Oxalates, Cyanogenic Glycosides, Flavonoids, and Flatus Producing Oligosaccharides

The phenolic compounds are made up of phenol rings attached to one or more hydroxyl groups which include flavonoids, phenolic acids, tannins, stilbenes, anthocyanins, xanthines and lignans. The antioxidant capacities of these phenolics are linked to the hydroxyl groups attached to phenolic rings. Phenolic compounds are highly reactive and can bind reversibly as well as irreversibly with proteins to interfere with their digestibility and bioavailability of amino acids. Tannins are complex phenolics and are structurally classified either as hydrolysable or condensed tannins. Tannins chelate with metal ions, particularly with iron and form complexes with proteins and starches lowering their digestibility and bioavailability (Singh et al. 2017a; Minatel et al. 2017; Singh 2017). High levels of tannins present in grain legumes have been shown to reduce their protein and amino acid digestibility (Serrano et al. 2009). However, tannins do not appear to have any significant adverse effect in human nutrition. Soybeans contain isoflavones, which have estrogenic properties and may cause safety concerns (Rizzo and Baroni 2018). The glucosinolates are sulphur containing compounds, which are mainly present in plants of *Cruciferae (Brassicaceae*) family such as cabbage, kale, brussels, sprouts, cauliflower, broccoli, and kohlrabi. Goitrogenic compounds are formed from the breakdown of glucosinolates by the enzyme thioglucosidase. The most common types of goitrogens are goitrins, thiocyanates and flavonoids. Glucosinolates can impair the thyroid functions by reducing its ability of hormone production and therefore interfere with iodine metabolism (Felker et al. 2016). Natural goitrogens present in legumes impede the uptake of iodine by thyroid gland and resist the production of thyroid hormones. Soybean foods containing goitrogens can increase the thyroid functioning by interfering with thyroid hormone action reducing the T4 absorption (Bajaj et al. 2016). Goitrins released from glucosinolates are mostly destroyed during household cooking processes and are leached out in cooking water. Pearl millet contains phenolic flavonoids, which may be implicated in the onset of goiter, because of iodine deficiency. The phytoestrogens are structurally similar to estradiol that can bind to estrogen receptors and disrupt the endocrine functions. The important compounds with estrogenic activity are isoflavones, lignans, coumestan, daidzein, glycitein, genistein stilbene and are mainly present in soybean, lentils, mung beans and their products (Nie et al. 2017; Patisaul 2017).

Although the cyanogenic glycosides (CNGs) do not play a role in seed germination and seedling growth, yet they protect the plant against the leaf herbivores. They have been reported to be present in wild lima beans (*Phaseolus lunatus* L.) (Cuny et al. 2019; Ballhorn et al. 2009; Cressey and Reeve 2019). Upon enzymatic degradation, cyanogenic glycosides release the toxic hydrogen cyanide that can cause hyperventilation, nausea, and vomiting. They are mainly present in bitter almonds, apricots, cassava roots, sorghum, lima beans (Cressey and Reeve 2019; Ballhorn et al. 2009). The exposure to CNGs from everyday diet could be from biscuits, juices, pastries, and cakes that can potentially contain CNGs (Schrenk et al. 2019). Saponins are bitter tasting, surface active agents, which are poorly absorbed and have intensive foaming activity that can cause hemolysis of erythrocyte by disrupting the cell membrane. Structurally they are triterpene glycosides or mono-, di-, tri-, or sesqui-terpenoids. Saponins have also been shown to form complexes with zinc and iron, and can limit their bioavailability (Mohan et al. 2016). Different types of saponins are present in food legumes (Shi et al. 2004). The major food sources are soybeans, sugar beet, peanuts, spinach, broccoli, potato, and apples. They have many health-promoting effects such as antidiabetic, hepatoprotective, anticarcinogenic, antimicrobial, cholesterol reducing, immune modulating, and anti-inflammatory properties (Singh et al. 2017b; Guzmán et al. 2020). In addition to protease inhibitors, grass peas (*Lathyrus sativus* L.) contain an endogenous neurotoxic nonprotein amino acid beta-N-oxalyl-l-alpha, beta-diamino-propionic acid (beta-ODAP). Long-term consumption of grass peas (*Lathyrus sativus* L.) is linked to lathyrism, a neurodegenerative disorder that can lead to bone deformations. It has been shown that grass pea cultivars free from ANFs can be developed through appropriate breeding programs (Xu et al. 2017, 2019). The nutrients and antinutritional contents of 18 pea lines (*Pisum sativum*) varied significantly. The highest TIAs were observed in peas with yellow cotyledons. The inositol hexaphosphate contents of peas varied with colour and size. The brown peas showed the highest amount whereas the bigger peas showed the lowest inositol pentaphosphate contents (Vidal-Valverde et al. 2003). Faba beans (*Vicia faba* L.) is an important legume that has not been properly utilized because of its ANFs (Rahate et al. 2021). Faba bean (*Vicia faba* L.) contains the pyrimidine glycosides vicine and convicine (v-c) that reduce its digestibility and feed efficiency and can cause favism, particularly in individuals having a genetically inherited deficiency of glucose-6-phosphate dehydrogenase (G6PD). Marker-assisted breeding programs have helped to reduce the levels of v-c in faba bean cultivars, which have been shown to be safer for G6PD-deficient individuals (Khazaei et al. 2019). Pulses and grain legumes contain some flatulence-producing oligosaccharides (inulin, raffinose, stachyose, verbascose and ciceritol and galactosyl cyclitols), which are not digested in the intestine due to the presence of α-galactosidic bonds as the human body lacks α-galactosidase that is required to break these bonds (Lahuta et al. 2018; Singh 2017). The anaerobic bacterial fermentation of undigested carbohydrates, particularly in the colon, leads to the production of gases (hydrogen, carbon dioxide, hydrogen sulfide and methane), which cause abdominal discomfort, bloating, belching and diarrhea (Singh 2017).

2.6 Antivitamins

Some vaguely defined ANFs may also appear to increase the requirements for vitamins A, B12, D, and E. Antivitamins are compounds that have similar chemical structures as the vitamins and compete with them in various metabolic reactions and decrease the effects of vitamins by modifying their molecular conformation. Thiaminases interact with thiamin-dependent enzymes and can obstruct the thiamine transport into the cells as well as the synthesis of thiamine diphosphate (Dolan et al. 2010; Tylicki et al. 2018).

2.7 Antihistamines and Food Allergens

Food allergens comprise a diverse group of proteins and glycoproteins, which induce allergic responses through different sensitization routes because of complex interactions between the protein and the immune system (Huby et al. 2000). The immunological and immunochemical methods are generally used to investigate them (Frøkiær et al. 1997). The major identified allergens present in plant protein foods are: Kunitz inhibitor, Bowman-Birk inhibitor, saponins, soya-cystatin, phytoestrogens (daidzein, glycitein, genistein), Maillard-reaction products, soybean hydrophobic protein, soy allergens, lecithin allergens, raffinose, stachyose, 2-pentyl pyridine (Csaky and Fekete 2004). Glycinin and beta-conglycinin are the two most important antigenic proteins in soybean, which are thought to enter the lymph and blood through the gaps between the intestinal epithelial cells from the undigested soy proteins. The glycinin and beta-conglycinin possess considerable antigenic activity and stimulate the immune system resulting in specific antigen-antibody reactions and T lymphoid cell-mediated delayed hypersensitivity. Certain immunomodulators, such as vitamin C and lipoic acid may block the immunoglobulin-E (IgE) -mediated anaphylaxis and may be effective in the prevention of soybean-induced allergy and perhaps other food allergies. Several peanut proteins have been identified as allergens, which can result in serious IgE-mediated type I hypersensitivity reactions (Yu et al. 2016; Al-Muhsen et al. 2003). The processing, particularly autoclaving at 2.56 atm, for 30 min, produced a significant decrease in IgE-binding capacity of peanut allergens (Cabanillas et al. 2012). The soybean allergenic proteins are considered as a public health problem particularly in infants and children. The immuno-reactivity of soybean proteins can be reduced through appropriate processing techniques or selective plant breeding programs and development of improved soybean cultivars with lower concentration of these allergenic proteins (Wang et al. 2014; He et al. 2015). Genetic variability affects the nutritional and antinutritional profile of pulses and grain legumes. Many toxic plant proteins (lectins, ribosome-inactivating proteins, protease inhibitors, ureases, arcelins, antimicrobial peptides and pore-forming toxins) isolated from leguminous seeds are currently being introduced into crop genomes to improve the plant's resistance against pathogens and diseases (Dang and Van Damme 2015). Further studies are however warranted to ascertain the biological role of these proteins, their mode of action, and potential therapeutic uses in disease prevention.

3 Methods to Reduce the Antinutritional Factors

Legumes are required to be processed to inactivate the ANFs and to improve their nutritional value for human consumption. Processing generally improves the nutrient profile of plant protein foods by increasing their nutrient bioavailability and digestibility. Without adequate and effective processing, the ANFs can limit the use of plant protein foods (Samtiya et al. 2020). The safety, nutritional quality, and palatability of plant protein foods can be improved by a variety of processing techniques. Most of the ANFs, such as the proteinaceous antinutrients like PIs and lectins, are heat-labile, and therefore, thermal treatments can be effective in removing their potential negative effects upon consumption. Simple processing methods (soaking, cooking, autoclaving, roasting, sprouting, fermentation etc.) reduce the

ANFs and may significantly improve the nutritional quality, bioavailability of nutrients and functional properties of proteins (El-Adawy 2002; Aryee and Boye 2017). Thermal treatment is the most common processing technique to inactivate the TIs and to improve the nutritional quality of plant protein foods (Aviles-Gaxiola et al. 2018).

VanderJagt et al. (2000) reported that a variety of wild edible plant foods from sub-Saharan Africa contain significant amounts of heat-stable TIs that did not get inactivated on boiling for 3 min. Consumption of these plant foods can therefore cause difficulties on the digestibility and bioavailability of their nutrients (VanderJagt et al. 2000). Some of the lectin phytohaemagglutinins (PHA) have also been reported to be somewhat resistant to heat denaturation (Shi et al. 2007; Zhang et al. 2008). The naturally occurring haemagglutinins (lectin) in red kidney bean was inactivated by extensive cooking of well soaked beans (Rodhouse et al. 1990). Different other processing techniques alone or in combination with thermal processing have been shown to be effective in removing or reducing the antinutrients (Aryee and Boye 2017; Burgos and Armada 2019). Heat treatments not only require high amounts of energy but may also affect the functional properties of foods and can trigger the loss of some nutrients such as amino acids and vitamins. Mild to moderate heat treatment can be advantageous but processing at high temperatures may have detrimental effects on the physicochemical properties and metabolic efficiency of proteins due to production of some toxic compounds (Liener 1996; Rudra et al. 2019). Proteins contain several reactive groups like amine, sulfhydryl, tyrosyl, and imidazole, which at high temperatures can go through physicochemical modifications and may affect the solubility, enzymatic activity, antigenic reactivity, electrophoretic properties of proteins (Neucere and Cherry 1982). Thermal and alkaline treatments of protein foods may yield Maillard reaction products like lysinoalanine (LAL), an unnatural nephrotoxic amino acid derivative. The formation of D-amino acids and lysinoalanine (LAL) during the alkaline heat treatment of lactalbumin, casein, soya proteins or wheat proteins have been shown to reduce the protein digestibility in animal studies (de Vrese et al. 2000; Gilani 2012). The data from model systems studies indicate that some caution should be observed with the use of alkalis in food processing, particularly with food products, which are susceptible to Maillard reactions (Oste 1991). Bollini et al. (1999) showed that the removal of phytohemagglutinins from beans (*Phaseolus vulgaris* L.) seeds resulted in a greater true protein digestibility (Bollini et al. 1999). Rezende et al. (2018) suggested that suitable processing techniques should therefore be applied not only to eliminate/reduce the antinutritional and flatulence factors but also to retain the nutritional attributes in Brazilian dry beans (*Phaseolus vulgaris* L.) (Rezende et al. 2018).

Controlling the processing conditions is therefore essential to remove or reduce the unwanted components (Anderson and Wolf 1995; Rudra et al. 2019). It has been suggested that the processing conditions like time and temperature must be optimized and should be within the optimum limits needed to eliminate the inhibitors. During processing, proteins interact with nutrients and non-nutrients present in the food and affect the bioavailability of nutrients. For example, tannins and phytates interact with minerals and vitamins and decrease their bioavailability. The processing practices like milling, dehulling, soaking, germination, fermentation, and cooking not only improve the nutritional quality of pulses but are also effective in lowering the toxic constituents in processed food products. The processing technique, processing conditions, and type of pulses all are related to the degree of removal of toxic compounds (Jain et al. 2009). Vadivel and Janardhanan (2000) concluded that appropriate processing method can effectively lower the ANFs like total free phenolics, tannins, L-DOPA, trypsin inhibitor and phytohaemagglutinins activities in velvet bean without affecting their nutritional value. They observed that the *in-vitro* protein digestibility of legumes ranged from 72.4% to 76.9% (Vadivel and Janardhanan 2000). It has been reported that if properly processed, the ANFs in seven different varieties of wild legumes from South India could not only be reduced to levels with no nutritional significance but can simultaneously improve their digestibility (Vadivel and Janardhanan 2005).

The amount of ANFs can be lowered through the application of mechanical, physical, thermal and chemical processing methods (Burgos and Armada 2019). Ibrahim et al. (2002) studied the effectiveness of soaking in water and bicarbonate solution, ordinary and pressure cooking, germination, and fermentation in reducing the ANFs, (like PIs, tannins, phytic acid and flatus-producing oligosaccharides, raffinose and stachyose), typically present in cowpeas (*Vigna unguiculata*). They showed that soaking for 16 h in bicarbonate solution resulted in significant reduction of ANFs. Pressure cooking was more effective than ordinary cooking. Cooking of pre-germinated cowpeas was the most effective, whereas the fermentation completely removed TIs, oligosaccharides and significantly reduced the phytic acid but tannins were markedly increased (Ibrahim et al. 2002). Kalpanadevi and Mohan (2013) showed that hydration, cooking, autoclaving, germination, and their combination (germination for 96 h plus autoclaving) reduced the antinutrients and improved the in-vitro protein digestibility of tribal pulse, *Vigna unguiculata* (Kalpanadevi and Mohan 2013). Ramakrishna et al. (2006) observed that the TIA of Indian bean (*Dolichos lablab* L.) decreased progressively with soaking time and was 51% after 12 h and reached to 17% at 32 h. The overall decrease in polyphenols was 70%, tannins 46%, phytic acids 36%, phytate phosphorus 30% and stachyose and raffinose 40–50%. The roasting was more effective in reducing the TIA and phytic acid contents, whereas boiling and pressure cooking was less effective in decreasing the polyphenols and tannins. They concluded that germination was more effective in reducing the TIA, tannins, polyphenols and phytic acid than the other cooking treatments (Ramakrishna et al. 2006). Biofortified germinated cowpea (*Vigna unguiculata* L. Walph) was found to have improved bioaccessibility and bioavailability of iron (Sant'Ana et al. 2019). Negi and colleagues (2008) reported that domestic processing and cooking methods like soaking (12 h), dehulling and germination (60 h), pressure-cooking and microwave cooking reduced the level of ANFs to varying extent in all four varieties of moth bean (*Vigna aconitifolia*). Germination markedly lowered the level of phytic acid and polyphenols whereas pressure-cooking and microwave cooking significantly reduced the TIA (Negi et al. 2008). The amount of active enzyme inhibitors can also be reduced through extraction, compositional modification through germination or inactivation by microorganisms. There are however some ANFs such as tannins, phytate, and saponins, goitrogens, phytoestrogens, flatus producing oligosaccharides, which are relatively heat stable. They can however be reduced or removed through the processes such as dehulling, soaking, germination, fermentation and/or combination of these processes (Muzquiz et al. 2012). Miranda et al. (2019) showed that the most efficient method for isolating the ANFs from the seeds of grass pea was by extraction with 50% isopropanol (Miranda et al. 2019).

Chickpea is a valuable global source of high-quality protein that presents much less allergenicity as compared to soybeans and has many health benefits (Jukanti et al. 2012; Gupta et al. 2017; Bar-El Dadon et al. 2017). Chickpea, pigeon pea and other pulses are cooked and consumed in a variety of forms. El-Adawy (2002) observed that cooking treatments and/or germination not only reduced the ANFs of chickpea but also caused significant decrease in some of the nutrients such as minerals and B-vitamins (El-Adawy 2002). Various types of cooking methods were more effective in reducing the TIs, hemagglutinin activity, tannins and saponins compared to germination. However, germination appeared to be more effective in reducing the phytic acid, stachyose and raffinose. Microwave processing of chickpeas was useful in retaining the B-vitamins and minerals as compared to traditional cooking and boiling methods. All processing treatments improved the *in-vitro* protein digestibility, protein efficiency ratio and essential amino acid index of chickpea. Based on these results, El-Adawy (2002) proposed that microwave cooking is the best alternative for preparation of legume in households and restaurants (El-Adawy 2002). Mittal et al. (2012) observed that different processing treatments (germination, boiling, pressure cooking and roasting) variably reduced different types of ANFs like phytic acid, polyphenols, tannins, saponins, oxalates and TIs in chickpea. Pressure cooking resulted in the highest reduction in ANFs such as 93.97% reduction in tannins and 87.71% in polyphenols. Germination increased the linolenic acid, Fe and K availability (Mittal et al. 2012). Olika et al. (2019) reported that Kabuli variety of chickpea had lower quantities of ANFs and better physicochemical characteristics, in particular lower cooking time than the Desi varieties. They observed that processing of various chickpea (*Cicer arietinum* L.) varieties not only decreased the ANFs and increased the digestibility but also enhanced the bioavailability of Zn, Fe, and Ca. Boiling was considered as the best method for reduction of antinutritional factors (Olika et al. 2019).

Similar results were reported by Hefnawy (2011), who observed that processing decreased the ANFs in lentils with simultaneous losses in some minerals and amino acids (lysine, tryptophan, total aromatic, and sulfur-containing amino acids). Lentils cooked in microwave resulted in lesser mineral losses as compared to boiling and autoclaving. Hefnawy (2011) suggested that microwave cooking not only improved the nutritional quality but also reduced the cooking time (Hefnawy 2011). Overall, cooking of presoaked chickpea significantly reduced the ANFs like α-galactosides and TIA and improved the available carbohydrates (Frias et al. 2000). Data from various studies revealed that a variety of processing treatments, and the use of enzymes (alone/in combination with processing) can influence the physicochemical, functional, and nutritional characteristics of lentils and improve the protein digestibility (Aryee and Boye 2017; De Angelis et al. 2021; Falade and Akeem 2020; Hefnawy 2011). Aryee and Boye (2016) observed that the *in-vitro* protein digestibility of raw and cooked lentil flours and protein isolates ranged between 22.3% and 94.4% (Aryee and Boye 2016). ANFs in raw lentil flour, cooked lentil flour and lentil protein isolate revealed distinct variations between the two flours and protein isolate that can be improved through processing. It was suggested that processing methods like dry milling, cooking and isoelectric precipitation can be used to produce the value-added lentil products with improved functional and nutritional attributes. It is expected that such processing approaches may markedly impact the nutritional value of lentils, diversify their use, and help to improve the popularity of the pulses (Aryee and Boye 2017).

Soaking, germination, and sour-dough leavening agents have been reported to lower the phytate content of plant protein foods (Arntfield et al. 1985). Soaking reduced the amount of phytate but did not improve the protein digestibility of common bean (Phaseolus vulgaris, L.) (Helbig et al. 2003). Phytates can also be removed from the protein isolates through the micellization process when protein is precipitated and isolated through salt extraction (Arntfield et al. 1985; Khalid and Elhardallou 2016). Phulia et al. (2018) observed that chemical treatment alone was not effective in removing the phytic acid and tannins but solid-state fermentation with *Aspergillus niger* fungus was significantly able to remove 100% phytic acid and 65.79% tannins from defatted Jatropha kernel meal (DJKM). However, all other treatment processes were likewise effective in 100% removal of trypsin inhibitors from Defatted Jatropha curcas Kernel Meal (DJKM). The solid-state fermentation was the most effective method for removal of ANFs from DJKM (Phulia et al. 2018). Montemurro et al. (2019) recently showed that fermentation was not only effective in reducing the ANFs but also improved the nutritional and functional profile as well as the sensory properties of sourdough fortified pastas (Montemurro et al. 2019). Sa et al. (2019) reported that the inactivation of ANFs through food processing such as cooking, autoclaving, germination, microwave, irradiation, spray-drying, freeze-drying, fermentation, and extrusion, may increase the plant protein quality (PPQ). However, additional studies are required to optimize the application of current non-thermal food processing techniques for the removal of ANFs with simultaneous increase in the digestibility by modifications in protein functionalities (Aviles-Gaxiola et al. 2018; Sa et al. 2019).

The efficiency of soybean protein utilization could significantly be improved by removing the allergic antigen proteins through appropriate processing techniques. Anderson and Wolf (1995) reported that type of processing affected the amount of the ANFs in various soybean food products such as conventional protein ingredients, flours, concentrates, isolates, and some of the traditional oriental soybean-based foods (Anderson and Wolf 1995). The processing of soybeans under severe alkaline conditions can lead to the formation of lysinoalanine, which has been shown to damage the kidneys in rats. However, it is not true for edible soy protein produced under milder alkaline conditions and therefore the allergenic response that may sometimes occur in humans on dietary exposure to soybeans-based food products should be considered with reference to other factors (Liener 1994). Bajpai et al.

(2005) demonstrated that soybean TIs and agglutinins were removed from soybean flour through aqueous extraction during the preparation of ANFs free soybean flour for its use in value-added products (Bajpai et al. 2005).

Traditional cooking processes, such as dehulling, soaking, boiling, microwave heating, germination, fermentation, fortification, extrusion, enzymatic treatment, and bioprocessing significantly reduced the ANFs in faba bean and increased its protein digestibility (Elsheikh et al. 2000; Rahate et al. 2021). Mubarak (2005) observed that dehulling, soaking and germination processes were less effective compared to cooking processes in reducing the TIs, tannins, and hemagglutinin activity in mung bean seeds (*Phaseolus aureus*). They reported that germination was more effective in reducing phytic acid, stachyose and raffinose and resulted in a greater retention of minerals compared to other treatments. All cooking processes improved the *in-vitro* protein digestibility and protein efficiency ratio. The chemical score and the limiting amino acids of mung bean varied considerably, depending on the type of cooking process (Mubarak 2005). The extrusion of hard-to-cook common bean cultivars (*Phaseolus vulgaris*, L.) significantly decreased their antinutrients such as phytic acid, lectin, alpha-amylase, and TIs and reduced the emulsifying capacity and eliminated foaming capacity, in different bean cultivars. In addition, the water solubility, water absorption index, and *in-vitro* protein and starch digestibility were improved by the extrusion process. These results indicate that it is possible to produce the extruded products with good functional and biochemical characteristics from these common bean cultivars (Becker and Yu 2013).

The anti-nutritional compounds like tannins, phytic acid, saponins, phytoestrogens, lipoxygenase, hemagglutinin, trypsin inhibitor, and allergens, can be removed during the traditional and industrial food processing. It was suggested that wet processing together with fermentation and/or germination was the most efficient way of removing the ANFs (Bennetau-Pelissero 2019). Dry fractionation and subsequent solid-state fermentation with *Autochthonous Pediococcus* spp. reduced the presence of ANFs (tannins, trypsin inhibitors, and α-galactosides) (raffinose, stachyose and verbascose) in chickpea sourdoughs with enhanced nutritional quality and functionality. Xing et al. (2020) suggested that chickpea sourdoughs can be used to fortify many bakery products (Xing et al. 2020). The nutritional characteristics of legumes can be improved through fermentation used as ingredient for their use in the preparation of novel foods (Curiel et al. 2015). Lectins and TIs can be isolated and removed from raw soybeans with the help of affinity chromatography techniques. Apart from the presence of ANFs, the digestibility of soybean proteins is limited because of its cell wall permeability to proteolytic enzymes. Food processing may modulate the cell wall permeability and hence the accessibility of protease enzymes to intracellular proteins. Cooking applied alone or with either germination or fermentation processes increased the cell wall permeability of boiled soybean cotyledon cells. Boiling combined with fermentation and/or germination improved the protein digestibility of soybean because of pre-digestion of the storage proteins and inactivation of trypsin inhibitors (Zahir et al. 2020). All types of processing methods like physical, thermal, biochemical and their combination can be applied to reduce the contents of ANFs. However, certain ANFs (Maillard reaction products, acrylamide, protein-bound D-amino acids and lysine-alanine (LAL), trans-fatty acids etc.) may be generated in food matrices during the *in-situ* processing or their concentration might increase on primary processing like milling or defatting. Since the processing is required to modify foods to a better metabolizable form, it is important to develop such food processes that limit the production of such antinutrients in processed foods to provide safe and optimum nutrition (Rudra et al. 2019).

As mentioned earlier, different traditional processing methods like soaking in water, boiling, roasting, microwave cooking, autoclaving, fermentation and micronization significantly reduce the ANFs in beans. Obizoba and Atii (1991) reported that cooking together with fermentation was more effective in reducing the ANFs and improving the nutrient quality and safety as compared to other processing methods (Obizoba and Atii 1991). Khattab and Arntfield (2009) observed that boiling caused the highest reduction in tannins followed by autoclaving and microwave cooking whereas autoclaving and fermentation were most effective in reducing phytic acid content. The highest reduction in oligosaccharides was observed on autoclaving followed by fermentation, while the least reductions were observed in roasted or micronized samples (Khattab and Arntfield 2009). Hydrothermal, thermal, and bioprocessing treatments were found to reduce the ANFs in all the cultivars of chickpea with simultaneous significant increase in the bioavailability of iron and zinc. The bioavailability of Fe and Zn was higher in kabuli cultivars (8.1% and 40.5%, respectively) as compared to desi cultivars (5.5% and 38.4%, respectively). The *in-vitro* bioavailability of iron and zinc was more distinct on autoclaving followed by microwave cooking, boiling, and roasting. Autoclaving resulted in highest reduction in ANFs and improvement in the availability of iron and zinc in all chickpea cultivars. They suggested that selection of suitable cultivars and proper processing conditions should be encouraged (Sharma et al. 2018). The processing of chickpea affected the composition, protein recovery and ANFs of protein concentrates. Defatting did not significantly affect the amount of ANFs in flours for both processes and the TIs content of the concentrates stayed high (Mondor et al. 2009). Kilonzi et al. (2019) observed that soaking, cooking, and germination significantly reduced the anti-nutrients like TIA in Dolichos lablab beans (*Lablab purpureus*) by 88%. They suggested that there is a need to investigate the combined effect of various processing methods on the nutrients and anti-nutrients contents of beans (Kilonzi et al. 2019). Sprouting was shown to reduce (up to 80%) the ANFs like phytate, trypsin inhibitors, cyanogenic glycosides, and oxalates. As the sprouting process supports the microbial and pathogenic growth, it can make the sprouts of various types of beans (mung bean (*Vigna radiata*) and chickpea (*Cicer arietinum*)), as potential sources of foodborne infections and intoxications. Development of combined treatment processes could be an effective commercial strategy for ensuring safety and marketability of such commercially processed legumes (Kumar and Gautam 2019). Processing techniques like dehulling, micronization, roasting, conventional, and microwave cooking, germination, and combination of germination and conventional cooking, and roasting significantly reduced the TIA and tannin contents and improved the in-vitro protein digestibility (IVPD) of Saskatchewan grown yellow field peas. Ma et al. (2017) suggested that the agri-food industry should work further to better understand the functionality of field peas and to improve the process efficiency to enhance nutritional and techno-functional qualities (Ma et al. 2017).

Extrusion processing has been shown to be a quicker, consistent, and effective way in reducing the ANFs and improving the nutritional quality of plant protein foods. It is the combination of controlled thermo-mechanical treatment processes in an extruder and depending upon the process conditions during the extrusion process, the ANFs can be reduced to varying degrees (Nikmaram et al. 2017). Extrusion processing at relatively low temperatures was shown to be effective in reducing the lectins, phytohemagglutinin activity (PHA) and oligosaccharides (raffinose and stachyose) in Navy and Pinto beans (*Phaseolus vulgaris* L.) (Kelkar et al. 2012). It is suggested that effective technical approaches and novel processing techniques like proteolysis, heating, gamma irradiation, and high-hydrostatic-pressure should be developed to either eliminate or reduce the adverse effects of lectins and other allergens (He et al. 2018). Siddhuraju et al. (2002) suggested that irradiation can be another possible processing method to inactivate and remove certain ANFs (Siddhuraju et al. 2002). Mallikarjunan and colleagues observed a 50% loss in the functional activity of phytohemagglutinin in dry and soaked seeds of kidney bean at the doses of 50 and 30 kGy, respectively (Mallikarjunan et al. 2014). Irradiation at 2.5 and 5.0 kGy has however been reported to cause significant losses in vitamins, particularly thiamin (Khattak and Klopfenstein 1989; Woodside 2015). The antinutritional factors in legumes, can also be eliminated through selective plant breeding techniques or genotypes or through post-harvest processing methods. The concentration of allergenic proteins can also be lowered through selective plant breeding programs and development of improved soybean cultivars with lesser levels of these allergenic proteins (Wang et al. 2014). The infrared heating has the potential to shorten the cooking-time of legumes. It has been suggested that infrared heating may offer a potential solution in the food industry to increase the consumption and utilisation challenges of African legumes and their flours (Ogundele and Kayitesi 2019). However, the seed size, the moisture content, surface temperature and time have been shown to affect the efficacy of the application of infrared heating (Liu et al. 2020). As the ANFs play important roles in the normal growth and development of plants and protect them against predators and environmental conditions, the entire removal of these compounds through improved plant breeding program or biotechnological techniques, even if possible, may produce plants with poorer growth and lower yield characteristics (Khokhar and Apenten 2003). Soybean is an outstanding source of plant protein and therefore development of improved cultivars are important to meet the growing global demand for protein foods (Luthria et al. 2018). Substantial variations have been reported to exist in Bowman-Birk chymotrypsin inhibitor and Kunitz trypsin inhibitor activities among grass pea (*Lathyrus sativus*) accessions that could also be exploited in breeding programs for the development of grass pea lines devoid of ANFs (Xu et al. 2019).

4 Protein Isolates, Concentrates and Hydrolysates

Protein isolates, concentrates and hydrolysates are the improved form of protein foods, which have higher digestibility as compared to original foods. The production of protein isolates, concentrates, and hydrolysates is of growing interest to food industry because of their functional properties and nutritional significance in the development of value-added food products. Several processes and techniques are used to obtain the protein concentrates and isolates with desired functional characteristics like improved gelling and emulsifying properties, which may help in the development of new food products (Garba and Kaur 2014; Klupšaitė and Juodeikienė 2015). They are generally produced from plant protein foods utilizing a combination of isoelectric and alkaline precipitation techniques employing H_2SO_4 and NaOH, followed by centrifugation to obtain pure isolates and concentrates. Soy protein concentrates and isolates are generally produced by acidic precipitation, ultrafiltration, electro-acidification and through combination of electro-acidification and ultrafiltration processes (Alibhai et al. 2006). The most effective way to produce protein hydrolysates with specified characteristics, is through the sequential action of endopeptidases and exoproteases coupled with the development of post-hydrolysis procedures (Clemente 2000). The protein concentrates and isolates are normally obtained using extraction methods with acid-base solvents, salting out methods with salt solvents, and modification by enzymatic hydrolysis techniques. The isoelectric point and protein solubility are the main concerns for maximizing the protein content of bean isolates and concentrates. The isoelectric precipitation generated isolates with higher surface charge and solubility as compared to those produced via salt extraction (Karaca et al. 2011). The use of protease enzymes has also been suggested as a useful way to improve the digestibility of protein isolates and concentrates (Kusumah et al. 2020b). The dried powdered form of protein isolates and hydrolysates can contain up to 90% of protein (Garba and Kaur 2014). The ANFs like trypsin and chymotrypsin inhibitory activities were very high in protein isolates prepared from Cassia uniflora Mill (*Cassia sericea* Sw) although they showed high (93.6%) in-vitro protein digestibility (Bhanu et al. 1991). The process of isolating protein from red and green beans produced protein powders containing 79% and 81% respectively with protein powder yield of 15% and 17%, respectively, on weight basis (Kusumah et al. 2020a).

Several plant-based protein powders are made from plant foods like peas, soy, and rice. Pea protein is one of the most popular plant-based options among vegetarians and vegans as it is high in fiber and contains all essential amino acids. The type of legume and processing technique of protein isolate production significantly affected the emulsifying and physicochemical properties of isolated proteins. Most of the functional attributes of these protein isolates were comparable to commercial soy isolates. The chickpea protein isolates showed high in-vitro digestibility and calculated protein efficiency ratio and contained most of the essential amino acids at acceptable levels compared to a reference pattern (Paredes-LÓPez et al. 1991). Protein isolates prepared from desi and kabuli chickpea cultivars by alkaline solubilisation followed by isoelectric precipitation and freeze drying processes showed considerable variations in their functional characteristics (Kaur and Singh 2007). Falada and Akeem (2020) showed that the thermal properties of the flours and protein isolates prepared from African mesquite bean (*Prosopis Africana*) varied depending upon the defatting and extraction methods (Falade and Akeem 2020). Protein isolates prepared from chickpea flour by micellization, and isoelectric precipitation techniques can have up to 88% protein. The protein isolates prepared by alkaline extraction followed by isoelectric precipitation or ultrafiltration differed in composition with respect to their globulins and albumins fractions. The method of preparation rather than the protein composition affected the gelling behaviour of protein isolates (Papalamprou et al. 2009). El-Adawy (2000) reported that acylated protein isolate from mung bean not only showed lower amounts of ANFs but also improved *in-vitro* protein digestibility (El-Adawy 2000). Steam injection heating process significantly reduced the antinutritional substances in protein isolates prepared from Jatropha kernel. The TIA in protein isolates was reduced by 90–97%, whereas the phytate, tannins and saponin contents were reduced by 90%, 85% and 98% respectively. The phorbol esters and cyanogenic glucosides were non-detectable. The improved *in-vitro* digestibility and other nutritional quality attributes of Jatropha protein isolate suggest its use as a potential protein source (Devappa and Swamylingappa 2008). Different forms of processing and biofortification practices such as cooked and oven-dried soaked beans, cooked and freeze-dried soaked beans, cooked and oven-dried beans without soaking and cooked and freeze-dried beans without soaking, contributed to high mineral profile of beans with increased bioavailability of iron and zinc (Brigide et al. 2019). Khalid and Elhardallou (2016) observed that no phytic acid and tannins were present in cowpea protein isolates (Khalid and Elhardallou 2016). The protein fractions (globulin and albumin) isolated from raw linseed meal contained several ANFs and showed lower protein digestibility because of higher trypsin and chymotrypsin inhibitors contents. Raw linseed consumption not only prompted negative impact on rat growth but also caused reduction in intestinal villi indicating that it should not be used as a sole source of protein because of enzymes inhibitors (Anaya et al. 2015).

The ANFs in protein isolates, concentrates and hydrolysates can be effectively reduced using appropriate processing methods. The amount of ANFs in three different types of legumes; pea (*Pisum sativum*), faba bean (*Vicia faba*) and soya (Glycine max), reduced significantly after the concentration process. The functional characteristics of pea and faba bean protein concentrates were comparable to soy protein concentrates and showed their suitability for use in food preparation (Cantoral et al. 1995). Considerably lower quantities of ANFs such as TIs and phytic acid were found in natural soy protein isolates. Chamba et al. (2015) demonstrated that high quality soy protein isolates can be manufactured using amaranth lye and lemon extracts instead of using the synthetic chemicals (Chamba et al. 2015). Protein isolates, concentrates and hydrolysates are the major sources of cheap proteins for athletes, bodybuilders, and vegetarians as well as for the development of infant foods. They are mostly used to prepare special nutritional formulations to meet the idiosyncratic protein requirements of patients and older people with specified nutrient needs. Because of their functional properties, they have gained wide application in food and beverages industries and therefore should be free from ANFs.

5 Conclusion

Plants produce thousands of chemicals compounds to protect themselves against different types of predators like herbivores, insects, pathogens, and microbes as well as to fight against the adverse environmental conditions. Depending on their structure and molecular weight, these secondary metabolites of plant protein foods can limit the utilization and bioavailability of their nutrients and may trigger variable adverse physiological effects in humans. How far these ANFs can impose threat to human health, still needs further evaluation. The safety, nutritional quality, and palatability of plant protein foods can however be improved by a variety of processing techniques. Humans have been probably processing the grain legumes to remove the ANFs partially or completely since the Neolithic period when these grains first became part of agricultural farming systems. The scientific data presented in this chapter indicate that it is possible to remove or reduce the level of these ANFs in plant protein foods using various processing/cooking methods either individually or in combination. Adequately and properly processed plant protein foods will practically pose no major threat to human health upon consumption. There is a need to inform people about the risks and consequences of consuming inappropriately processed plant foods containing high concentrations of ANFs. Simultaneously it is also needed to develop and exploit the improved cultivars with lower levels of noxious chemical compounds through plant breeding programs or biotechnological techniques in meeting the growing global demand for protein foods.

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Chapter 15
Meat Replacers and Meal Plans Based on Plant Protein Isolates for Human Consumption

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1 Introduction

Meat is a source of good quality protein with high nutritional profile and appreciable taste. Meat protein plays a significant role in providing all the essential amino acids and also imparts special functional properties to the processed food products (Xiong 2004). The protein functionality also positively influences the sensory properties like texture, appearance and mouthfeel of meat products. The presence of high quality biological protein, vitamins and minerals also adds to the nutritional value of meat and meat products (Asgar et al. 2010).

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2 Global Need of Meat Replacements

Meat consumption has been increased more than twofolds from the year 1961 to 2007 and prompt growth is reported in developed countries as compared to developing countries. It is foreseeable that by 2030, the demand of meat will rise more than 72% due to increased urbanization, industrial development, population growth and rise in income (Steinfeld et al. 2006). There also seems an evident increase from 5% to 13% in meat production during last decade. Profound increase in the production of animal products to 9.1 billion by 2050 from 229 billion in year 2000 has been expected (Steinfeld et al. 2006; Bruinsma 2009). Numerous environmental concerns (environmental pollution, depletion of natural resources and deforestation), ethical or animal welfare issue and public health issues associated with the production and consumption of meat and meat products has been identified newly (Mayfield et al. 2007; McEachern and Schroder 2002; Kumar et al. 2017).

Fundamentally, animals are reared and then slaughtered for the production of meat and rearing of food animals involves the modification of plant based protein into animal protein by means of food animals. The rearing of animals for production of meat requires more involvement of natural reserves as compared to the production of vegetables crop. Thus meat production is referred as uneconomical and waste of natural resources for conversion of plant protein into animal protein. Similarly, in context of increasing world population and limited natural resources, utilization of plant based protein is suggested as it is economical, requires less natural resources and implicates less greenhouse gas emissions in comparison to the animal foods (Kumar et al. 2017).

Overconsumption of meat products is of great public health significance as responsible of causing many diseases including cancer, coronary heart diseases, autoimmune and inflammatory diseases. Coronary heart diseases instigate about is 1.8 million causalities annually. The risk of colorectal cancer reported to increase significantly due to the per day consumption of 30 g of processed meat and 120 g of red meat (Larsson and Wolk 2006). Moreover, lot of food borne illness and food borne outbreaks associated with meat and meat products due to prevalence of food borne pathogens such as Commpylobacter, Salmonella and E. coli causing millions of deaths and illnesses each year (Kumar et al. 2017; CDC 2012). So, ineffective usage of valuable natural resources to produce meat in replacement to stable grain is questionable (McMichael et al. 2007). So, there is need to replace meat majorly due to the concerns associated with the production of meat as environmental, animal welfare and public health issues (Kumar et al. 2017). Other reasons that trigger the need to replace meat include:

- More variation in diet is demanded by meat eaters
- Increase in the number of vegetarians with the growing awareness about the health risk associated with the consumption of meat
- More inclination of peoples towards convenience food products

- Emerging trend of healthy diet which including plant based food (Dekkers et al. 2018).

Accordingly, modification in the current eating pattern through the incorporation of plant based meat replacers prepared from the combination of varied plant based proteins could be deemed as effectual and sustainable approach (Sadler 2004).

All these issues associated with the consumption of meat have intensified the need to fabricate the plant based protein rich food that can be used in replacement of meat and has palatable and acceptable sensory and nutritive properties. These alternatives to meat are known as meat replacers or meat substitutes. Presently, meat replacers are at very initial phase of development and occupies very minute portion (1–2%) of market (De-Bakker and Dagevos 2010). However low cost of plant based protein along with nutritive valve guarantees the increment in the utilization of meat replacers as alternative to the expensive animal protein (Kumar et al. 2017). Plant proteins have the capability to replace the functionality and nutritive value of meat protein in diverse meat products. Plant proteins derived from wheat and soybean are most commonly utilized in meat products all around the globe. Possibly, other plant proteins such as corn, canola, rice, pea, chickpea and protein from legumes are expected to be accessible in markets in near future. Globally, soy protein are extensively utilized to impart functional properties like gelation, emulsification, and water binding, textural and nutritional characteristics to the meat products processed in the industries. Wheat proteins are also of great significance as frequently utilized in processed meat products and contribute the water binding, emulsification and structural properties to the product. Potato proteins and non-genetically modified pea proteins are extensively known in Europe but they are relatively novel to the processing industry and are recently at preliminary phase of commercialization. Plant proteins can be available as meat analogues or can be derived from specific plant in powdered and dray textured form. In majority of countries the utilization of plant protein as meat replacers in meat processing industry is controlled by regulations vary from one country to another and must be monitored prior to their utilization. Most commonly utilized plant proteins as a replacer of chicken and uncured red meat are recommended to be having low nitrite and nitrate level in order to prevent cured meat color reaction in uncured meat products (Egbert and Payne 2009).

Plant protein isolates are pure form of protein with high concentration of protein fractions generally greater than 85% on wet basis (Aluko 2017). Currently, the usage of plant proteins in food and non-food market has been increased resulting in the increased attention of industry towards the preparation of plant protein isolates. In this regard, European Union is extremely focused to grow protein crops of their own in order to lower the reliance on accessibility of protein (Sánchez-Vioque et al. 1999). Isolation of protein is achieved through the various procedures including solubilisation of protein as preliminary step followed by ultrafiltration or isoelectric technique for further recovery. However, “salting out” technique is also used to obtain highly concentrated plant protein isolates (Sandberg 2011).

3 Potential Health Benefits of Plant Protein Replacements

Plant protein isolates are very economical source of protein and also impart the functional and nutritional properties of product. However, protein from vegetables except from soybean seed has limited application in global trade market (Sánchez-Vioque et al. 1999). Moreover, numerous health benefits are associated with the consumptions of plant based protein foods as given in Table 15.1.

Additional benefits associated with the ingestion of plant based foods (nuts, vegetable, cereals and grains) include considerably lesser risk of cardiovascular disease (CVD) and chronic kidney disease (CKD) (Joshi et al. 2020; Hu 2003). There are numerous advantageous nutrients present in these foods such as vitamins, minerals, mono-& polyunsaturated fatty acids, $n-3$ fatty acids, antioxidants and plant protein that are responsible for their protective effects against various diseases (Hu 2003). So, it is extremely recommended to incorporate plant based foods with high protein content in regular meal plans.

4 Major Ingredients Used in Meat Replacement Products

4.1 Legumes

Legumes are rich sources of food proteins belong to the family "Leguminosae" also known as "Fabaceae". The first important ingredient is referred as legumes which are edible seeds of Leguminosae family. The members of this family are leguminous plants and comprise of pulses (lentils), beans and other podded plants like peas (Oboh et al. 2009; Asgar et al. 2010). Since the earliest of civilizations, these plants are good and cheap alternatives to animal products for protein source and known as Novel protein foods (NPFs). In history, plant foods (legumes) were supposed as the food for the poor's and exerted undesirable gastrointestinal effects on their consumption. So, due to their image consumers declined their usage since the end of World War II. But, in recent era, the concept towards sustainable production of plant based protein enrich foods have changed the mind set of consumers (Sandberg 2011).

The legumes contain significant amount of protein, called as albumins and globulins. The proportion of proteins varies between 17% and 30% and its content and quality is dependent on origin of legume. On albumins (10–20%) and globulins (60–90%) composition, a variety of meat free vegan products and dairy products are available in the market (Soderberg 2013). In the developing countries, malnourished children are fed with legumes part of the daily diet to fight against diseases due to their high quality protein content (Butt and Rizwana 2010).

They occupy a vast area worldwide and are mainly grown for their edible seeds or pods also called grain legumes. Grain legumes are used as pulse (dhal), chick peas, peas, huge variety of beans and also the peanuts. Legumes' protein is an economical but amazing substitute of animal-based protein. Legumes contain 17–30%

Table 15.1 Plant based proteins and their role in the body

Plant	Protein/Peptide	Biological activity	References
Soybean	Beta-conglycinin (7S globulin) and glycinin (11S globulin)	↓ Serum cholesterol ↓ Body fat ↑ Serum insulin Prevent CVD	Friedman and Brandon (2001) Jooyandeh (2011)
	Bowman–Birk inhibitor (BBI)	Anti-carcinogenic Anti-diabetic	
Cereals Wheat	Low-molecular-weight glutenin, High molecular weight glutenin, alpha-gliadin, gamma-gliadin, omega-gliadin	Antiamnestic Angiotensin-converting enzyme(ACE)-inhibitor Antioxidant Hypotensive DPP-IV inhibitor Activate ubiquitin-mediated proteolysis	Cavazos and Gonzalez (2013)
Oat	11S Globulin/ 12S Globulin/N9 Avenin B-hordein precursor/C-hordein/D-hordein/		
Barley	Globulin		
Nuts	Arginine	↓ Gastro-intestinal issues ↑ Immune system ↑ Male fertility ↑ Skin, joints & muscles health Antiaging Regulates hormones Regulate blood sugar	Duggan et al. (2002), Arya et al. (2016)
Pea	Globulins, lectins	↑ Weight loss ↑ Immune system Anticancer Antihypertensive Antioxidant ACE-inhibitor	Dahl et al. (2012)
Pulses/legumes (pea, chickpea, lentils)	Lectins	Anti-cancer Anti-obasity ↓ Human hepatoma (H3B) ↑ Immune system	Roy et al. (2010)
	Protease inhibitors	Anti-inflammatory Anti-cancer	
Mushrooms	Ribosome inactivating proteins, Laccase	↓ Hepatoma (Hep G2 cells) proliferation ↓ Breast cancer (MCF-7 cells) proliferation ↓ HIV-1 reverse transcriptase activity	Xu et al. (2011)
	Lentin	Antifungal ↓ Leukemia cells proliferation	

proteins based on their origin. The proteins found in legumes are 60–90% globulins and 10–20% albumins (Iqbal et al. 2006). In order to present a best substitute of red meat, the excellent choice will be legumes. Legumes are extremely beneficial in replacing red meat.

The consumption of legumes was declined in the past since the end of World War II due to multiple reasons including their association as “food for the poor” and some unwanted gastrointestinal effects that occur due to their consumption. The recent interest towards sustainable production of protein rich foods may however change this trend. As the world is now taking interest in production of high protein foods and the need of sustainable protein source is shifting the trend of people towards consuming vegetable source of protein. Foods from plants in this category are Novel Protein Foods (NPFs) which are the best possible alternative to replace animal protein and it will be environment friendly as well (Sandberg 2011).

4.1.1 Soy Bean

Soy is considered as cheapest source of protein to meet the deficiency of animal source of meat which is relatively much expensive. Among all the others proteins sources soybean provides more options for food variety through value addition (Adams et al. 2004; Ferreira et al. 2015). Proteins are stored in protein bodies or aleurone grains. The size of these bodies ranges between 15 and 20 μm in diameter and most of them have a lower diameter ranging between 5 and 10 μm. 60–70% of total proteins found in soybeans are stored in these protein storing bodies. The pH at which the minimum solubility occurs for these proteins is 4.2, which corresponds to the iso-electric point of major proteins. About 90% of extracted proteins are precipitated when the aqueous or dilute sodium hydroxide extracts of defatted meal is adjusted to a pH of 4.0–4.2 (Lampart-Sczapa 2001).

The storage proteins that are found in soybeans are mainly composed of two proteins; globulins including β-conglycinin (7S) and glycinin (11S) which form approximately 80% of the total proteins. The 7S is composed of three subunits linked through hydrophobic interactions and chemically is a quaternary trimeric glycoprotein. There are two intermolecular disulfide bonds present in β-conglycinin and have considerable amphiphilic properties for flavor binding and good surface activity. In Glycinin, two pairs of disulfide linkage and 6 hydrophobic interactions bind acidic and basic subunits. The iso-electric point of glycinin is at a pH of 6.0 and it shows limited solubility limit at this point. There are two free thiol groups present at its surface which can contribute in thiol-disulfide exchange (Jiang et al. 2011). The functional properties of soy proteins are mainly due to these proteins (Kanauchi et al. 2015). Glycinin is a hexamer molecule and has six subunits each of which have basic and acidic polypeptides linked through each other by disulphide bonds (Saeed et al. 2016). Beta conglycinin included in 7S globulin is a trimer having three subunits α, α′, and β and they exist in the form of several combinations (da Silva et al. 2011).

Soy whey proteins (SWP) During preparation of soy protein isolate and cheese, whey is separated out as a waste product but it also has marvelous nutritious components. Soy whey proteins are an efficient source in food applications as long as a superb emulsion activity, solubility, stability, and foaming capacity. SWP contains nutraceutical value because of its anti-hypertensive quality. In western countries, these proteins are used as a substitute source of animal-based proteins. SWP shows properties even more than soy protein, SWP comprises of high molecular weight and hydrophobic in nature and effective in cholesterol lowering and binding bile acids (Lassissi et al. 2014).

Soy products are getting attention all over the world as their increased consumption indicates. These products are used as meat alternative and are found in the form of **soy protein isolate, textured soy protein and tofu.**

The most refined soy protein product available across the globe is Soy protein isolate **(SPI)**. The composition of **SPI** is more than 90% protein on dry basis and contains glycinin 11S and β-conglycinin mainly 7S which are soy storage proteins and form up to 70% of the total protein found in SPI (Taski-Ajdukovic et al. 2010). In most cases, the production of SPI is done by extracting water from dehulled and defatted soybean flakes. To inactivate the trypsin inhibitors, this water is then heat treated.

The usage of **SPI** in meat-free sausages, chicken-style nuggets, chicken chunks etc. and in other products which have resemblance with sliced cooked meats. The production of **Textured** *Soy Protein (TSP)* is carried out by using defatted soy flour, which is free from soluble carbohydrate, and the remainder is textured by spinning or extrusion. This textured flour is then dried which results in a sponge like texture. After this, flavors can be added to it and are molded to look like chunks or granules. The commercial use of textured soy protein is found in meat-free sausages and vegetarian burgers, and some other meat-free products. It is also found as an ingredient for home cooking. The protein content of textured soy protein is second to soy protein isolate. **Tofu** is the soy-based product which has the lowest protein content as compared to the SPI and textured soy protein. It is also called pressed soy curd which is prepared using the coagulated soy. For coagulation of soy, calcium sulphate or calcium chloride is used as coagulating agent. Tofu needs to be flavored either by smoking or marinating, as it lacks the taste and feels quite tasteless. Use of tofu is found in tofu burgers and tofu pate.

However there are some health related disorders due to consumption of Soybean allergy is one of the most common food allergy especially among children reported about 0.4% of young children (Barni et al. 2015). The soy proteins are very specific and almost 28 soy proteins are to bind with IgE in people who are soy-allergic patients, however, only a few of these proteins are considered magic to soy (Kattan et al. 2011).

Fibrous Vegetable Protein is a soy-based product that is produced by Tivall. It is designed to give mouth feel and texture like that of muscle meat therefore it is different from other forms of soy. It is used in vegetarian products that may include sausages, burgers, and cold cuts and in beef/chicken-style home kitchen ingredients (Sadler 2004).

4.1.2 Pulses

Food and Agricultural Organization (FAO) of the UN defines pulses as, “limited to crops harvested solely for dry grain, thereby excluding crops and harvested green for food which is classified as vegetable crops” (Havemeier et al. 2017).

The energy level is moderately low in pulses as they provide about 1.3 kcal/g. They are known to be the good source of digestible proteins. In most of the pulses, the protein content varies from 17% to 30% on dry weight basis proteins which are found in pulses have both nutritional as well as functional properties such as solubility, water and fat binding capacity and foaming which make their use favorable in food formulation and food processing. Researches have shown that some of the functional characteristics of pulse proteins can be compared to soy or whey proteins which are known to be the frequently used proteins in food processing industries. These proteins are used to impart functional characteristics in the preparation of bakery products, soup preparation and ready to eat snacks (Boye et al. 2010). The essential amino acids such as lysine and threonine are found abundantly in a value of 64 mg/g of protein and 38 mg/g of protein respectively which is much higher than the value found in other plant-based protein sources. The other essential amino acids such as methionine, tryptophan, and cysteine are found in lower quantities in pulses. Due to this reason they are regarded as lower quality source of proteins and compared to the other plant-based products such as soy and legumes. Hence they should be used in conjunction with other meat/animal or plant-based protein sources so that it can make a high protein meal (Havemeier et al. 2017). Pulses are milled to flours and fractions and these products are used as ingredients in the manufacturing of meat products such as sausages, nuggets and burgers. Their use is also found in manufacturing of pasta products (Farooq and Boye 2011).

Generally, the cross-reactions of pulses have maximum chances of occurrence in protein sensitive people. Nowadays, the concern about allergic reactions of lupin is increasing. Lupin is also a pulse in the Fabaceae family and a member of the genus Lupinus. It is reported that allergic reactions of lupin can occur in the children having peanut allergy. Many studies have reported the allergic reactions of lupin and raised concerns to add it to the priority allergens. However to overcome this issue many allergen-free food products have been developed by food professionals (Boye et al. 2010).

Beans

The Nordic Nutrition Recommendations were reviewed and new recommendations were made to decrease the consumption of animal-based protein sources (such as pork and beef); and recommended to increase in the consumption of vegetable sources such as legumes. Researches have shown that the intake of beans based meal increases appetite sensations and energy intake as compared to animal based meals having similar proteins and calorie values. It is interesting to know that a meal prepared from vegetable source having higher digestibility and gratifying as a meal prepared from animal protein sources that can be due to dietary fiber and palatability of plant based meal (Kristensen et al. 2016).

Fava Beans: Legume crops such as fava bean is rich source of vegetable protein and it becomes a part of different meals in different combinations for increasing protein quality and quantity with fava proteins. The protein quantity is approximately 250 g per kg of fava seed (Macarulla and others 2001) and it provides the energy of about 320 kcal per 100 g on dry weight basis (Ofuya and Akhidue 2005). The production and ingestion of fava bean is increasing with awareness of consumer towards therapeutically and functional benefits of vegetable protein isolates and its utilization in food and drink industry (Duranti 2006; Vioque et al. 2012). The fava bean accumulates high amount of protein during its seed development (Duranti 2006) the major storage protein in seed is "globulins" which is about 69–78% of total seed protein and comprise of 2 high-molecular-weight proteins; legumin (11S) and vicilin (7S) (Multari et al. 2015).

Fava bean could be used in combination with other plant-based foods to improve the quality and quantity of the proteins provided in a meal. The intake of these crops should be further encouraged in developing countries where meat can be scarce, as it provides some essential amino acids required for growth and repair of body tissues.

Mung Bean: Mung bean (*Vigna radiata*) is an important dry legume and considered as the most significant and inexpensive protein (20–27%) source for people of low incomes. It is used to substitute meat protein for assuaging protein energy malnutrition. It has been shown that mung bean protein isolate has different functional and physicochemical properties. Mung bean protein isolate has excellent functional properties like gelation, foaming, water and oil absorption capacity thermal stability 157.90 °C, lysine content (140.19 mg/g) and these properties are comparatively higher than soy bean protein isolate (Branch and Maria 2017).

Peas

Peas are a type of legumes, and are a rich source of proteins. They are quite suitable for the preparation of novel protein foods for human consumption which can be used as meat substitute. The proteins obtained from peas are used in development of protein foods both due to their functional characteristics as they improve texture and stability of products; due to nutritional values and for having higher protein content in economic prices. The processing of peas for obtaining higher protein content should be in controlled conditions to minimize formation of undesirable compounds that can interfere with the nutritional value, sensory and functional properties of the final product (Sandberg 2011). Green peas are consumed worldwide as an important green vegetable both in the form of fresh and canned product. Peas flour is used in diet to make many different varieties of savories, such as soups and curries (Asif et al. 2013). High levels of protein content and digestible carbohydrates are found in pea seeds. Also, insoluble dietary fibers are present in high concentrations and fat in low concentrations.

Other than proteins peas are also rich in vitamins, starches, minerals, and fibers. They are used in the manufacturing of snacks, sprouts, soups etc. Higher protein contents are found in pea protein concentrate (PPC) and pea protein isolate (PPI) (Choi and Han 2001). Pea protein concentrate (PPC) is made by removing proteins from starch using air from pea flour, which produces a protein content of 47%. The preparation of pea protein isolate (PPI) which have 80% protein content, is also done by using pea flour but by the method of aqueous extraction and isoelectric precipitation of pea flour (Söderberg 2013).

The protein content in peas is approximately 25%, but this is highly dependent on pea variety (Aluko et al. 2009). The proteins found in peas are vicilin (7S), legumin (11S), and albumins (2S), having 11S and 7S in abundant quantities (O'Kane et al. 2005). The amino acid composition and subunit structure of legumin and vicilin is similar to the glycinin and β-conglycinin of soy proteins (Söderberg 2013). Pea proteins have high content of lysine and threonine and are a good source of essential amino acids, but deficient in Sulphur-containing amino acids like other legumes. 65–80% of the pea proteins comprises of globulins. The digestibility of pea protein is found to be varying between 83% and 93% as assessed by rat assays (Sandberg 2011). The pea is known to be the natural and traditional food, and the products of pea protein can fulfill nutritional requirements of human body.

Among legumes, Peas (*Pisum sativum*) are being cultivated at least 4000 years ago in New world and in the Near East since 6000 BC. Now a days, it can be produced from non-GMOs (nongenetically modified organisms) and very popular in Europe (Egbert and Payne 2009). These are assumed as suitable for development of NPFs like soups, stews, puddings, snacks, stews, and bakery products in the replacement of meat. It can also be used in animal feed as in therapeutic products due to peptide structure of its proteins (Hoang et al. 2012). The inactive protein peptides exhibit immune-modulatory, antihypertensive, antimicrobial and antioxidant potential and these can be converted into active on by enzymatic treatments (Sandberg 2011).

They are comprised of low concentrations of fat (12 g/kg) and high levels of digestible carbohydrates (225 g/kg), insoluble dietary fibre (63 g/kg) and protein (440 g/kg). So, humans can utilize them as dry or fresh whole seeds for protein production with 83-93% rate of digestibility (Sandberg 2011; Salam et al. 2011). The pea proteins are named as albumins (2S), legumin (11S) and vicillin (7S) and contribute upto 25% in total dry matter wt of seed. Although research on pea protein is still limited, but its nutritional quality, and functional characteristics are similar to soy protein (another member of leguminous plants) as confirmed by food scientists (Chavan et al. 2001; O'Kane et al. 2004; Barac et al. 2010; Toews and Wang 2013). The reason of its popularity could be less quantity of anti-allergic substances than soy protein, relatively cheap source of essential amino acids (threonine, lysine) and contain significant amount of vicillin (7S) and legumin (11S) as reported by Gwiazda et al. (1979) and O'Kane et al. (2004). These proteins are very soluble between pH 2 and 7.3 and less solubility is noted between 4 and 6 pH (Sandberg

2011). On rising demand of food industry, pea proteins are converted into PPI (pea protein isolate) and PPC (pea protein concentrate) with ~80% and 47% protein contents, respectively (Choi and Han 2001; Sosulski and McCurdy 1987). The production methods for PPI and PPC differ just from one process. Both are prepared from pea seed flour and proteins for PPI have removed by air-classification from the starch granules, whereas for PPI proteins are by isoelectric precipitation and aqueous extraction method (Owusu-Ansah and McCurdy 1991).

Furthermore, digestion and nutritional profile of proteins can be improved through heating process. This cooking tends to destroy trypsin and chymotrypsin inhibitors (lectins) and also help to remove phytic acid. The heat labile nature of lectins require soaking (18 h) and autoclaving of peas at 121 °C for 5 min, result in 65% reduction of its activity (Bender 1983). This nutronal quality can be assessed by protein efficiency ratio (PER) method followed by cooking for 1 h 38 (Deo et al. 1986; Sandberg 2011). Beside this, other biological techniques such as addition of endogenous enzymes and fermentation assist in substantial reduction of the anti-nutritional factors and significant increase in digestion absorption rate of iron and zinc in protein based meals (Brune et al. 1992).

The European Union funded a project named as New Technologies for Improved Nutritional and Functional Value of Pea Protein and abbreviated as NUTRIPEA to use advance technologies for development of pea protein based products. The objectives of this program are to verify the technical feasibility of a bioprocess for preparation of protein products, to check the nutritional value of foods prepared with increased proteins, to solve the safety and legal issues related to these products, to determine the anti-nutritional factors in finished innovative products and to give final decision about pea proteins to be utilized as meat replacers (Sandberg 2011).

The functional properties are those physicochemical properties that deal with behaviour and organoleptic attributes of food during preparation, storage and consumption (Heng 2005; Soderberg 2013). These properties are affected by matrix nature, ionic strength, temperature and pH of food materials to make food acceptable for consumers (Adebowale and Lawal 2004). Some of these characteristics of pea proteins have been discussed below;

Solubility

This property is supposed as function of temperature and pH. In pea proteins, PPI exhibit high solubility rate than PPC and present a u-shaped curve on fluctuation of environmental and processing parameters (Habiba 2002; Adebiyi and Aluko 2011).

Emulsifying Properties

This property is combination of emulsion activity (EA) and emulsion stability (ES) of the protein's (Boye et al. 2010). PPC exhibit ES of 65.3 and EA of 60.6% as reported by Gwiazda et al. (1979). These are also affected by temp and ionic strength of food and demonstrate the inverse relationship with temp and NaCl concentration (Tian et al. 1998).

Foaming Properties
It is measure of foam expansion (FE), foam capacity (FC) and foam stability (FS) of the proteins.

The FS of PPI is comparatively better than SPI and also affected by pH, temp and nature of food materials (Toews and Wang 2013).

Gelling Properties
According to the study of O'Kane et al. (2004) the pea protein forms supplementary unstructured gels in comparison to soy protein, but the gelling property of pea protein is not good as soya protein. Akintayo et al. (1999) described in its study that concentration of (72% protein) pea protein had small gelling properties. Although a study revealed that pea protein isolate formulates a paste instead of a forming a rigid gel (Adebiyi and Aluko 2011). Nunes et al. (2006), showed that the pea protein work as an alternative of egg and dairy proteins to form a gelled in vegetable dessert. The study results revealed that pea proteins formed good gels that were highly appropriate for food product.

The disadvantages of using legume proteins in replacement of meat are that they are limited in sulfur containing amino acids as well as "hay-like" and "beany" flavor that is tough to disguise and ultimately reduce the consumer acceptance in the market (Leterme et al. 1992; Owusu-Ansah and McCurdy 1991; Heng 2005). This flavor is due to volatile (saponins) and non-volatile (ketone and aldehyde) compounds due to auto-oxidation and lipoxygenase activity (Rackis et al. 1979; Aspelund and Wilson 1983).

The pea proteins also have anti-nutritional factors (ANF) like protease inhibitors, tannins, lecitins, phytates and saponins (Liener 1994) which disturb the bioavailability and digestibility of proteins in a negative approach. Their content is ANF components produced during legume proteins processing (Gilani et al. 2005). Different methods using physical (dehulling), chemical (soaking, irradiation, heating) and biological (germination and fermentation) means can be employed for the inactivation purpose (Bhat and Karim 2009; Asgar et al. 2010). The significant amount of non-protein components and anti-nutritional factors (polyphenols, saponins, raffinose, phytate and oligosaccharides) restrict the direct utilization of pea proteins in foods (Sandberg 2011).

Chickpeas

Chickpea protein is the chief plant protein source. Chickpea is categorized in two major types: Kabuli and Desi. The size of Kabuli varieties is large, is cream-colored and has a thin seed coat whereas desi varieties have small size, darker color, have a thick seed coat. Desi varieties usually contain higher amounts of proteins than that of Kabuli varieties. The protein contents found to be ranging from 20.9% to 25.27% in different varieties of chickpea. The major forms of proteins were albumin, globulin, prolamin and glutelin and range between 8.39–12.31%, 53.44–60.29%, 3.12–6.89%, and 19.38–24.40%, respectively. Some other studies shown that globulins were present in chickpeas in higher percentage of (41.79%) the total protein, then albumin (16.18%), glutelin (9.99%), and prolamin (0.48%) respectively (Boye et al. 2010).

Pea, bean, chickpea and lentil are not categorized as major allergens. Even though the proteins found in these pulses can cause allergic reactions but they are limited mostly to the European regions, Asia and the Mediterranean, this may be due to higher rate of their consumption in these areas. Severe allergic reactions due to pulse consumption are rare that is the reason they are not included in the list of major allergens. These allergic reactions can occur due to multiple proteins found in pulses which are mostly thermo-stable. Furthermore, cross-reactive characteristics of pea, lentil, bean and chickpea allergens are found (San Ireneo et al. 2000).

4.1.3 Peanut

The main oil crop in China is peanut, the edible seeds peanuts or groundnuts are actually legume. As compare to other serving beans, peanuts have higher level of protein than any other nut. Peanut protein along having appealing aroma and whitish color is enriched with numerous essential amino acids which makes it supercilious over soybean protein and easily absorbable in human body. So its usage in plant industry as source of plant protein is becoming common. Some physicochemical modifications have been applied on peanut protein to enhance its application in food processing industry (He et al. 2014). The remaining meal called defatted flour left after oil extraction is actually less costly protein rich source mostly exploited peanut product having superior quality protein about 50%. The production of **peanut protein concentrate (PPC)** protein flour that is defatted can dispense the food industry with an innovative superior quality protein food ingredient for protein fortification and product development (Yu et al. 2007).

Peanut contain higher level of protein called Barginine and contain all twenty amino acids in variable proportion. Peanut and legume protein such as soybean protein have much significance for human health and body growth as they are nutritionally equivalent to meat and egg protein as per Protein Digestibility Corrected Amino Acid Score (PDCAAS) (FAO 2002). Protein meal amino acid profile depicts that it can be used for protein fortification Unlike animal protein, the plant based protein in peanuts carries additional components with it that provide beneficial compounds such as fiber, certain bio actives. Peanut protein possesses some beneficial properties such as emulsifying ability and stabilization of emulsion, water holding capacity, good solubility. Moreover its role in food industry as an ingredient for product formulation and protein fortification can never be denied (Wu et al. 2009). On the basis of recent observations, peanut protein is used in infant formulas (Nimsate et al. 2010) and noodles (Wu et al. 2009).

However the use of peanut as a protein source may cause some allergic reactions that are related to action of immunoglobulin E (IgE) and other anaphylatoxins, which act to release histamine and other mediator substances from mast cells (degranulation). The histamine prompts vasodilatation and constructs bronchioles in the lungs, called bronchispasm. The symptoms of this allergic reaction are vomiting, urticarial, diarrhea, angioedema (swelling of the lips, face, throat and skin), exacerbation of atopic eczema, asthma, anaphylactic shock. By using some new techniques if allergy is countered, peanut can be used for nutrition to all as it is highly dense with essential nutrients. Some emerging techniques recently used includes; Anti IgE therapy, Chinese medicine, probiotics use, oral desensitization, cellular meditator, soy-based Immunotherapy, Immunostimulatory sequencing, engineered allergen immunotherapy, Oligodeoxynucleotide-Based Immunotherapy, Plasmid DNA Immunotherapy and Bacterial adjuvant (Nowak et al. 2011). There is some research required as these methods are on initial stages and have long way to get approved for regular practical implementation.

4.2 Cereal Grains

Cereals are considered as chief food crop worldwide. They are consumed in form of seeds (rice, maize barley and oats,), flour (wheat, maize and rye), or flakes (barley, maize and oat). Their protein content is calculated on the basis of dry matter percentage and its amount varies in all types of cereals. The wheat comprises 8–17.5%, barley (7–14.6%), maize (8.8–11.9%), oats (8.7–16%), rice (7–10%), and rye (7–14%) of protein (Shoaib et al. 2018). These protein fractions are named as globulins, gliadins, albumins, and glutelins (Singh and MacRitchie 2001; Asgar et al. 2010).

Among these, gluten is the most lavishly found protein in wheat, rye, and barley, and may cause celiac disease to some individuals. The consumption of this protein as alternative of meat is in area of research for industrialists and product development experts (Sadler 2004). In processed meat applications, these impart purposeful properties as structure improvement, nutrient supplement, processing aid, formulation aid, stabilizer, finishing agent, thickener, surface and texturizing agent, maintenance of emulsification and enhance water binding capacity (Egbert and Payne 2009). All these effects are achieved by interaction between glutenins and gliadins (Asgar et al. 2010; Kumar et al. 2017; Ren et al. 2020).

Gliadins are the monomeric proteins with intramolecular disulfide bonds with low or medium molecular weights. By using electrophoresis mobility, they are separated on polyacrylamide gels at acid-PAGE, and distribute wheat gliadins into four major groups as α (fastest mobility fractions), β, γ, and ω groups (lowest mobility) (Woychik et al. 1961). Gluten is made up of higher molecular weight (HMW) wheat fractions of glutenins and low molecular weight (LMW) glutenins (Stevenson and Preston 1996). In wheat, proteins in form of Gliadins and glutenins account 80% of total mass of seed (Mondal et al. 2016).

Recent research indicated that gelling properties of wheat gluten may be improved through combinations of transglutaminase and heat treatment (Wang et al. 2007; Egbert and Payne 2009). Gluten is available in many functional forms such as (1) native, vital and devital cereal protein, (2) solubilized forms by deamination or enzyme hydrolysis (Alimentarius 2001). In current era, gluten is extensively being used in combination with soy proteins to produce meat analogs such as meat batters (Patana-Anake and Foegeding 1985), and restructured beef steaks (Miller et al. 1986) and coarse ground meat applications.

In a study, Olavarria (1981) patented protein binder compositions for texturized proteins which were composed of 10–20% gluten, 10–20% whey proteins, 1–5% albumin and sufficient quantity of milk or water. The Nguyen (1988) scientist, patented meat analogue resembling to chicken breast prepared from wheat gluten and soy flour. For this, 80% wheat gluten, 11% wheat flour and 9% vegetable oil were mixed, and water sprayed on the mixture. Dough was prepared and heated by conventional or microwave means. It was noted that finished product was as per perceptions of consumers.

The study conducted by Kumar (2009) and Kumar et al. (2012) reported that incorporation of wheat gluten in analogue of meat (nuggets) improved all sensory attributes. The concept was further strengthened by Kumar et al. (2017) and concluded that addition of 10–20% wheat gluten in meat substitutes has increased the flavour and colour scores of the product. Researchers have observed and recommended that protein supplementation with cereals of rich in lysine could provide a balanced mixture of amino acids (Wondimu and Malleshi 1996; Asgar et al. 2010; Singh and Sidhu 2014).

4.3 Nuts

These have an obvious place in the archetypal vegetarian diet known as 'nut cutlet' due its provision to high levels of protein. These can be grown in different parts of the world, and include tree nuts (almonds, brazil, and cashew nuts) and ground nuts (peanuts). Although, USA produces nuts about two million tonnes a year and import 100,000 tonnes to UK (BNF 2002). It is noted that less peanuts are eaten in the UK as compared to America (Taylor and Hefle 2006).

A number of allergenic food proteins have been characterized in peanuts, soybeans, lentils, common beans, mungbeans, chickpeas, peas, and tree nuts (Singh and Bhalla 2008; Riascos et al. 2010). The soybeans and peanuts are mainly responsible and supposed as common sources of food allergens. Peanut bases allergies can cause acute and severe reactions, but allergies to soybeans are assumed not to cause severe reactions (Van Boxtel et al. 2008). An allergy is basically an adverse immune-mediated hypersensitivity by environmentally harmless substances called as allergens. This induces a specific immune response in genetically predisposed individuals through the production of elevated levels of a specific immunoglobulin E (IgE) (Singh and Bhalla 2008). In recent years, due to awareness about healthy life style, nuts are being used as important ingredient in meat-free convenience products such as nut roasts as meat analogues (Sadler 2004; Goodman et al. 2013; Asgar et al. 2010).

4.4 Mushroom

These belong to class of fungi and their nutritional benefits arise from its chemical composition. It may be a valuable supplement for cereal-based and legume-based diets. During the 1960s, nutritionists and politicians across the world were concerned that the predicted growth in the world's population would lead to global protein shortages in the future. Food scientists were seeking interest to develop a microbial protein source that would be inexpensive and palatable during this period (Asgar et al. 2010).

It is thought, cell walls of the hyphae (cells) are cheap source of dietary fiber (chitin and glucan). Their cell membranes are source of polyunsaturated fat and cytoplasm is the source of high-quality protein (Hoseyni and Khosravi-Darani 2010). The amino acid composition of mycoprotein indicates the presence of all essential amino acids to suggest it as economical meat replacer. The natural protein of mushroom is fibrous in nature and provides chew ability to the products (Katya et al. 2014). The mycoprotein can also reduce the harmful LDLs (low density lipoproteins) and enhances the beneficial HDLs (high density lipoproteins) in consumers.

In 1967, an organism *Fusarium venenatum* (*F. venenatum*) was identified as first primary source of mycoproteins in Marlow, Buckinghamshire and U.K. and was eventually exploited to produce mycoproteins (Denny et al. 2008). Mycoprotein is the generic name given to the ribonucleic acid (RNA)-reduced biomass, comprising the hyphae of *F. venenatum* ATCC PTA-2684 in a continuous fermentation process while using glucose as substrate for 6 weeks (Denny et al. 2008). The CO_2 evolution rate, biomass concentration, determines the flow rate of the protein. During the production process, biomass cultures are maintained at 28–30 °C with a pH of 6.0, and mycotoxins are also tested after 6 h intervals to ensure that the mycoprotein is mycotoxin free. The RNA content of the fungal biomass must also be reduced during production to meet required safety standards (Wiebe 2002). After harvesting from the fermenter, the culture broth is subjected to a short heat treatment to reduce its RNA content from 10 to ≤2%, which is achieved by heat activation of the endogenous RNAse enzymes (Denny et al. 2008). This fungal biomass is heated in a separate tank to temperatures above 68 °C (optimal 72–74 °C) for 30–45 min. The heat-treated culture broth is then centrifuged and recovered as a paste (Wiebe 2004).

The research to confirm the safety status of *F. venenatum* ATCC PTA-2684 strain concluded that it did not produce mycotoxins (O'Donnell et al. 1998) and growth conditions used should be suitable for mycotoxin production (Johnstone 1998). Furthermore, *F. venenatum* has been approved for sale as food by the Ministry of Agriculture, Fisheries and Food in the United Kingdom in 1984. A panel of experts evaluated the suitability of mycoproteins for food use in the United States. Four studies were performed to assess the tolerance of humans to mycoproteins, and the results demonstrated that mycoproteins are well tolerated by humans and has an extremely low allergenic potential. The GRAS safety status of this class also allow it to be used as meat analogues commercially for burger patties, sausages, fillets, cold-cut slices, nuggets, burgers, ready meals, pastries, and pies (Miller and Dwyer 2001; Kumar et al. 2017).

The meat alternatives are prepared proteins by mixing the fermented mushroom with egg and other seasonings/ flavourings. The meaty flavour of these products attributed to the presence of sulfur containing amino acids and glutamic acid in fungi (Trinci 1994). The taste of mushroom derived products is better than other plant derived products. Based on this advantage, these are widely used in European countries as alternative to beef and chicken. However, the wide spread public perception of the Fusarium as pathogenic and not a true mold has forced the researchers and food technologist to search a better alternative in last decades. Chevrolet and Vitroculture (1987), revealed a composition for making of meat like products by using the edible mushroom to serve as gelling agent (polysaccharides undergoing gelation), texturing agent (proteins) and flavorings. Kumar et al. (2011) studied that 22.5% mushroom replacing texturized soya protein increased the sensory attributes of meat like nuggets due to increase in flavour and overall acceptability.

The use of mushroom/ fungal protein is preferred over bacterial and vegetable proteins to exert the following advantages as flavour of its protein is more acceptable as compared to beany flavour of textured soy protein, it tend to give convincing meat, poultry and fish analogues by alignment of the protein filaments with fibrous or flaky texture, these mycoproteins are tasteless, colorless and unpalatable with texture similar to meat fibre but with added flavoring and coloring it could also be made into a passable imitation of fish, chicken, veal or ham and exhibit potential for antitumor and other health effects.

Conclusively, mushrooms can be recommended to fulfill our daily requirements of protein, minerals and vitamins (Kumar et al. 2017) (Fig. 15.1).

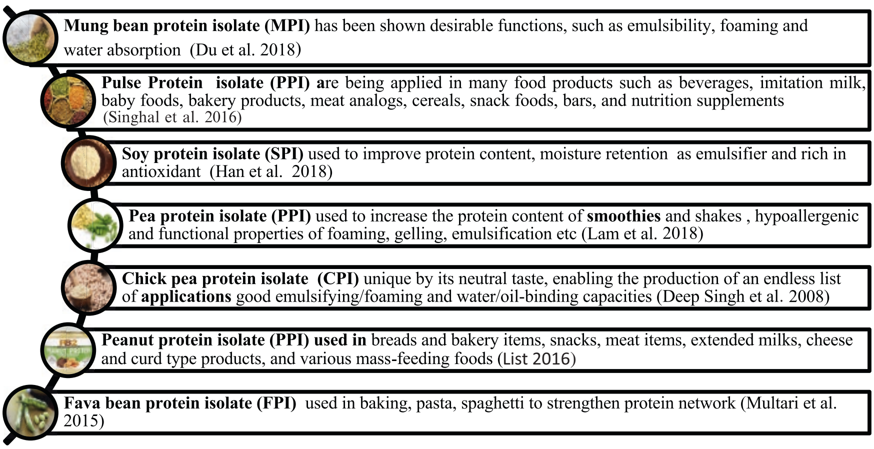

Fig. 15.1 Application of vegetable protein isolates and their functional properties

5 Meal Plans Based on Plant Protein

A meal plan is defined as a guide to design daily meals and snacks routine. It is designed in a manner to provide the consumers with good nutrient balance to maintain health. The purpose of meal planning varies from desire of healthy eating, weight management to weight loss. Meal plan is designed keeping in view a person's daily nutrient requirement, which varies with age, activity level and health condition. These meal plans usually provides appropriate amount of food from all the basic 5 food groups. Meal planning is an important phenomenon in predicting the lifestyle and possible health related medical outcomes of individuals. The adoption of healthier meal plans is the most appropriate way for enjoying healthier lifestyle. The meal planning plays a key role in maintaining and sustaining healthier lifestyle due to balanced and managed diet intake (Opperman et al. 2004). Food requirements are diversified and variable for individual to individual depending upon age, physical activity, environmental conditions and medical sufferings. So, keeping in view all these aspects, meal planning needs certain concerns for developing diversified meal plans (Brunstrom 2014). Hence, prevalence of these aspects regarding protein isolates is summarized in Table 15.2.

Usually there are two categories of healthy meal plan that are used to fulfil daily caloric and food intake requirements. Traditional meal plans and vegetarian meal plans; a traditional meal plan is based on food items from all food groups whereas a vegetarian or vegan meal plan does not include fish, poultry and meat-based diet.

Table 15.2 Requirement of protein isolates/meal sourcing from vegetable proteins during Childhood and Young Adolescent

	Age											
	0–4 years			5–9 years			10–14 years			15–19 years		
Protein Isolates	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)
Fava Beans	4.1	7.3	6.2	9.1	12.2	10.8	9.6	13.4	15.4	21.0	25.4	18.6
Black Beans	3.3	6.8	5.5	10.2	11.1	9.2	8.8	12.5	14.7	20.3	23.4	18.4
Red Beans	4.4	6.7	5.1	8.8	13.1	10.3	9.3	13.2	16.5	19.9	24.5	17.7
Lima Beans	2.9	5.2	4.8	7.1	10.9	11.1	9.9	12.4	15.7	15.9	21.4	19.9
Mung Beans	4.0	4.6	4.0	5.4	12.4	8.7	10.3	11.3	14.2	20.9	22.5	20.5
Kidney Beans	3.5	4.8	4.2	4.9	9.2	10.4	9.9	14.2	17.4	18.5	23.5	19.4
Navy Beans	1.9	3.8	3.7	3.9	10.5	9.8	12.5	13.0	13.2	19.5	25.6	17.6
Chick Peas	2.2	3.2	3.8	5.0	12.5	9.3	9.3	10.0	12.3	16.6	22.2	18.4
Lentils	4.8	6.0	3.9	10.5	10.8	12.5	13.0	13.2	18.5	26.6	18.3	18.9
Peas	5.5	6.5	4.3	11.7	10.8	15.8	12.1	10.3	11.4	15.7	18.7	22.4
Black Eyed Peas	2.8	6.4	5.5	10.4	11.4	16.5	13.7	12.5	10.6	19.5	16.4	20.4
Soy Beans	3.7	4.0	4.0	5.4	9.4	9.5	10.1	11.1	12.5	19.5	20.3	20.5

Where *B* Breakfast, *L* Lunch and *D* Dinner
Jean-Michel and Stéphane (2016)

In Vegetarian Meal Plans protein requirement is fulfilled by plant protein isolates and meat substitutes. However, The USDA Food and Nutrition Service (FNS) permits the use of alternative protein product (APP) to replace meat and meat-based products in designing meal plans but these substitutes must fulfill certain requirements particularly when implemented for Child and Adult Care Food Programs, School Breakfast Program, National School Lunch Program and Summer Food Service (United States Department of Agriculture, Food and Nutrition Service). The set criteria for inclusion of alternative protein product in daily meal plan is based on the biological protein quality, which can be determined by evaluating the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (Schaafsma 2000)

$$\text{PDCAAS} = \frac{\text{mg of limiting amino acid in 1 g test protein}}{\text{mg of same amino acid in 1 g reference protein}} \times \text{fecal true digestibility}$$

The alternative protein product must have a Protein Digestibility Corrected Amino Acid Score of at least 0.80. There are some of the plant proteins with poor protein digestibility that do not meet the minimum requirement of nutritive protein content. Protein quality is important in meal planning as in developing countries due lack affordability of meat protein usually minimum daily requirements for protein are not being met. As per standard of World Health Organization, daily requirement of protein for a person is 27 g, whereas in developing countries like Pakistan public is consuming 17 g a day only. Therefore, we are already consuming less animal protein as per required standards (Tables 15.3 shows requirement of protein isolates/meal sourcing from vegetable proteins in adults: ages from 20 to 44 and Table 15.4 shows requirement of protein isolates/meal sourcing from vegetable proteins in adults: ages from 45 to 64).

According to a study conducted by Nath (2011) for addition of plant-based meat substitutes in diet plans of Australian population, observed that these meat substitutes are a valuable aid for designing meat-free diets. As these meat replacers act as social facilitators for the consumers that prefer plant proteins to part of their daily meal routine. With the increasing trends vegan diets, plant proteins are now being used in festive meals (Nath and Prideaux 2011).

In another study conducted in Netherlands, Schösler (2012) found that most of the consumed meat substitutes, which are becoming substantial part of daily meal plan include nuts, pulses and lentils. But still these substitutes are ranked lower in the preference list of dietary protein sources, stating that pulses and other alternative protein sources have to break through a challenging pathway in order to disrupt familiar meal formats. To familiarize the consumer with meat replacers it requires active effort to break from the existing conventions where meat provides the structural aspect to the meal. To analyze the consumption trends of meat replacers an audit was conducted, and it was observed that in 2/3 of the products legume proteins are used but in order to meet recommended requirements, current levels of legume intake must be raised by 470% (Baghurst and Magarey 2011). But the prime barrier to that withholds the inclusion of legumes in daily meal planning is lack of knowledge regarding their preparation methods and time constraints (Figueira et al. 2019)

Table 15.3 Requirement of protein isolates/meal sourcing from vegetable proteins in adults: ages from 20 to 44

	Age														
	20–24 years			25–29 years			30–34 years			35–39 years			40–44 years		
Protein Isolate	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)
Fava Beans	19.3	26.7	21.5	22.6	30.1	19.6	21.6	28.4	20.0	21.0	29.5	19.7	23.5	16.4	15.1
Black Beans	20.3	24.7	20.5	19.6	29.5	18.7	20.6	27.4	21.0	20.6	27.6	17.9	21.5	17.4	16.1
Red Beans	21.3	25.5	22.4	21.6	28.7	19.5	17.8	28.8	20.0	22.3	25.4	18.8	20.5	18.4	17.6
Lima Beans	20.4	26.5	19.6	20.7	27.7	20.8	16.5	26.9	18.7	19.4	26.5	18.8	22.2	17.9	18.8
Mung Beans	18.8	24.4	18.3	20.8	30.9	16.4	22.4	29.6	19.9	20.7	30.3	29.9	21.4	18.9	16.5
Kidney Beans	16.6	25.5	20.7	22.5	26.6	17.9	19.9	25.8	21.5	18.3	27.7	19.8	20.3	18.4	17.4
Navy Beans	18.9	20.1	19.7	18.0	20.4	14.4	18.6	24.5	22.3	22.4	29.9	16.6	19.7	16.4	18.7
Chick Peas	16.2	21.2	18.9	20.9	16.8	16.3	20.8	19.3	20.7	21.4	23.3	20.1	19.7	18.8	17.9
Lentils	20.1	18.7	18.0	20.2	14.4	18.6	24.5	22.3	21.3	27.7	17.5	18.6	18.3	19.4	20.9
Peas	18.6	20.2	24.4	20.8	20.5	26.6	19.4	18.4	26.8	19.0	21.3	24.3	19.7	19.4	17.9
Black Eyed Peas	19.4	19.4	23.5	19.8	17.5	24.4	21.5	21.5	23.4	25.5	19.5	22.5	20.5	18.7	18.3
Soy Beans	18.1	23.1	19.1	16.8	26.6	18.3	21.4	28.5	17.7	20.7	27.3	27.9	20.4	17.7	17.3

Where *B* Breakfast, *L* Lunch and *D* Dinner
Amol et al. (2014)

plant-based meat substitutes such as seitan, tempeh, tofu may offer a convenient and surreptitious way to increase intake (Gilham et al. 2018).

Similarly, this study showed that 20% of the burgers contained on an average 8 g of whole grains per serving, presenting a distinct opportunity to help consumers to reach their 48 g daily intake target. Ingredients like brown rice, buckwheat, quinoa, and other on-trend grains could be considered when formulating new options. In this respect, plant-based meat substitutes could become a vehicle for increasing the whole grain consumption (Schösler et al. 2012).

6 Conclusion

The nutritional importance of grains and legumes cannot be denied due to higher percentages of both micro and macro nutrients. Proteins play an important role in understanding their amino acid composition, which can be then easily balanced in

Table 15.4 Requirement of protein isolates/meal sourcing from vegetable proteins in adults: ages from 45 to 64

	Age											
	45–49 years			50–54 years			55–59 years			60–64 years		
Protein Isolate	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)	B (g)	L (g)	D (g)
Fava Beans	19.8	27.6	18.3	17.3	28.3	19.6	15.2	22.3	15.2	12.1	20.9	14.7
Black Beans	18.2	24.9	16.3	18.7	26.9	18.6	17.7	19.9	16.5	14.6	19.8	13.9
Red Beans	19.9	22.5	18.9	19.8	25.4	20.6	20.5	22.6	14.6	11.4	17.9	15.6
Lima Beans	19.6	21.1	17.9	17.9	23.0	19.9	18.8	20.9	15.8	14.8	16.5	14.9
Mung Beans	17.7	25.2	16.5	18.7	24.5	17.6	16.4	20.9	14.8	13.6	18.6	17.6
Kidney Beans	15.3	24.3	18.5	19.7	24.4	19.4	18.3	18.9	11.4	9.8	13.5	12.5
Navy Beans	18.9	23.6	14.5	13.4	20.5	17.7	14.5	21.4	20.4	12.5	18.0	16.4
Chick Peas	18.3	20.2	17.8	12.1	22.4	19.4	9.7	15.4	10.4	11.1	14.5	10.5
Lentils	22.4	16.3	14.2	21.8	18.5	16.8	22.2	23.5	13.4	18.8	15.4	3.9
Peas	18.5	18.8	20.4	18.6	19.0	24.3	20.2	21.4	22.5	14.7	12.7	16.9
Black Eyed Peas	20.3	19.5	20.4	21.6	15.6	19.8	17.6	18.5	19.4	10.3	10.5	8.5
Soy Beans	17.5	25.0	17.4	15.5	22.4	18.1	17.1	20.6	14.1	10.4	14.3	7.9

Where *B* Breakfast, *L* Lunch and *D* Dinner
Millward (2018)

diet. The components found in grain and legumes contribute in human well-being and play positive role in preventing and treating different diseases. Hence their use should be put forward in diet to have a healthy life. There is a scarcity of protein of high biological value due to rapid increase in the world population and limited natural resources. As meat is a good source of protein of high biological value but converting the vegetable protein into animal protein is not economical. With this increase in population and an ever-increasing demand for animal protein, there will be a greater need for plant proteins to fill the gap between world animal protein production capabilities and the world demand for protein-based food products. This will result in increasing the use of low-cost vegetable protein such as textured proteins, mushroom, wheat gluten, pulses etc. as a substitute for animal-protein. The availability and use of plant proteins is needed to continue to grow into the future as the world population grows and as this population becomes more prosperous and their meat consumption patterns increase. The functional properties of the current plant proteins should be improved in order to meet the opportunities for future growth in the protein-based food marketplace. The marketing prospects of meat analogue is very bright due to its inherent qualities of very cheap source of protein, suitable for health-conscious non-vegetarians, lactose intolerant people, persons following rules of religion, or to address ethical qualities and nutritional issues for vegetarians. Thus, meat analogues have a far better chance of success than other products as some consumer desire organoleptically attractive and nutritious product entirely free of meat. Meat processors begin to work closely with the manufacturers of plant-based proteins to ensure that their future needs are met from a functional protein standpoint as well as from a technological knowledge base with regard to the use of plant proteins in their processed meat systems. Further studies have to be carried out on optimization of formulas for these kinds of products in order for them to gain consumer acceptance.

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Chapter 16
Environmental Aspects of Plant Protein Foods

Poritosh Roy, Takahiro Orikiasa, and Takeo Shiina

1 Introduction

The global meat demand has grown by 58% over the last two decades to reach 360 million tonnes (Whitnall and Pitts 2019), and is projected a rise of 15% by 2027 (FAO 2018). In 2018, the global meat market was $1000 billion (Gerhardt et al. 2020).

Meat is considered to be the primary source of protein. In 2019, per capita meat consumptions in the World, developed, and developing countries were 34.5, 68.6, 26.6 kg, respectively (OECD/FAO 2020). Livestock production contributes 14.5% of global greenhouse gas (GHG) emissions (Gerber et al. 2013). Approximately 83% of the World's farmland is currently used for the production of animal products, which supplied only 37% of protein and 18% of calories (Poore and Nemecek 2018). The global demand for protein/protein-rich food is driven by socio-economic changes (Henchion et al. 2017). The growing meat consumption is not only responsible for climate change but also creating health problems (Henchion et al. 2017; Godfray et al. 2018). Nowadays, plant-based proteins are being produced in an attempt to combat rising GHG emissions from the food sector (Green 2020).

Food is consumed not only for filling the stomach and meeting the taste/satisfaction but also to get adequate energy and nutrients (Roy et al. 2009) to stay healthy

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as well as to avoid lifestyle related chronic diseases (Wirt and Collins 2009). The nutritional component of food is one of the most important quality indicators, such as protein and minerals. Both fresh and processed food are consumed to meet the dietary needs. However, minimum or unprocessed food (raw/or without additives) is known to be superior to processed food (industrial foods usually, contain artificial colors, flavors, or other chemical additives), which can contribute to chronic diseases (Harverd 2020), although there are exeptions such as foods from designer foods project (Sugarman 1991; Rajasekaran and Kalaivani 2013). Food production, distribution, preparation, and preservation consume a considerable amount of energy, contributing to greenhouse gas (GHG) emissions.

The growing health and environmental concerns led countries or regions to look for an alternative to meat. In addition, the national dietary guidelines advise reducing consumption of animal products, especially red meat (HealthCanada 2016). Consequently, enormous efforts are underway in the development of alternatives to meat, such as plant protein-based meat and cell-based meat (Slade 2018; Green 2020), to meet the increasing demand for meat of the rising population (Heusala et al. 2020b). This initiative encompasses lab-grown (cell-based meat, i.e., *in vitro* product, hereafter referred to cultured meat), plant-based, insect-based and fungi-based products (Asadollahzadeh et al. 2018; Dekkers et al. 2018; Ismail et al. 2020).

The consumer demands for plant-based food are growing (Nielsen 2017; GoodFood 2020) due to lesser environmental impact compared with animal-based products (Veeramani et al. 2017; Berardy et al. 2019; Green 2020). For example, in the USA, plant-based product sales increased by 29% in the last 2 years (reached \$5 billion), which replaces animal-based products (GoodFood 2020). The US plant-based protein market will reach \$450 billion by 2040 (Gerhardt et al. 2020). The plant-based meat sector is projected to contributing about \$3 billion by 2030 in Australia (Lawrence 2019). However, the emerging plant-based meat/protein may disrupt the present food supply chain, especially the livestock industry. Although plant-based meat is claimed to be environmentally benign, environmental impacts and consumer preference compared with their counterparts are yet to be elucidated. This study summarized the consumer preference and environmental impacts of meat alternatives, i.e., alternative protein sources and compared with animal sources.

2 Dietary Patterns and Sources of Protein

Malnutrition (deficiencies of micronutrient, undernutrition), as well as metabolic syndromes (overweight, obesity, etc.), are the persistent global challenges humankind face today. Although the dietary guides for a healthy diet were developed by various nations (MHLW 2015; HealthCanada 2016), food choices are often steered by the culture, religion, geography, availability, affordability, as well as social and behavioral motives. Uncontrolled food consumption is identified to be one of the major reasons of many health-related problems (obesity, chronic diseases) in both developed and developing countries. In addition to the food guide, Japan has

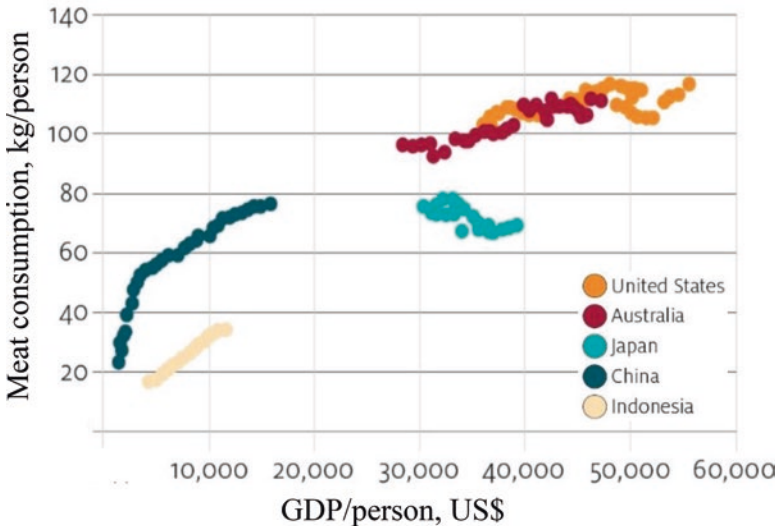

Fig. 16.1 Effect of gross domestic product on meat consumption (OECD forecast) (Whitnall and Pitts 2019)

introduced the Metabo law (requires men to maintain a waistline of less than 33.5 inches and for women it is less than 35.4 inches along with several health risk indicators including blood glucose level, blood lipid level, and blood pressure) in 2008, in an attempt to eradicate some of the health-related problems. Figure 16.1 depicts that meat consumption increased with income growth in most of the countries, except Japan. The world health organization (WHO) recommends 10–15% of human energy intake from proteins and animal protein should contribute 10–25% of dietary protein for a healthy diet (WHO 2003), which indicates that animal protein should contribute only 2.2% to total calorie intake. Even in Japan, where the meat consumption is less, animal protein contributes to about 10% to total calorie intake in Japan (Roy et al. 2012). In Japan, the recommended energy intake from protein is 13–20% (MHLW 2015). On the other hand, recommended energy intake from protein was 10–35% (Dubé 2018). Animal-based proteins are known to be complete as they contain all of the essential amino acids, while plant-based proteins lack one or more of these amino acids (Hoffman and Falvo 2004; Brown 2017).

The major sources of protein are meat, dairy products (predominantly, milk and yogurt), and pulses. Wheat provides 20% of global protein for human consumption (Tilman et al. 2011). On the other hand, animal products provide 25% protein in the global food supply (Mottet et al. 2017). The recommended safe protein intake level is 0.8 g/kg body weight/day (WHO 2007; Berardy et al. 2019; Gunnars 2020). However, protein intake in most of the developed countries is found to be more than the recommended intake. In 2007, daily per capita protein consumption in the World, developed and developing countries was 77, 103, and 70 g, respectively (ChartsBin 2011). In the same period, protein consumption in Canada, China, India,

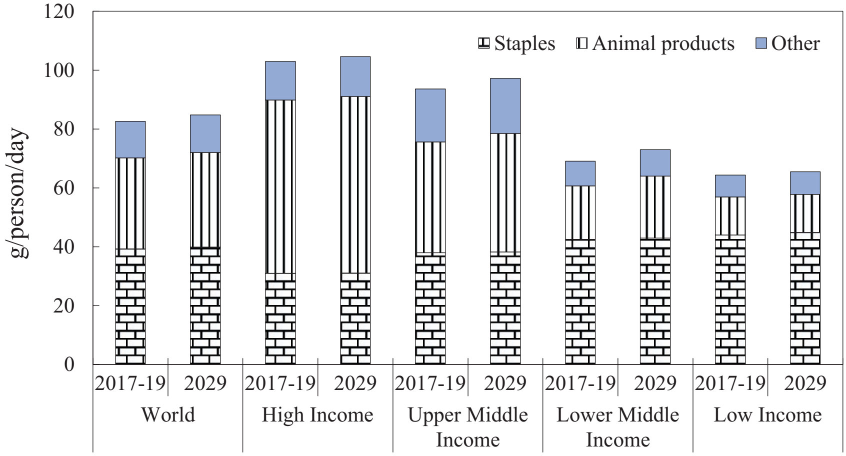

Fig. 16.2 Protein consumption by various income groups (OECD/FAO 2020)

and Japan was reported to be 105, 89, 56, and 92 g, respectively (ChartsBin 2011). The protein intake was found to decrease in Japan while increase in the developing countries as well as in some of the developed countries. In most of the developing countries, plant-protein is the major contributor to total protein intake. On the other hand, animal protein is the major contributor in the developed countries. Figure 16.2 represents protein consumption from different sources by various income groups. Although meat consumption or protein intake slightly decreased in Japan (compared with 1990 consumption level), yet the consumption is greater than the WHO recommended safe level of protein intake from meat, like other developed countries.

3 Consumer Preference

Consumer preference is reported to be dependent on the culture, price, taste, texture, health risk, environmental footprint, and animal welfare (Bryant et al. 2019; Whitnall and Pitts 2019). The availability, affordability, and convenience are also recognized as a major determinant of food choices. The consumption patterns are also noted to be dependent on income growth and meat supply (Whitnall and Pitts 2019), except in Japan (Fig. 16.2). In the last two decades, global meat consumption increased by 85% in the developing countries whilst Chinese consumption increased by 72%; however, meat consumption decreased in Japan (Whitnall and Pitts 2019).

The consumer acceptance of cultured meat in China, Thailand, and Vietnam is reported to be about 26%, 34%, and 52%, respectively (YuGov 2018), while consumer acceptance is reported to be 40% in the USA and 18% in the UK (Nutraceutical 2018). Animal health and ethical treatment to the animal, and environmental concerns are the main motivators for the selection of plant-based foods in the USA, UK, and Canada (Fox and Ward 2008). On the other hand, in another study, the acceptance of cultured meat is reported to be 29.8%, 59.3%, and 56.3% in the USA, China, and India, respectively (Bryant et al. 2019). However, the acceptance of plant-based meat is noted to be 32.9%, 62.4%, and 62.8% in the USA, China, and India, respectively (Bryant et al. 2019). The consumer preference for fungi-based meat burgers, cultured-meat burgers, and hamburger is reported to be 26%, 22%, and 51%, respectively (Hellwig et al. 2020). The taste was noted to be the most important criteria in this consumer preference. However, consumer acceptance also seems to be dependant on the supply, such as in the developing countries shortage of meat supply might led to a greater acceptance of meat alternatives.

A survey among more than 1800 US consumers was conducted to determine the consumer choices on meat alternatives (alternative to conventional meat products). Burger patties of conventional beef, lab-grown meat, and plant-based alternatives (pea protein and animal-like protein). The consumer choice for farm-raised beef-, pea-protein-, animal-like protein, and lab-grown meat burger-patties was reported to be 72%, 16%, 7%, and 5%, respectively (Van Loo et al. 2020). The authors also noted that farm-raised beef burger patties maintain the major share in the market even after a 50% price reduction for its alternative products. It seems that plant-based meat has better acceptance than cultured meat, insect-, and fungal-based meat. Consequently, the main hurdles that have to overcome in the commercial production of meat alternatives (especially cultured, insects-, and fungi-based meat) are ethical, public health, and social acceptance.

4 Development of Meat Alternatives

The development of plant-based proteins is led by the increasing demand for protein by the growing population, growing concerns about human health, animal welfare, and the environment. Although some of the plant-based proteins (tofu, soybean meal, etc.) are being used since the ancient period, the development of first-generation plant-based meat was initiated in the 1960s (He et al. 2020) and got enormous attention in recent years. Texturized vegetable protein emerged in the mid to late twentieth century (Lawrence 2019). The meat alternatives are also being developed from fungi (Asadollahzadeh et al. 2018; Singh et al. 2020) and insects (Smetana et al. 2015; Ismail et al. 2020). In addition to plant-based alternatives, cultured meat has also been developed and marketed. However, several start-up companies are involved in the development process of cultured meat, which is yet to get approval, except for a recent case in Singapore. Recently, Singapore Food Agency has approved cultured chicken meat developed by Eat Just for marketing in Singapore, which is known to be the first in the World and met the standards for poultry meat (Lucas 2020). However, food components in protein-rich foods vary depending on their sources and processing conditions (Tables 16.1, 16.2, and 16.3). Usually, extrusion and shear-cell techniques are used in production process of plant-based meat alternatives. Currently, *in vitro* technique has been widely used in the

Table 16.1 Food components per 100 g of ground meat (Roy et al. 2012)

Type of meat	Water, g	Protein, g	Lipid, g	Iron, mg	SFA, g	PUFA, g	Vitamins A, μg	E, mg	B_1, mg	B_2, mg	Energy, kJ
Chicken	69.8	20.9	8.3	1.2	2.35	1.29	40.0	0.2	0.10	0.21	695
Pork	65.4	18.6	15.1	1.1	5.71	1.72	12.0	0.4	0.62	0.22	925
Beef	64.5	19.0	15.1	2.3	Tr	Tr	4.0	0.5	0.08	0.20	937

Source: Food Composition Table 2000, Japan

Table 16.2 Food components in some food of plant origin (per 100 g)

Type of grains	Water, g	Protein, g	Lipid, g	Iron, mg	SFA, g	PUFA, g	Vitamins A, μg	E, mg	B_1, mg	B_2, mg	Energy, kJ
Peas (boiled)	63.8	9.2	1.0	2.2	0.12	0.3	93	2.4	0.27	0.06	619
Lentil (boiled)	57.9	11.2	0.8	4.3	0.09	0.25	31	3.1	0.2	0.06	711
Corn	14.5	8.6	5.0	1.9	1.01	2.24	373	5.1	0.3	0.1	1464
Corn meal	14.0	8.3	4.0	1.5	0.8	1.79	384	5.5	0.15	0.08	1519
Wheat	12.5	10.6	3.1	3.2	0.56	1.53	–	–	0.41	0.09	1410
Tofu	88.9	6.6	4.2	0.9	0.68	2.21	–	4.7	0.07	0.03	310

Source: Food Composition Table 2015, Japan (MEXT 2015)

Table 16.3 Nutrient content in some edible insects and plant products

Insects	Component, % (dry matter basis) Protein	Fat	Fiber	NFE	Ash	Energy, kCal/100 g	Reference
Orthoptera (crickets, grasshoppers, locusts)	61.32	13.41	9.55	12.98	3.85	426.25	Lee et al. (2020)
Odonata (dragonflies, damselflies)	55.23	19.83	11.79	4.63	8.53	431.33	Lee et al. (2020)
Coleoptera (beetles, grubs)	40.69	33.40	10.74	13.20	5.07	409.78	Lee et al. (2020)
Lepidoptera (butterflies, moths)	45.38	27.66	6.60	18.76	4.51	508.89	Lee et al. (2020)
Soybean meal	47.5	1.8	–	–	7.0	–	Asadollahzadeh et al. (2018)
Fish meal	63.9	12.0	–	–	21.0	–	Asadollahzadeh et al. (2018)

development process of meat alternatives (Dekkers et al. 2018). The selection of protein sources is noted to be very important in improving the biological and chemical safety, flavor and appearance of plant-based meat alternatives (He et al. 2020) as well as ensuring the essential food components for a healthy diet.

5 Environmental Impacts of Proteins

LCA study requires to define a functional unit to refer to the environmental impacts of the product, process, or service. Food LCA often uses multiple functional units such as mass (kg), volume (L, m^3), land-use area (ha), energy (MJ), etc. as well as the mass of the nutrient content, i.e., mass (kg) of protein (Haas et al. 2001; Basset-Mens and Van der Werf 2005; Hayashi 2006; Roy et al. 2009, 2012; Sonesson et al. 2017). Usually, the environmental impacts of protein-rich food are greater than the carbohydrate-rich products (Ozawa and Inaba 2006). However, the environmental impacts of protein-rich food are also reported to be dependent on the source of protein that includes in the diet (Berardy et al. 2015). The carbon footprint of plant-based protein substitutes is reported to be 34%, 43%, 63%, 74%, 87%, and 93% lower compared with farmed fish, poultry meat, pig meat, farmed crustaceans, dairy herds-beef, and beef herds, respectively, whilst tuna and insect had a smaller carbon footprint than that of plant-based protein (Santo et al. 2020). The authors also noted that the carbon footprint of protein depends on the processing intensity. For example, compared to plant-based protein, tofu, peas, and other pulses have 1.6, 7.0, and 4.6 times lower GHG emissions, respectively (Santo et al. 2020). In contrast, the environmental impact of plant-based meat is noted to be greater than that of beef raised on well-managed pasture (Van Vliet et al. 2020). Food processing often produces multiple products; thus, the allocation method (mass/economic value/energy content/protein content, etc.) also affects the life cycle environmental impacts of the products (Roy et al. 2012; Heusala et al. 2020b). Figure 16.3 shows the environmental impacts of meat and meat substitutes (protein-rich products). The global warming potential (GWP) of a product depends on the type of products, protein content, and geographical locations, thus resulting in a wide variation. Another reason behind this variation might be depend on the selection of functional unit or allocation method. It reveals that some of the plant-based protein sources have lower GWP compared with animal-based protein sources.

5.1 Impacts of Plant Proteins

Impacts of plant proteins are dependent on the sources of proteins as well as the processing intensity (Fig. 16.4, Table 16.4). Plant-based proteins are reported to be more sustainable compared with fishmeal or soya concentrate (Green 2020). Digestible indispensable amino acid score (DIAAS) has been adopted in a food LCA to incorporate bioavailability of protein and determine the environmental impacts of various protein sources (Berardy et al. 2019). The authors confirmed that the environmental impacts of animal-based food are greater than that of plant-based food (Berardy et al. 2019; Heusala et al. 2020b). For example, oat- and faba protein exhibit 50% and 80–90% lower carbon footprint, respectively, compared with dairy protein (Heusala et al. 2020b). Some of the plant-based protein undergoes various

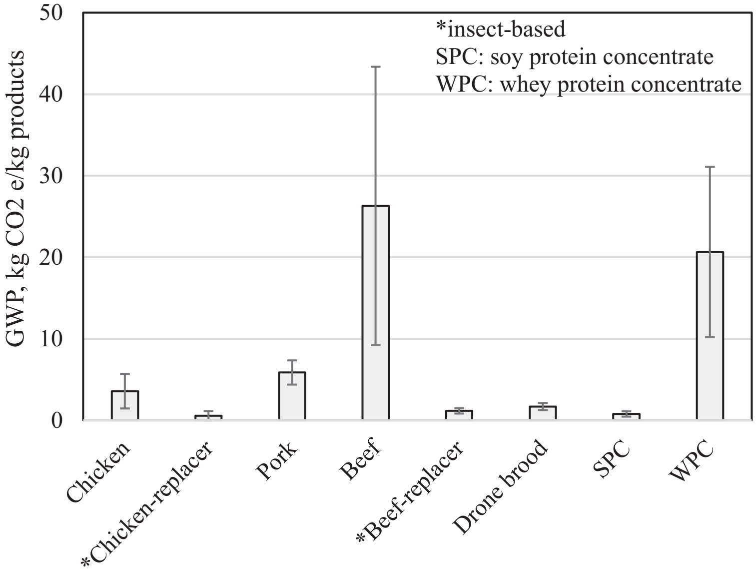

Fig. 16.3 Environmental impacts of meat and meat substitutes (Data sources: (De Vries and de Boer 2010; Roy et al. 2012; Thrane et al. 2017; Van Mierlo et al. 2017; Bacenetti et al. 2018; Ulmer et al. 2020))

chemical and mechanical treatment for improving the nutritional value and texture of plant-based meat (protein), thus has greater environmental impacts (Berardy et al. 2015; Bacenetti et al. 2018). For example, plant-based meat produced as soy protein isolate has greater environmental impacts compared with unprocessed chicken, pork, or even beef except for freshwater eutrophication (Berardy et al. 2015). Figure 16.4 confirms that the environmental impacts of plant-based proteins are also dependent on the sources of protein and degree of processing. For example, the environmental impact of plant-based protein concentrate is lower than that of protein isolate, which requires more processing (Blonk 2020).

5.2 *Impacts of Animal Proteins*

Usually, meat, egg, and milk are the primary sources of animal proteins for human consumption. However, with the increasing concerns on human health, environmental impacts, and rising demand for animal products/animal protein, various meat alternatives have also been developed. With the increasing demand for protein for the rising population, insects are also identified to be a potential source of protein for food and feed (Halloran et al. 2016). Among the sources of animal proteins, insects are reported to have the potential to be converted into animal protein food or feed and can be an environmentally friendly choice compared with their

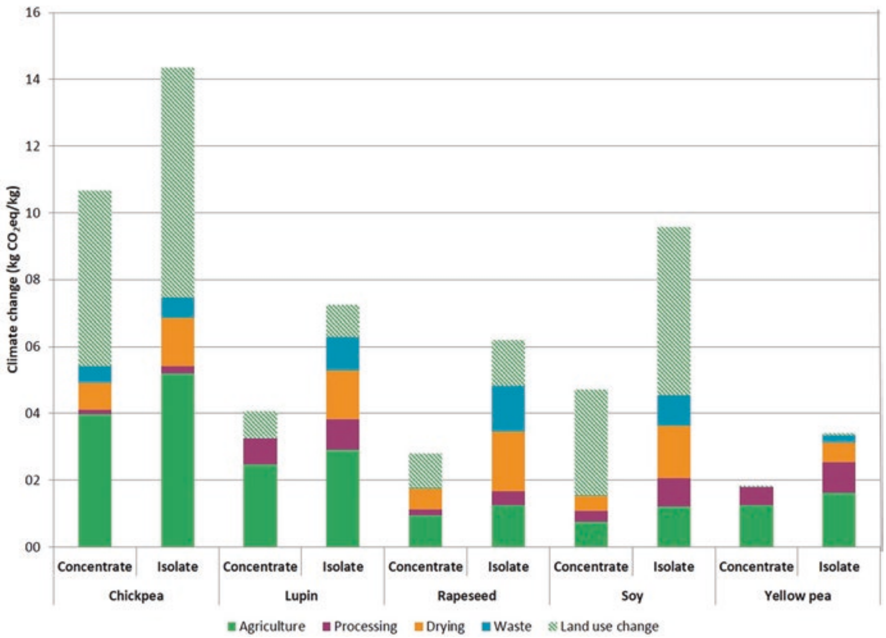

Fig. 16.4 Environmental impact of the life cycle of some plant proteins (Blonk 2020)

counterparts (Van Huis et al. 2013; Smetana et al. 2015; Halloran et al. 2016). For example, GHG emission from the life cycle of protein produced from mealworms is reported to be 2.65 kg CO_2 eq./kg protein (Oonincx and De Boer 2012), which is lower compared with meat sources (chicken, pork, and beef) (Table 16.5). Table 16.6 represents the environmental impacts of fishmeal (used as feed) from different sources, which confirms that the environmental impacts of protein feed also depend on the source of protein. The difference in system boundary, assumption, and processing intensity might also responsible for the varied results. For example, the GWP of protein from dairy cows (beef) varied from 45 to 150 kg CO_2 eq./kg depending on the raising methods, geographical locations, and the system boundary (Thrane et al. 2017; Ulmer et al. 2020).

6 Discussion

Although enormous efforts are underway for moving toward a sustainable alternative to conventional meat and meat products, especially for plant-based proteins, the nutrition transition seems to be affected by the economic growth, environmental and health concerns, ethical choices, and animal welfare as well as availability/supply. From an environmental and health perspective, plant-based proteins need to be reinstated in place of animal-based protein consumption, especially in the regions where

Table 16.4 Life cycle impacts of plant proteins

Source of proteins	System boundary	Impact category/kg product						Reference
		GWP, kg CO_2 eq.	OD, CFC-11 eq.	AP, kg eq. SO_2	EP, kg eq. PO_4^+	Water use, m^3	Land use, m^2	
Soy protein isolate	Cradle to gate	20.22	–	–	–	38.95	–	Berardy et al. (2015)
Soy protein isolate	Cradle to gate	6.10	–	–	–	–	–	Thrane et al. (2017)
Soymeal	Cradle to gate	0.901	0.00024	0.0041	0.0038	–	–	Dalgaard et al. (2008)
Gluten powder	Cradle to plate	3.81	–	–	–	–	–	Smetana et al. (2015)
Mycoprotein	Cradle to plate	5.85	–	–	–	–	–	Smetana et al. (2015)
Oat protein concentrate	Cradle to gate	3.3	–	–	–	–	3.2	Heusala et al. (2020b)
Oat protein contentrate	Cradle to gate	8.8	–	–	–	–	8.6	Heusala et al. (2020b)
Faba protein concentrate	Cradle to gate	1.1	–	–	–	–	0.8	Heusala et al. (2020b)
Faba protein concentrate	Cradle to gate	1.9	–	–	–	–	13.3	Heusala et al. (2020b)
Microalgae	Cradle to gate	14.7–245.1	0.9–19.8	260.5–1407.5	40.6–105.3	–	1.7–5.4	Smetana et al. (2019)

GWP Global warming potential, *OD* Ozone depletion, *AP* Acidification potential, *EP* Eutrophication potential, *HTTP* Human toxicity potential

humans are at risk from some chronic diseases due to the increasing consumption of red meat. However, sources of plant-based proteins have to be selected carefully to maintain quality and avoid intensive processing.

Despite enormous efforts in the development process of meat alternatives to meet the rising demand for proteins, their appearance, texture, flavor, and palatability are yet to meet the standard of livestock-based traditional meat. Cultured meat is likely to be similar to livestock-based meat, but the growth rate and the production cost are the main commercialization constraints (Lee et al. 2020). In addition, meat alternative lacks consumer acceptance; thus demands continual improvement in

Table 16.5 Life cycle impacts of animal proteins

Source	System boundary	Impact caterory/kg product						References
		GWP, kg CO_2 eq.	OD, CFC-11 eq.	AP, kg eq. SO_2	EP, kg eq. PO_4+	Water use, L	Land use, m^2	
[a]Milk protein	Cradle to gate	0.032	3.5E-09	–	–	0.0013	4.95	Gesan-Guiziou et al. (2019)
Skim milk powder	Cradle to gate	23.0	–	–	–	–	–	Thrane et al. (2017)
Whey[b]	Cradle to gate	38.05	0.0036	–	–	–	–	Bacenetti et al. (2018)
Whey[c]	Cradle to gate	39.17	0.0037	–	–	2.08	–	Bacenetti et al. (2018)
Whey[d]	Cradle to gate	40.65	0.0038	–	–	3.90	–	Bacenetti et al. (2018)
Whey protein	Cradle to gate	20.0	–	–	–	–	–	Thrane et al. (2017)
Whey concentrate	Cradle to gate	7.48	3.33	56.6	37.3	–	0.26–8.27	Smetana et al. (2017)
Whey[b]	Cradle to gate	38.05	0.0036	–	–	–	–	Bacenetti et al. (2018)
Lab-grown meat	Cradle to plate	24.27	–	–	–	–	–	Smetana et al. (2015)
Fresh insect	Cradle to plate	2.93	–	–	–	–	–	Smetana et al. (2015)
Mealworms	Cradle to gate	2.65	–	–	–	–	3.56	Oonincx and De Boer (2012)
Mealworms protein	Cradle to gate	14.0	–	–	2.491		18.0	Oonincx and De Boer (2012)
Protein (insect)	Cradle to grave	15–29	–	–	–	–	1.2–17	Ulmer et al. (2020)
Protein (Pork)	Cradle to grave	77.88	–	0.675	2.491	–	55.00	Zhu and van Ierland (2004)
Protein (pork)	Cradle to grave	22–53	–	–	–	–	39–75	Thrane et al. (2017), Ulmer et al. (2020)
Protein (Chicken)	Cradle to grave	10–30	–	–	–	–	23–40	Thrane et al. (2017), Ulmer et al. (2020)
Protein (beef, dairy cows)	Cradle to grave	45–150	–	–	–	–	37–210	Thrane et al. (2017), Ulmer et al. (2020)
Egg protein concentrate	Cradle to gate	23.4	1.01	4000	139	–	40.1	Smetana et al. (2017)

GWP Global warming potential, *OD* Ozone depletion, *AP* Acidification potential, *EP* Eutrophication potential, *HTTP* Human toxicity potential

[a]Impacts/L; [b]protein concentration 35%; [c]protein concentration 60%; [d]protein concentration 80%

Table 16.6 Life cycle impacts of fishmeal as a source of proteins

Source	System boundary	Impact category/kg product			Reference
		GWP, kg CO_2 eq.	AP, kg eq. SO_2	EP, kg eq. PO_4^+	
Poultry byproducts	Cradle to gate	0.49–3.55	0.002–0.048	0.001–0.02	Maiolo et al. (2020)
Microalgae	Cradle to gate	15.37–27.09	0.039–0.08	0.014–0.03	Maiolo et al. (2020)
Insects	Cradle to gate	1.02–2.45	0.01–0.047	0.007–0.051	Maiolo et al. (2020)

quality as well as to develop the methods and standards for evaluation of plant-based meat (He et al. 2020).

The consumption of large quantities of processed meats and red meats are associated with an increase in cancer risk (Godfray et al. 2018; IARC 2018). Other risk factors include lifestyle and dietary habits (Ksouri 2019). High levels of conventional meat consumption cause various chronic diseases (Lawrence 2019). Although various alternatives (fungi-based meat, insect-based meat, plant-based meat, and cultured meat) to conventional meat are available in the market, their production process is relatively complex and costly as well as have limited consumer acceptance (Dekkers et al. 2018; He et al. 2020). It seems that plant-based meat could be a potential alternative to conventional meat compared with fungi- or insect-based meat because of their complex production process and consumer acceptance for the latter two. However, the plant-based protein products contain insufficient essential amino acids and trace elements which are reported to be the most challenging to meet the nutritional value of conventional meat-based protein (Ismail et al. 2020). Transitory food consumption while maintaining a healthy diet and avoiding health risks would be the big challenge humankind might have to overcome in the near future. Therefore, adequate amount of health-beneficial components and energy for maintaining sound health need to be ensured while abating GHG emission from the food sector. The health effects of meat alternatives are yet to be determined, which need to be considered in the development and commercialization process of meat alternatives.

The growing concerns on health and environment may lead to changes in the perception of the consumer and the development of meat alternatives; however, their role will depend on consumer acceptance and health benefits. In addition, the acceptance of meat alternatives seems to be guided by taste, texture, price, health risks, environmental impacts, and animal welfare. It is essential to outline the activities (research, production, marketing, consumption, and health) in food systems involving all the stakeholders, which may enable to avoid any unwanted risk in the food sector and mitigate environmental impacts. For a smooth transition to meat alternatives, enormous social, institutional, and technological efforts are required depending on the type of meat alternatives (Fig. 16.5).

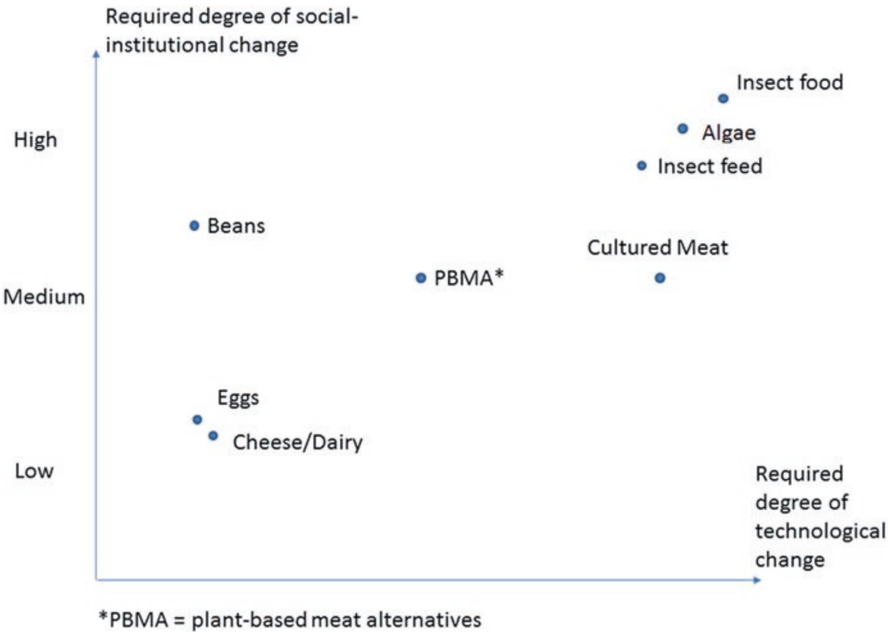

Fig. 16.5 Required social-institutional and technological change for meat-alternatives (van der Weele et al. 2019)

Processing contributed 75% to the total environmental impacts of the life cycle of oat protein concentrate (Heusala et al. 2020b), which indicates that plant-based protein that can produce with minimum processing would reduce environmental impacts. It is also worthy to mention that environmental impacts vary depending on the allocation method (Roy et al. 2012; Heusala et al. 2020b). Although enormous efforts have been placed in evaluating the life cycle impacts of different protein sources, only a few studies have considered the quality of protein (Sonesson et al. 2017; Berardy et al. 2019), which affects the LCA results. In most of the LCA studies, environmental impacts of meat/meat substitutes are determined for a certain mass of the products, then expressed in terms of protein content in them (Table 16.7). It seems that the environmental impact of both the plant- and animal-based protein productss depend on the protein content in them. For example, the environmental impact of whey concentrate that contains a greater amount of protein is noted to be higher than the whey concentrate with lesser protein content (Table 16.7). This table also depicts that environmental impact depended on the types of product, which might be the result of the protein content in primary materials. Consequently, protein-rich materials would be a good choice for producing meat substitutes or plant-based proteins. However, the contribution of protein to the total energy supply varies depending on the protein content in meat or meat substitutes. For example, protein in oat starch (protein content 6.32%) as a protein source supplied only 18.9% of energy to the total energy (Heusala et al. 2020a). It is also worthy to note

Table 16.7 Protein contents in various products and their life cycle environmental impacts

Product	Protein content, %	GWP, kg CO_2 e/kg		Reference
		Product	Protein	
Oat starch+Oat protein concentrate pasta	6.3	1.0	12.4	Heusala et al. (2020a)
Oat protein concentrate	37.0	3.3	8.8	Heusala et al. (2020b)
Pea protein concentrate	55.0	1.3	2.2	Heusala et al. (2020b)
Soy protein isolate	87.0	5.3	6.1	Thrane et al. (2017)
Whey protein concentrate	80.0	16.0	20.0	Thrane et al. (2017)
Whey protein concentrate	35.0	13.3	38.1	Bacenetti et al. (2018)
Whey protein concentrate	80.0	32.6	40.7	Bacenetti et al. (2018)
Beef	19.0	35.6	187.6	Roy et al. (2012)
[a]Beef	–	–	45–150	Thrane et al. (2017)
Pork	18.6	6.9	37.3	Roy et al. (2012)
Chicken	20.9	6.0	28.6	Roy et al. (2012)

GWP global warming potential; [a]Dairy cow

that not only energy supply but also other food components vary and provide various health benefits. Consequently, the life cycle of proteins needs to be evaluated, considering the quality of protein as well as other health beneficial food components in protein-based food for a better comparison.

If the food sector fails to provide adequate food containing balanced nutrients to meet human needs, human health problems will severely increase. Insufficient supply of even one essential nutrient for a long period will result in a dire health consequence (Graham et al. 2001). Consequently, food systems face persistent challenges, thus human health. Japan is one of the most successful countries in health outcomes (Wang et al. 2017; Yoneoka et al. 2019), which might be the result of the promotion of healthy dietary guidelines. Thus, it seems that a system approach is required to integrate research and development in nutrient supply, consumer behavior, circularity, and climate change for the sustainability of the food system (Fig. 16.6).

7 Conclusion

Despite enormous efforts in the development process of meat alternatives to meet the rising demand for proteins, their appearance, texture, flavor, and palatability are yet to meet the standard of livestock-based traditional meat in most cases. The reliable production of cost-competitive quality meat alternatives, ensuring their safety for consumption, would be required to enhance the market and acceptability of

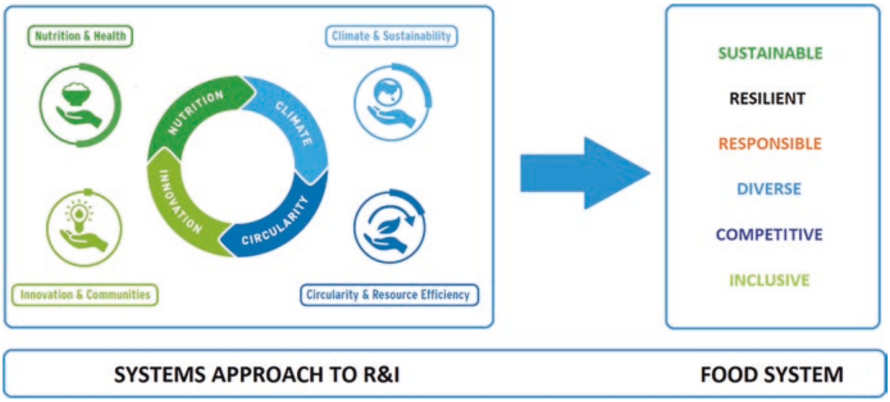

Fig. 16.6 Conceptualization of system approach and future-proofing food systems (Gill et al. 2018)

these products, which may mitigate the environmental impacts from the food sector. However, the development of meat alternatives should consider a comprehensive sustainability assessment to avoid any risk of investment and health.

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Chapter 17
Market Drivers and Barriers for Plant-Based Protein Foods

Dana McCauley

1 The History of Plant-Based Protein Consumption

Whether a company is selling plant-based proteins or other food products, success in the food business depends on understanding people. The food science innovators and product developers who will have impact in the plant-based protein category will spend time before they start product development studying the drivers and barriers behind purchase decisions; they will research both the conscious and unconscious factors that influence a consumer to buy a plant-based protein product and develop a deep understanding of which consumer will respond to which features and benefits the product offers. The process sounds straight forward: figure out what problems people want to fix; develop a product that solves that problem; create communication vehicles (such as label design, package claims and websites) to motivate consumers to identify your product as a solution to their problem and success will follow. Yet, so often new products fail. Depending on which sources you consult, the number of new product launches that fail to gain market traction ranges from 80% to 95% (Kocina 2017) suggesting that it is simply not enough to create a unique product that tastes good. In this chapter we'll explore what innovators in the plant-based protein space need to know to be counted among the small 5–20% of successful food product launches.

To understand the modern plant-based consumer, we need to look back at the history of eating meat and why that practice became a dominant part of not just our diets but a ritual part of our lifestyles (Tannahill 2008). Only once we understand

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these factors can we unpack the place that plant-based proteins can fill in the marketplace. No one really knows how humans developed an omnivorous diet; however, we do know that until about 10,000 years ago, humans likely subsisted on a predominantly plant-based diet until farming and systematic cultivation became common. Foraging for wild fruit and edible roots and shoots required less risk, fewer specialized skills, and lower energy output than hunting. One can speculate that because meat was difficult to obtain, that our societal opinion that meat is valuable and that eating it conveys status was established around this time. And, until the innovation of creating and controlling fire (Scott 2018) became common knowledge, even good hunters likely satisfied their dietary needs most often with more easily obtained plant-based foods. Eating meat daily is a relatively modern habit. After the development of domestic heat based cooking, daily animal protein consumption evolved slowly. Dairy and eggs were byproducts of farming and became dietary staples before meat. Regardless of how meat came into a household – whether hunted in the wild or purpose raised by humans for food – eating meat regularly simply required too many resources. It remains more efficient even today to maintain an animal and consume what she produces than to continually replace animals that you have eaten. So, even after farming and heat-based cooking were common parts of human life, meat eating was still aspirational, reinforcing its symbolic status and embedding in our collective consciousness an opinion that economic success and meat eating are linked. This deeply seated belief continues to influence global protein trends.

In numerous cultures, meat consumption is entwined with rituals and rules that bind communities together. Catholics, for instance, abstain from meat on Fridays as a remembrance of Christ's sacrifice. Catholics also serve meat as a celebratory food at Easter. Yet, fish, (an aquatic meat protein) is a permissible food for Catholics to eat on Fridays and during Lent, another period when meat eating is not allowed. Similar, meat consumption rules and restrictions cascade through different cultures. Muslims, Jews, and Hindus eschew certain meats for religious reasons, too. And, in the Buddhist faith, eating meat is 100% verboten for compassionate reasons. Buddhism prohibits the taking of a life of a person, animal or sentient being as the act of killing is believed to 'cut off great compassion' (Ohlsson 1998). Buddhist tenets led to the development of culinary traditions in several cultures where the consumption of plant-based proteins is ubiquitous and influencing today's product developers. In China, Buddhist cooks long ago refined the practice of using plant derived ingredients to make foods like 'mock duck' and 'mock chicken' which cunningly simulate meaty textures and umami flavours. In an essay published in 2018 in Tastecooking.com (Erway 2019), Cathy Irwin wrote that "in China, the origins of both tofu and wheat gluten are somewhat linked to their use as meat replacements. In Mandarin, *mianjin*, or wheat gluten, means literally 'wheat meat'. And tofu, a food that dates to prehistoric times in China, was popularly known as "small mutton" in the tenth century." Since the 1960's Western innovators have been intrigued with wheat gluten which now is commonly sold as *seitan*, a term coined by George Ohsawa, the creator of the macrobiotic diet. For many centuries, Japanese Buddhist law prohibited the consumption of four legged animals but allowed poultry and fish to be included in diets which influenced the development of that country's cuisine. Today, even though laws were loosened in the nineteenth century allowing the Japanese to consume meats from quadrupeds such as beef and pork, Japanese cooking tends to skew heavily toward fish and chicken with red meat being used just sparingly as a garnish or augmentation to recipes. In India, where Buddhist meat use was more restricted, people who follow that faith have little interest in new plant-based protein products that mimic the taste and texture of meat. "For many Indian vegetarians, I don't think a meat substitute would even register since meat was never a reference point to begin with." says Brooklyn based food entrepreneur Chitra Agrawal (Erway 2019).

In religions and cultures where meat was included in the diet, meat from four legged animals often became a celebratory food. Feasts held after hunters returned to their villages with their spoils, often included rituals that expressed thankfulness to divine entities. Sacrifices and complex rules about who ate which cuts and in what order further reinforced that meat held more than nutritive value. These practices extended into agricultural times and often dictated when and how domesticated livestock could be slaughtered and consumed. Lambs and other high value livestock were common choices as sacrificial animals and their slaughter was carried out with specific rituals that conveyed respect and subjectivity to the prevailing gods. Ancient Greeks, for instance, normally dined on red meat only when the animal had been first ritually sacrificed (Visser 2008). Immediately after killing, the liver of the animal would be examined as it was believed to hold prophetic messages, then this organ would be eaten by the local priests. Such rituals raised the importance of animal protein to a holy level.

Until the economies of scale achieved in farming in the last century (King n.d.), the complexity and expense of raising domesticated food animals also made animal protein precious. For Europeans subsistence farming was the norm and famines were a frequent reality until at least the eighteenth century. King Henry (Encyclopedia.com 2021) the fourth of France acknowledged that meat eating was not a daily dietary practice in the sixteenth century. While trying to cultivate support among his subjects he declared his hope for widespread prosperity by saying "I want there to be no peasant in my realm so poor that he will not have a chicken in his pot every Sunday." The slogan was resurrected by Herbert Hoover's presidential campaign team in October 1928 when they used it in ads designed to remind voters that Republicans had "…put a proverbial 'chicken in every pot' (INDC 2019). And a car in every backyard, to boot…" (Encyclopedia.com 2021). This connection between regular meat consumption and economic affluence continues to influence meat consumption, in developing markets such as India and China where meat consumption, is not historically high. Among the growing middle classes in these markets, there is a desire to emulate developed nations who equate meat eating with affluence and status.

2 Current Plant Protein Facilitators

The focus on plant protein centric diets and products is driven by different trends in different geographic locations. In North America, the European Union and United Kingdom, sustainability started to influence protein decisions as long ago as 1971 when the iconic book Diet for a Small Planet (Moore 2021) by Frances Moor Lappė brought the environmental impact of large-scale animal agriculture into popular discourse. Simultaneously, movements such as People for the Ethical Treatment of Animals (PETA) emerged and initiated activities to persuade the public to consider compassionate reasons for foregoing meat. While conversation and debate ensued, sustainability didn't significantly impact consumption rates of plant-based proteins until recently when the effects of climate change started to be more generally acknowledged by politicians and the media. Given that veganism and vegetarianism levels are relatively static, the rise in plant-based protein consumption and greater product choice in the form of meat alternates, milk alternates and even egg substitutes since 2010 suggests compassion for cows is not driving the trend. Instead, successful strategies shifting consumption include prompts for people to make small changes to contribute to environmental change. Movements such as Meatless Monday (The Monday Campaigns 2020) are a good example. Meatless Monday is a community minded campaign that encourages families and individuals to skip eating meat one day a week to help reduce greenhouse gas emissions (Meatless Monday 2017). The organization has not only raised awareness but created a tribe of true omnivores willing to commit to plant-based foods on a part-time but regular basis. A US survey conducted in 2020 revealed that while only 3% of Americans identify as vegans, 36% of consumers identify themselves as flexitarian (Baker 2021), meaning that they consume animal proteins as well as vegan or vegetarian meals.

While sustainability is a driver behind plant-based protein acceptance for all ages, it is Generation Z (GenZ) who will prioritize sustainability in purchase decisions. Born from the mid 1990's until around 2010, the GenZ cohort is the next wave of consumers who will enter the economy. This cohort expects more from grocers and food manufacturers and in the process accelerate plant-based protein trends significantly. Even whilst some conservative governments will not admit that climate change is man-made, many school curriculums now emphasize the impact industrialization and globalization have had on the environment. With this exposure to facts, ideas and knowledge, young people are becoming increasingly passionate about sustainability. Research shows that today 59% of consumers who currently say that it's important to them that their food is produced in an environmentally friendly way (Malochleb 2020) and as GenZ matures, that number is likely to grow. As the largest, most digitally connected and most highly educated generation they have all the tools required to affected significant change. Swedish teenager Greta Thunberg is the most famous embodiment of this generational persona. Starting as a local protester, Greta captured international attention and prominence as a policy influencer. Greta may be just one person, but she embodies the culmination of many social, economic and feminist trends that have been building for decades and that will position her peers to fuel a surge in plant-based protein consumption that will transform cooking and eating long term.

The female portion of the GenZ demographic is rising as ambassadors of the environment. She will accelerate the plant-based protein movement over the next 30- to 40-years when she wields her household buying power to make dietary choices for her family. According to Morgan Stanley, in 2019 women were responsible for an estimated US$31.8 (Entrepreneur Surveyed by Morgan Stanley 2019) trillion in consumer spending. Although more men are active participants in household chores and child-rearing than ever before, globally 89% of women control or share in daily shopping decisions, compared to only 41% of men (Neilsen 2019). As the person in the household who will write the shopping lists and manage the children's diets and health, GenZ mom will be important to grocers. Her commitment to the environment and her strength of conviction will empower her to insinuate her values and commitments into the fabric of the family dynamic. Her education and confidence will likely see her wield significant power in boardrooms and government where she will influence policy and industry more than any other female demographic in history.

A shift in attitudes toward globalization is also boosting the popularity and availability of plant-based protein. As we exit the COVID19 pandemic many countries are concerned about the extent to which their economies can be self-sufficient to ensure food system resiliency in times of crisis. Singapore has given themselves a deadline of 2030 to have 30% of their nutritional needs supplied locally – a 20% improvement over their 2020 status (Earth Org 2021). As a country with less than 1% of its land devoted to agriculture, their 30% goal cannot be achieved without urban farming, agri-tech innovation, and food science. Demonstrating commitment to their goal, in December 2020 the Singapore government approved the first commercial cellular agriculture protein product for human consumption; the product, chicken nuggets made from poultry cells, became available to Singaporeans just weeks later, launching at a private members club called Restaurant 1880. Although this product innovation is not technically a plant-based protein, the country has many plant-based protein projects following fast tracks to market and they are cultivating global attention to create partnerships with far-flung innovators who can help them fulfill their protein needs.

Singapore's timing is good. We are in a 'golden age' of food science and investors are willing to commit millions of dollars to create plant-based proteins that are nutritionally and hedonically like their animal counterparts. While new technologies are being developed quickly, there is no reliable information on how many universities, research centres and entrepreneurs are funneling brain power and money into solving the complex food science problems that will result in more progress in the development of plant-based protein products that will satisfy the global craving for meat. But, according to the Good Food Institute, global investments in plant-based, cell cultivated and fermented protein companies reached $3.1 billion in 2020, significantly higher than in 2019, when investment levels were near $1 billion (Nunes 2021). With such money at stake, the race to innovative is fast-paced, making this an exciting category for food business insiders and consumers alike.

3 Who Is the Plant-Based Protein Consumer?

Misunderstanding of the plant-based consumer abounds. At conferences, meat and dairy producers voice concern about losing their share of the marketplace and warn that vegetarian interest in implementing radical change from animal agriculture to plant-based agriculture will be disruptive to people, the planet and economies. However, these groups often speak before doing sufficient research. If they did review the facts, they would learn that plant-based protein consumers are not typically vegans or even vegetarians. Accurately estimating the number of vegans and vegetarians in the world is not simple and most estimates skew heavily toward data captured in developed economies; however, the commonly reported global number of vegans (those who consume no products derived from animals) is just 3% of the population and vegetarians (those who eat no meat but may eat dairy and eggs) is 5% (Williams 2020). While the number of vegans and vegetarians is expected to grow, the key consumer for plant-based proteins is actually meat eaters who crave variety. In fact, the adults who make the decision to give up meat to become vegans or vegetarians, generally find products that mimic the shape, flavour, and texture of animal protein distasteful; they chose a meatless lifestyle because the idea of eating meat is abhorrent. This consumer is much more likely to choose an old-fashioned veggie burger, complete with chunks of beans and corn, over a facsimile product that mimics the taste and texture of beef or chicken.

While animal protein commodity groups can breathe a sigh of relief that they are not being put out of business, food marketers are left with a puzzle: how can they create innovative new products that mainstream consumers will want to buy not just occasionally, but frequently enough to generate a payback for the incredibly high R&D costs required to bring new food science driven plant-based innovations to market? Companies like In Impossible Foods seem to have cracked the code. In 2020 they disclosed to the media that nine out of 10 people who eat Impossible products are meat eaters (Applegren 2021). Omnivores are people who eat the wide range of foods including meat and fish of many kinds; they can be segmented into several sub-groups. One of the most important is the baby boomer omnivore. Born post World War II until around 1960, baby boomers who choose plant-based proteins want options that are as similar as possible to the item they are replacing. Typically, this demographic reduces their intake of meat for health reasons. They have no intention of becoming vegetarians. With cardio-vascular diseases and diabetes being more prevalent in this age group, many change their diets upon the advice of health care professionals. In other words, they choose plant-based proteins as substitutes for their first choice which is meat. As a result, they are a key target market for plant-based foods that replicate the taste, texture and aroma of real meat. This is also the consumer whose shopping cart will include plant-based meat substitutes for healthy weekday meals and choice of steaks or roasts they'll serve when entertaining or celebrating special occasions.

Studies of families with young children show that these households truly exemplify omnivorous behaviour. The Guelph Family Health study surveyed a limited number of millennial aged consumers with children under 5-years of age, revealed that parents often keep both dairy based milk and plant-based dairy alternatives on hand (Topakas et al. n.d.). The facilitators that led to such behaviour were interesting. Parents believed that dairy milk and plant-based dairy alternatives both deliver nutritional benefits and they agreed that plant-based dairy alternates add variety to their family diet. Plant-based dairy won a place in their shopping carts by easing their ethical concerns about dairy farming but failed to deliver the fun, child-friendly products (such as cartoon themed packaging) offered by the well-established dairy product producers. Concerns the parents expressed about plant-based dairy alternates included the environmental impact of production, use of pesticides in field crops and high sugar content. Clearly, neither dairy nor plant-based dairy alternatives are solving all their problems, leaving significant room for innovators to work.

Even while plant-based purchases rise, meat consumption grows, indicating that people are eating more protein in general. According to Canada's Food Price Report (Charlebois n.d.), for the year to December 2020, meat prices increased overall by 2.5% and even with price spikes of 10% or more during the summer months, meat continued to sell at normal or close to normal rates. Experts attribute the spikes to COVID-19 shutdowns at processing plants as well as to the seasonal summer grilling demand surge. Co-author of the Price Report, Dr. Simon Somogyi, the Arrell Chair in the Business of Food at the University of Guelph, says "…grocers know there isn't a lot of substitutability for meat so increases in meat prices typically don't impact overall demand."

That said, as the world exits the pandemic, there is a growing segment of price sensitive consumers. After Covid-19 shutdowns put many people in hospitality, tourism and service-based businesses out of work, a strong trend toward value grocery shopping has emerged. Agricultural economist Dr. John Cranfield, Associate Dean for External Relations at the University of Guelph's Ontario Agricultural College, notes that "…price still tops the list of what matters when making a choice at the meat counter". Will cost drive some consumers to the less expensive plant-based protein options likes beans and lentils? Perhaps. We can be confident that few value shoppers will buy items like Beyond Meat plant-based hamburger which in 2020 cost more than twice the price of ground beef at a US grocery store (Piper 2020). As economies of scale are achieved in plant-based protein production, this dynamic will change significantly. After heavily funded companies earn back their pre-revenue outlay on R&D, marketing and lobbying and when more competitors enter the category prices will fall but how much remains to be seen. Consider that Impossible Foods spent $270,000 US (Barkho 2019) to lobby the government to gain regulatory approvals for its novel ingredients, and it's obvious that payback times will not be short.

But what about the premium consumer? That demographic is thriving; since lockdown and travel bans were imposed in March 2020, there is a tier of professionals who successfully transitioned to working from home at full salary. According to a Toronto Star story published in January 2021 (Hall 2021), "Canadians accumulated a pool of disposable income last year that is about four times larger than exists in normal times." The trend extends to other countries with good broad band

Case Study: Lessons Learned by a Plant-Based Start-Up

This interview records the sales and marketing learnings that the founders of Neophyto Foods, a science driven food company that strives to make it easy for consumers to eat delicious meals that don't harm the planet, learned in the first 2 years of running a plant-based protein business.

The Neophyto origin story began in 2018 when the two founders, then University of Guelph graduate students, entered a campus-wide contest to create a soy-based product. Their interest was fueled by a personal desire to make a great tasting alternative to dairy-based cheeses, something not available on the market at the time. Little did they know that this extracurricular activity would propel them on an entrepreneurial journey.

Buoyed by the successful reaction their fermented soy-based cheese entry gleaned during the contest, masters of food science student Jane Ong and masters of ecology student Kamil Chatila-Amos wondered whether they could sell such a product in grocery stores. They joined the University's incubation program which supports academics who want to test commercial options that put their knowledge into real world use. The program required extensive research and the founders gained understanding not just about the demand for their product but also the technical feasibility of scaling it to serve commercial demand. The duo analyzed their learnings and decided to pivot; Covid-19 had shuttered both their foodservice clients and their production facility. Likewise, the plant-based cheese space was becoming crowded and scaling this product would require extensive capital investment. They put their original cheese concept aside but didn't quit. Instead, they developed and launched a unique shelf stable plant-based ground meat alternative called Neokit which allows foodservice outlets and home cooks to swap out ground meat to make plant-based versions of their favourite recipes. The product took off quickly via ecommerce and today the company is testing retail and foodservice channels. I interviewed Jane and Kamil via video conference in May 2021 just as the Covid-19 vaccines were being rolled out; we discussed what they'd earned about the consumer drivers and barriers faced by plant-based protein businesses. Note: their answers have been combined to simplify the reading experience.

Q: Was all the time you spent working on your cheese product wasted?

A: We still hold out hope that we can commercialize our cheese in the future but even if that doesn't happen, developing that product was a good use of time. We honed our entrepreneurial skills and gained highly valuable transferable consumer insight.

Q: *Although your team has won prize money at numerous pitch competitions and has been awarded grant money to continue solving the complexities of the food science that allows you to create unique products, you have not raised venture capital to grow Neophyto. So, tell me, is it truly possible to be innovative in the plant-based space without spending millions of dollars on equipment, staff, and marketing?*

A: We can't compete with larger players when it comes to the amount of funding we have access to. It's very difficult for us to be innovative from a process standpoint because that requires large capital investment. However, there are other ways to drive innovation. It is only difficult if you approach the business the same way everyone else is approaching it. We can't compete with big players on their playing field. When our cheese product proved technically difficult and hard to scale with a traditional co-packer, we shifted to new ideas. Now our goal is to create our own category. We've turned our energy to filling gaps in the market where we can succeed using innovative thinking instead of pricey innovative technology.

Q: When the COVID-19 pandemic struck and it became obvious that your cheese product was not going to come to market unless you could travel and tour manufacturing facilities, why did you persevere and develop a plant-based meat alternative product?

A: The pandemic took away a lot of the things we enjoyed about the job – being in the lab perfecting our product, going places, and seeing people enjoy our food. It would have been easy to give up except that we had employees and we needed to take care of them. Our resiliencies are born of frustration; we felt like the whole world was against us, and we had to prove them wrong.

Q: Your current product is unlike any other plant-based meat alternate on the market in that it is dry and needs to be rehydrated to become a versatile substitute for ground meat. How have you educated consumers about this new product and made it a compelling choice for them?

A: Our strategy has been to use recipes and great food photography to drive online conversations and awareness of Neokit on social media and that has worked well for us. Although we feature many vegan recipes, we've consciously chosen to target omnivores who will still use cheese in their lasagna but skip the meat to make dinner a bit more healthful and sustainable.

The key to getting people to our e-commerce store is that we champion the simplicity of our product to position it against the heavily processed products that have been criticized in the news. We proudly call out that Neokit contains just 5 ingredients, contains 0 preservatives, and is made in Canada – three claims that bring online searches to our website often.

Online market development can be tricky though. Our messages seem to attract a hostile minority of carnivores who sometimes hijack our conversation threads and sideline our messaging efforts. What's interesting is that the way social media algorithms work ensures that people who engage with certain kinds of content get fed more of the same. So, the irony is that these vigilant defenders of meat who tell us they never want to see meatless products on their plates just get to see more plant-based advertising and content!

We cope with this problem by taking the high road and not engaging with them. Interestingly, our customers often call out the trolls and deal with them for us.

Q: When you developed your products, I recall that you had identified a younger millennial market – those in their late 20's and early 30's – as your target consumer. Have you been able to validate that assumption?

A: We were a little surprised to learn that our e-commerce demographic is primarily women over fifty. These mature women are looking for plant-based protein products they can use in their cooking. They often tell us that they want to make meals that please their husbands while also keeping them healthy. These families aren't vegetarian, and they often blend our plant-based ground protein with animal derived items like dairy products. The other consumer who has surprised us is dads looking for products they can use to cook for their vegan children. Neither of these personas were ones we thought we'd be speaking to in our marketing when we started this company. We've had to adjust our messaging accordingly.

Q: With so much competition from meat and other plant-based protein products, how hard is it to get an innovative plant-based protein product onto a grocer's shelf?

1. Selling at retail is hard! Getting into the store is not the most difficult part of the process; when buyers see that our product is unique and has online support and sales, they usually want to stock it. The real puzzle is merchandising – how do you put your unique product in the right part of the store in a package that provides all the right info to motivate a shopper to pick it up and put it in their cart? Stores are so crowded with products and visual distractions that without sampling programs it's really hard to educate consumers about new things. We find that tasting is believing so until in-store sampling is an option again, we plan to put most of our focus on e-commerce and foodservice channels. Our hope is that foodservice partners will introduce their patrons to our product so that when those people are in the grocery store they'll see it and be ready to buy it.

connectivity as well. Unlike the value consumer, this shopper is very interested in the story behind the meat products they purchase because they are not making an economic choice but an emotional one. Shoppers in this category want to avoid imported foods with opaque supply chains or that originate in countries they distrust. Many are also affected by news stories about local foods going unused or are motivated by an urge to support businesses struggling to stay in business amid COVID-19 restrictions. Farmers, butchers, and online companies report that affluent consumers are actively seeking out local meat and meat products and are willing to pay for delivery to obtain it, proving that access to products may be a key to ensuring demand.

The rapid growth of in plant-based restaurants in urban centres contributes to the impression that vegetarianism is growing like wildfire. According to vegetarian restaurant tracking website happycow.net (Happy Cow 2020), from 2007 until 2019 the number of vegan restaurants in Europe numbered 85. By 2014 that number had grown almost tenfold to 755. Vegetarian restaurants grew just as dramatically during that time; in 2001 there were 517 restaurants advertised as vegetarian and 3816 in 2019. Contributing volume to the trend were fashionable cities such as London and Berlin. But where things get interesting is the 5-year period between 2015 and 2019 when the number ballooned to 2662! Consumer cravings for variety and novel eating experiences are more likely to be the driver leading this food service segment's popularity, not because there are more vegetarians but because more consumers are interested in variety and the novel experience of trying something new. Often this urge to dine out on plants is buoyed by the healthy halo of the vegetarian lifestyle; however, the highly processed nature of many of the more innovative plant-based meat alternates such as the Beyond Burger led to a media backlash which made some consumers reevaluate their opinions.

4 Marketing and Product Development Opportunities

It's not enough to know that consumers are open and willing to try new products and that plant-based proteins are of particular interest to them as ways to support their values, maintain their health and add variety to their menus. For this category to truly flourish and go beyond a trend to become a longstanding category, product developers and marketers will need to deeply understand their consumers and not buy into assumptions that are based on opinions and short-term behaviour. To truly succeed they will need to have deep empathy for their customers (the retailers' distributors who buy these products to sell to others) as well as for their consumers (the end users – including foodservice operators) who will make a purchase choice. Customer insight is the easiest piece. Generally, retailers, restaurant owners and distributors take on new products for just two reasons: one to sell more to their existing customers; and two to attract new customers who are either unserved or going elsewhere to purchase the products that solve their food problems. Communicate how a product can do either of those two things for their business and you're likely to get a sale.

Consumers on the other hand are a trickier puzzle to solve. Not only do their needs change with life stage, economics, and other factors, but they often don't truly know how to articulate their problems. The famous industrialist Henry Ford put it well. If he had asked people what they wanted, they would have said faster horses. Instead, his key to success was understanding that people wanted to move quickly from place to place, have reliable transportation and convenience. He then turned to technology to apply his creativity to solving these problems with a solution that people found simplified their lives – cars only needed to be fed when they wanted to go someplace, unlike the horses who need to eat everyday regardless of whether a trip was planned. Then he used his creativity to envision new applications for this technology that would not only solve these consumer's problems but do so at a price they were willing to pay and that would make him a profit. This is the same problem that entrepreneurs and established food companies who want to capitalize on the plant-based protein opportunity face today. It is not enough to create a product that tastes good and delivers protein without using animal derived ingredients. These products must solve problems for three stakeholders: the business creating them must be able to sell them for a price that makes them money; the retailer needs to be able to at least make more money or ideally also steal market share from their competitors; and the consumer must see the product as a solution to a problem that they are willing to pay to fix. And ideally, the consumer must want to solve that problem often so that they buy the product repeatedly so that a cycle of benefit emerges.

Many of the new plant-based protein products emerging on the market have a communications challenge as well. As discussed above, Impossible Foods invested heavily in getting regulatory frameworks in place to launch their products. Although marketing and communications figures can't be easily pinpointed, it is safe to assume that they spent at least the same amount – and likely more- on communications. As we have learned from the world-wide debates on genetically modified foods, vaccines and many other topics, consumers are not sure who to trust for dependable scientific information. Any sliver of doubt about the claims offered by those bringing new science driven products to market can become polarized discussions. Often these conversations start in social media and then grow to become newspaper headlines, conference topics that zap resources from all parties. Examples abound. I'm reminded of the GMO debate that escalated in the early part of the century to the point of where anti-GMO activists were sabotaging crops and hiring lobbyists. Companies like Monsanto made a huge mistake in not communicating more about the history and science behind these optimized seed stocks when they launched. Then they perpetuated the problem by not facing the controversy head on. It took them years to reach out to Bill Nye, the famous television personality, and a vocal critic of the GMO movement. But finally in 2017, an opportunity for this trusted influencer to sit down with scientists and learn about the complexities of the topic was arranged and Bill Nye changed his mind about GMO's (Pommeroy 2016) and created a doorway in the conversation that relied more on science than on fear and confusion. Imagine if such a communications program had been created when GMO seed products were first launched?

More recently and in the plant-based protein market, Beyond Meat faced a similar problem when it launched into North American foodservice outlets in 2019. The product was positioned as a healthier alternative to meat which was somewhat true. Beyond Burgers do contain a lot of fibre, a nutrient often lacking in western diets, but they also contain a lot of sodium, and the ingredient list is long and complex. As a result, many consumers felt duped by the product and the company had to invest time and money in defending itself. A much better communications strategy would have been to position the product differently – why not call out the benefits of fibre it can offer that beef cannot? – and to explain the food science breakthroughs which allow the product deliver other benefits such as meaty taste and texture without the need to kill an animal. In other words, if they had spent some time trouble shooting what could go wrong as they launched this product and prepared their marketing to speak to the top of mind needs of their target market as well as to their less obvious secondary concerns, they may have had a smoother launch and garnered many more favourable headlines.

A classic innovation mistake in all food categories is to pin your sales strategy on launching cheaper versions of existing products instead of pinning your growth strategy to filling unmet consumer needs. Although short term gains can be made by launching the cheaper version of the original, invariably, retailers will play the two brands off one another to get the lowest prices they can, eliminating the margin for the manufacturers. Instead, innovation leads in plant-based companies should leverage their consumer insights, their company's technological assets and the talent of the product development team to make unique products that can get out in front of the competition.

Many new food manufacturers celebrate when they get a listing or join a distribution network; however, market penetration is just one step toward the final goal: subsequent orders. Regardless of how great a new product is and how well the team controls the conversation and answers consumer questions about the science and benefits of a new plant-based product, a sell through strategy that will get consumers to not just buy your product once but buy it often is essential to a successful product launch. Common ways to support achieving household penetration and volume sales abound and these strategies are well documented in many excellent marketing books. Such books expand on strategies such as the value of recipe programs that give consumers and foodservice operators more ways to use value-added products and the effectiveness of instore sampling to drive trial and volume sales for new products.

Anyone who takes the time to stroll the aisles in grocery store will observe that most of the current plant-based product offering is designed for everyday consumption and often packaged and marketed as an alternate for people within households who have particular dietary needs and preferences. In Western society we lack plant-based options designed for sharing and celebration. Feasts for holidays like New Year's, Christmas, Easter, and Thanksgiving are invariably associated with whole turkeys and geese, or larges ham, legs of lamb and beef roasts. As mentioned earlier in this chapter, these main course choices are deeply rooted in our history and harken back to much earlier rituals. But rituals must start somewhere, and I believe that there is an opportunity for plant-based innovators to be working on strategies that focus on a horizon where there is large format, inclusive plant-based protein options for celebrations that will be just as special as the meat options that currently trigger our holiday emotions.

We've talked a lot in this chapter about understanding consumers and communicating to them in a way that serves their needs and solves their problems. I'd be remiss if didn't point out that in most developed market economies regulations that are said to protect and serve consumers actually create a barrier for new plant-based innovations to easily communicate the value of their products to consumers (Buchwald 2019). Animal product commodity boards have had at least a century to work with government regulators to define terms like meat, milk, cheese, and eggs. As a result, products that consumers buy to do the dietary and culinary jobs of traditional animal derived products are cryptically labeled as 'soy beverage' or egg-substitute. This is not just a big challenge for plant-based product companies but also not ideal for consumers. They're left wondering if they can use 'soy beverage' on a bowl of cereal or to make pancakes or if it is just a drink. These rules are particularly complicated to navigate for plant-based product companies who sell products in many countries as they rule, and wording allowed on packages will vary from place to place. There is much debate about the right way to move forward. Scientists often focus on technical definitions such as the fact that milk is produced by a mammary gland while marketers focus on the consumer and what will make their purchase decision simpler. The solution likely falls somewhere in the middle where the nutrient content of these items is compared and foods – regardless of their relationship to animals – that provide the similar nutrients and applications get named accordingly. However, this situation seems like a very far off reality as this text is being written in 2021.

5 What Factors Will Limit the Adoption of a Plant Based Lifestyle?

Regardless how quickly scientists advance the technical quality of plant-based proteins and no matter how diligent marketers are in understanding the consumer, their customer and the competition, plant-based proteins will still face challenges as they strive to become a mainstream.

Societal doubt and fears about the intentions and practices of processed foods will be a problem especially as competitive products and fear mongers intentionally fuel their self-interests by spreading disinformation and start conversations that create fear and distrust. Cost of production infrastructure will also limit some plant-based food manufacturers from achieving scale. Unless these companies have the capital to invest in specialized equipment, they are unlikely to find manufacturing partners who have the equipment and facilities required to make new products that rely in innovative technology. And, given the margins in the grocery and foodservice sectors, they are unlikely to earn back their investments very quickly; therefore, it will only be the well-financed companies who can handle a very long return on their investment who will achieve scale in this space.

As discussed above, as consumers become more accustomed to buying plant-based products and start to treat them as dietary staples, they will delve more deeply into the nutritional value of plant-based meat and dairy alternates and expect them to offer the same nutritional compositions as their meat-based counterparts. In other words, if parents don't believe that a glass of almond milk will nourish their children equivalently to a glass a of cow's milk, they will continue to look for new solutions. Likewise, culinary functionality will be key to making plant-based beverages, cheeses and meats normal pantry items. Non-vegan home cooks want plant-based ingredients to come as close to recreating their favourite recipes with plant-based proteins as possible. So, plant-based milk and cheeses that can be used to make macaroni and cheese just like grandma used to make will be around in 20 years while those that don't will likely disappear from the marketplace.

Finally, to truly capture the heart and wallet of Generation Z, plant-based food products will have to deliver on sustainability. This will require food companies to scrutinize their supply chains, simplify their logistics and be willing to show consumers what their products are made from, how they are made and delivered to store shelves. Companies that succeed will be able to leverage other trends to tell this story. For instance, is there a local story to tell? Are your products made from a low number of ingredients? Was the labour working along the production value chain treated equitably? All these supporting marketing messages will divide the winners from the losers in the plant-based sector.

6 The Future

What will someone writing a chapter of this book in 50-years' time say about the history of plant-based protein products? Will they have mainstreamed to be globally popular products? Will animal-based proteins be relegated to special occasion food only available to the rich? One could make a case for almost any of these scenarios as well as many more. What we do know is that the developing economies of China and India will wield tremendous buying power as their populations and prosperity grows. We also know that many citizens in these countries equate success with the ability to emulate the lifestyles of Western cultures. With celebration and status tightly bound up in Western and European thinking about meat, it isn't surprising that animal protein consumption has become an important emotional purchase and choice driver in the developing world where it is common to equate economic success with the ability to emulate North American and European lifestyle practices. It's interesting that as plant-based protein consumption gains support in developed Western and European cultures, it is losing ground in these other markets. China is currently the world's largest consumer of meat; in fact, their consumption of pork (Low 2020) alone is predicted to be more than double that for all European Union Countries combined in 2020 and the republic's demand for beef and chicken is expected to be higher than for any other country apart from the USA. That said, per capita consumption of meat products in China is still far behind that of developed markets. The annual average per capita consumption of beef in the U.S. is 26.3 kg, compared with just 4.1 kg in China. But, since Asian cuisine has popularity that reaches far beyond its own borders, savvy food companies are increasing efforts to create products that will satisfy not only Asian consumers but also others who reside in the West and love Asian flavours (Watson 2021).

Despite the aspirations of China's middle class to emulate Western prosperity, the plant-based protein market is also very important in that economy. The country's "free from meat" market, which includes plant-based meat replacement products, has grown 33.5% since 2014 to be worth $9.7 billion in 2018, according to

Euromonitor. It predicts that the industry will be worth $11.9 billion by 2023 (Low 2020).

Only time will tell how this category develops, but if food science (Lamas 2021) continues to advance at its current pace, it is highly probable that plant-based proteins will be able to satisfy consumer demand for taste, price and sustainability to become dietary staples.

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Chapter 18
Challenges in Promoting Plant Protein Food Consumption

Roya Daneshmand

1 Introduction

Red and processed meat are the main sources of proteins in western countries. Healthy lifestyle including healthy diet quality is associated with lower risk of cardiovascular disease (CVD) and cardiometabolic risks (Atkins et al. 2014). Among different food components, red and processed meat are associated with higher risks of ischemic heart disease, cardiovascular disease, cardiometabolic risks, and cancer (Key et al. 2019; van der Spiegel et al. 2013).

During recent years, meat analogues as well as a flexitarian diet have become an interesting topic for researchers. Although the nutrition guidelines such as Canada's Food Guide (Health Canada 2019) emphasizes on reducing the animal based protein and increasing the plant based protein, the available data indicated that the plant based protein consumption is lower than the recommended levels (Mudryj et al. 2012).

Plant based protein has positive effects on human health through ingredients such as high soluble fiber content, polyunsaturated fatty acids, sterols, isoflavonoids, and reduced saturated fatty acids content. Therefore, plant based proteins are one of the essential food groups in many recommended healthy diets in reducing risks of CVD and CVD risk factors such as high blood pressure (Tielemans et al. 2014). The results of previous study indicated that by replacing animal based protein with plant based protein, there is a significant reduction in fasting blood sugar, fasting blood insulin level, hemoglobin A1c, and overall this improves glycemic control in patients with diabetes (Viguiliouk et al. 2015). The studies also pointed to the other beneficial effects of plant based protein such as soy proteins consumption

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on total serum cholesterol and low-density lipoprotein cholesterol (LDL) level due to their high isoflavonoids content (Hermansen et al. 2003). Soy protein consumption also has an improving effect on serum high-density lipoproteins cholesterol (HDL), decreasing serum triglycerides, and improving HDL/LDL-cholesterol ratio compared to the animal based protein consumption (Hermansen et al. 2003).

Healthy diet including replacing animal-based protein with plant-based protein not only decreases the risk of morbidity and mortality, but also decreases the healthcare cost. In 2017, a Canadian cost of illness analysis indicated that regular consumption of legumes by including 100 g legumes per day in diet in 50% of the population and following the low glycemic index diet or high fibre diet would decrease the CVD and type 2 diabetes related healthcare cost (Abdullah et al. 2017). The total savings in annual health related costs of type 2 diabetes and CVD is reported between Can$6.2 (95% CI $2.6–$9.9) to Can$62.4 (95% CI $26–$98.8) and Can$31.6 (95% CI $11.1–$52) to Can$315.5 (95% CI $110.6–$520.4) million respectively (Abdullah et al. 2017).

Although recommendations and guidelines emphasize on reducing animal-based protein consumption and increasing plant-based protein intake, and while health benefit of plant based protein especially on chronic disease is well documented, the consumption is still below the recommended levels, especially in the Western countries (Lang et al. 2003; Stables et al. 2002).

2 General Barriers in Changing Eating Habits

Changing in the diet is one of the cornerstones in the prevention and treatment of chronic diseases, but changing in eating habits is challenging. Changing food habits is complex and is affected by multi-component factors including previous exposure experiences, acceptability of the product appearance, taste and texture, willingness to try new food, food literacy (e.g., preparation and cooking skills), and other interpersonal and intrapersonal determinants.

Few available frameworks with a list of underlying determinants associated with individual food choice exist. The Determinants of Nutrition and Eating (DONE) framework (Stok et al. 2017) is one of the most comprehensive frameworks with an interdisciplinary overview of determinants associated with the food choices. The determinants are divided into individual, interpersonal, environmental, and policy categories (Stok et al. 2017). Among individual determinants, demographic characteristics (e.g., socioeconomic status, age, gender, food knowledge and skills) and psychological determinants (e.g., mood, emotions, and attitudes) could have an effect on an individual's eating pattern. Social and cultural determinants such as social and peer influence, family food culture and family food habits are considered as interpersonal factors with possible effect on eating behaviour. Food availability and accessibility and eating environment can also affect the eating behaviour (Stok et al. 2017).

Furthermore, personal motives are effective in an individual's food choices as well. A study assessed the association between personal motives and food choices using Eating Motivation Survey indicated that 15 motives potentially impacts individual's food choice including liking the food, visual appeal, pleasure, affect regulation, need/hunger, sociability, social norms, social image, weight control, health, price, convenience, habits, traditional eating, and concern for nature/ethical aspects (Renner et al. 2012).

3 Barriers in Plant Based Protein Consumption

So far, little is known about determinants and potential barriers associated with plant-based protein consumption particularly in regions where plant-based protein sources are not part of the food culture. To facilitate the transition to more plant-based protein intake and less animal-based protein, researchers need to seek the motivations and determine potential barriers associated with low plant-based protein consumption. This provides more insight into potential effective interventions to promote plant-based protein intake.

3.1 Food Literacy

Food literacy, which encompasses the knowledge and skills to choose, prepare, and cook healthy food items, has an important effect on plant based protein consumption. Among few studies which focused on determining barriers in pulse (dried beans, peas, lentils) consumption, a marketing study in Canada in 2010 with 1100 participants aged 18 and older addressed a list of the barriers and facilitators in pulse consumption (Ipsos-Reid 2010). The study, which was based on online questionnaires and 230 interviews with participants who were living in Canada for 20 years or more in the format of focus groups, suggested that lack of cooking/preparation skills was one of the most important barriers of not eating pulses (Ipsos-Reid 2010).

A result of another study which was conducted in IOWA in low income women, pointed to the lack of knowledge about the healthy effect of dried beans on the human body as one of the main barriers in pulse consumption (Palmer et al. 2018). The result of a cross sectional survey in Australian adults, which was conducted with the aim of collecting relevant data for future marketing campaigns and consumers guidelines, pointed to the lack of cooking skills and time restrictions associated with cooking legumes as the two important barriers in legume consumption (Figueira et al. 2019). Another study in Australia that focused on the barriers and benefits associated with plant based food, pointed to lack of cooking skill as the most relevant barrier associated with legumes consumption (Lea et al. 2005).

3.2 Sensory Appealing

Recently many companies joined the meat alternative movement and produced plant-based protein products with sensory attributes similar to meat (Fiorentini et al. 2020). However, complete meat replacement with plant-based protein while keeping the same sensory attribute is challenging. Flexitarian, which refers to partially replacing meat with plant based protein while maintaining the sensory appeal, can be a practical solution to improve in plant based protein consumption and a strategy to reduce meat consumption (Dagevos 2021). According to the US survey 89% of the participants chose taste as the most important determinant in intention to buy meat alternative products (IFIC 2019). Shape, color, and appearance have a greater effect on meat alternative acceptance compared to the texture and taste (Elzerman 2011). Results of the survey from 1039 German participants indicated that in order to increase meat alternatives consumption, their taste, texture, and ease of preparation should be comparable to meat (Michel et al. 2021). Therefore, to increase the consumer's acceptability, it is important to develop meat alternative products with similar taste, texture, juiciness, smell, and appearance to the meat (Szejda and Parry 2020). Also providing meat alternatives in the dish format that is usually consumed by consumers such as rice, soup, vegetables as well as using familiar seasoning and sauce have an important effect on their acceptability (Fiorentini et al. 2020). Consumers have a greater interest in trying food with familiar shape, taste, appearance, and preparation method than the novel ones (Szejda and Parry 2020). This is particularly important in the consumers with food neophobia, a psychological determinant in food choice which acts as a barrier in trying new food.

3.3 Food Neophobia

Food neophobia, which is described as a fear to try unfamiliar food items, is another potential barrier in plant based protein consumption especially in those with lack of meat alternative exposure due to their food culture and food habits (de Koning et al. 2020). A study conducted in China, USA, France, UK, New Zealand, Netherlands, Brazil, Spain, and the Dominican Republic to assess the consumers' attitudes around meat-alternatives including plant based protein and their willingness to try, buy, and pay for meat alternative proteins (de Koning et al. 2020). The results of the study based on 3091 responses indicated that food neophobia is the main barrier towards consumers' intentional behaviour towards meat alternatives (de Koning et al. 2020). Conversely, the increased knowledge about benefits of the meat alternatives as a result of increasing knowledge on nutritional benefits, health benefits, environmental effect, and the sensory attributes, had an improving effect on consumer's willingness to try, buy, and pay for meat alternatives (de Koning et al. 2020). In this study the expectation of the nutrition value of animal based protein was the strongest barrier in intention to try, buy and pay more for plant based protein (de Koning et al. 2020).

3.4 Meal Context

Meal context appropriateness refers to the effect of social and cultural factors in consumers' purchase decisions (Szejda and Parry 2020). The results of a German survey based on 1039 participants highlighted the effect of eating situations on the meat alternative acceptance. Using meat alternatives was accepted while eating alone; however, the acceptance was low when having meals with other family members, at a restaurant, for a business meal, or in a social gathering (Michel et al. 2021). These findings were in accordance with the result of previous studies which highlighted the sociability (Renner et al. 2012) and effect and pressure of the peers (Higgs 2015; Renner et al. 2012) as the two motive determinants in an individual's food choice. A study conducted in Canada in 2015 to determine the facilitators and barriers in lentils consumption pointed to both lack of knowledge and the unfavorable attitudes of other family members towards lentils consumption as the two outstanding barriers in lentil consumption among non-consumers (Phillips et al. 2015). The results of the Beans, Lentils, Peas Survey which was implemented in the United States, pointed to different barriers in beans, peas and lentils consumption including the effect of other family members' food preferences (Perera et al. 2020).

3.5 Price Adjustments

Price is a key element in an individual's purchasing decision. Providing the plant based protein with a similar price or less expensive than their counterparts is another potential effective strategy in promoting plant based protein consumption (Szejda and Parry 2020). Results of the survey from 1039 German participants indicated that in order to increase meat alternatives consumption they need to be offered at the competitive price to their counterparts (Michel et al. 2021). Providing incentives such as coupons was suggested as an effective tool to encourage consumers to try meat alternatives and to become regular consumers eventually (Szejda and Parry 2020).

3.6 Convenient to Prepare and Cook

Questions are still abounding about what strategies are needed to increase plant-based protein consumption. One of the potential barriers in plant-based protein intake is being not convenient to prepare and cook compared to their counterparts

(Szejda and Parry 2020). Providing new products which are easy and quick to prepare and cook can improve the consumers' intention to buy plant based proteins. Increasing the availability at the point of purchase by distributing the convenient, healthy food items with plant based proteins in popular grocery stores, placing them in highly visible, high traffic areas of the store are important strategies in promoting plant based protein consumption (Szejda and Parry 2020). Including simple, easy, quick, and familiar recipes on product packages is another useful strategy in promoting meat alternative intake (Szejda and Parry 2020).

3.7 Other Important Barriers

Other barriers associated with low plant based protein consumption are environmental factors, such as low accessibility (Ipsos-Reid 2010), digestive issues and feeling gassiness (Perera et al. 2020), and time constraints (Lea et al. 2005; Phillips et al. 2015).

4 Conclusion

Although available recommendations and guidelines emphasize on increasing plant based food and reducing meat consumption, overall consumption is still low especially in countries where plant based food is not a part of cultural food habits. Some of the important barriers in increasing plant based protein consumption are at the individual level such as lack of food literacy, lack of time to prepare and cook, and not willing to try new food items. Whereas some of the available barriers are environmental barriers including being costly, not easily accessible, lack of sensory appeal, and not being accepted as an appropriate food item by peers and other family members.

Making healthy changes in an individual's eating habit is not easy. There are different factors which affect an individual's food choices. Therefore, understanding these determinants can help to recognize potential barriers and possible strategies. Although some studies focused on potential barriers in plant based protein consumption, there is a need for research to address some practical strategies to translate available knowledge about potential barriers and facilitators into practice. These strategies can be addressed by including suggestions and recommendations from not only consumers but also different related stakeholders and experts including but not limited to health sectors, retailers, food marketing, nutrition science, food science and food technology, and product development.

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