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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A. J. Hernández-Álvarez
School of Food Science and Nutrition, Nutritional Science and Epidemiology Research Group, University of Leeds, Leeds, UK
e-mail: A.J.HernandezAlvarez@leeds.ac.uk

M. G. Nosworthy
Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada
e-mail: matthew.nosworthy@agr.gc.ca

M. Mondor (✉)
St-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada
e-mail: Martin.mondor@agr.gc.ca

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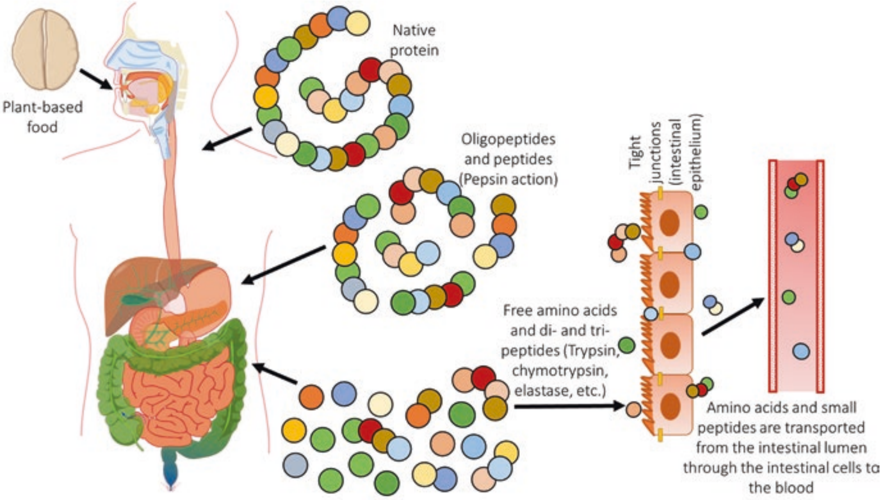

Fig. 12.1 Protein digestion and absorption

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 $\mathrm{PER}_{\mathrm{Experimental}}$ by the $\mathrm{PER}_{\mathrm{Casein}}$ 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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