Chapter 9
Hemp Seed as a Source of Food Proteins

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Abstract The galloping population growth is leading to a significant increase in the protein demand. Conventional protein sources, particularly those of animal origin, will no longer be sufficient to meet this demand. The use of plant proteins, less costly in terms of resources and with a much lower environmental impact, is an interesting alternative to meet future societal and environmental challenges. Hemp seed is an undervalued co-product resulting from the cultivation of industrial hemp. This plant resource has significant contents in protein and oil of nutritional value, about 26% and 36% respectively, that may help to meet the challenges of sustainable food. The specific valorization of hemp proteins for human consumption is a recent issue. Through this chapter, we showed the current state of knowledge on hemp proteins in terms of composition, nutritional aspect, extraction, and physicochemical, functional and biological properties. Different extraction routes have been proposed to recover the main hemp protein fractions from oil press cakes in general. Extraction yields generally vary from 34% to 51%, and the protein purity of the resulting protein isolates from 87% to 94%. The proteins, usually extractible from hemp meal, belong to the globulin and albumin families. Most of hemp protein isolates contain predominantly globulins, mainly the 11S edestins. The hemp proteins have an interesting essential amino acid profile and have a high digestibility of about 90%. Many authors have highlighted that hemp protein hydrolysates possess a wide range of health biological activities such as antioxidant properties, metal chelation, antihypertensive, hypoglycemic properties... Hemp proteins also have techno-functional properties such as gelling, emulsifying and foaming properties adapted to the development of new plant-based foods. Different treatments of hemp proteins can improve their functional properties, such as enzymatic and chemical modifications or pH- and heat-induced denaturation. Despite limited solubility, hemp protein ingredients represent an alternative to current cereal and legume protein materials in human diet in the future.

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Abbreviations

HEC Hemp Enzyme Concentrate, hemp protein concentrate obtained by enzyme-assisted extraction
HMI Hemp Micellized Isolate, hemp protein isolate obtained by micellization
HPH Hemp Protein Hydrolysate
HPI Hemp Precipitated Isolate, hemp protein isolate obtained by globulin isoelectric point precipitation
SPI Soy Precipitated Isolate, soy protein isolate obtained by globulin isoelectric point precipitation

9.1 Introduction

According to the forecasts of the Food and Agriculture Organization of the United Nations (FAO), the world's population is expected to reach 9.1 billion by 2050, requiring a 70% increase in world food production. In 2017, 11% of the world's population was undernourished and 13% of adults were obese (FAO 2018). Feeding the planet by fighting against global warming are the major issues that will have to be faced, especially as global warming is leading to a decrease in crop yields each year (FAO). Ovovegetarian food is known to require less energy, land and water resources than meat food (Pimentel and Pimentel 2003). The consumption of more plant proteins is a solution to face these problems. Global protein demand was expected to increase by 40% between 2010 and 2030. This increase was expected to be 33% and 43% for animal and plant protein demands respectively (Ozanne 2014).

Cereals, legumes and oilseeds are the main sources of plant proteins. It is estimated that only 35% of the total proteins produced by the agricultural sector is used for human consumption. The rest is used for livestock feed, non-food applications, or simply treated as a waste. Soy proteins or corn and wheat flours, are already widely used. In contrast, "conventional" sources of protein should no longer be sufficient to meet the global demand for protein in the coming years. Finding alternative sources of protein is strongly recommended (Sari et al. 2015).

Hemp is an annual herbaceous plant, highly harvested worldwide, mainly for its fibers in stems (Ash 1948). Asia is the biggest producer of hemp, and cultivates nearly 75% of the world's hemp. In Asia, production is mainly shared between China, which grows 50% of the world's hemp, and North Korea. Europe is the second largest hemp growing continent. In 2004, it produced 14% of the world's hemp, but it has seen a renewed interest in hemp cultivation since 2011. The area under cultivation has increased from 8,000 ha in 2011 to 33,000 ha in 2016. France is the European leader and produces nearly half of European production, placing it in

third position worldwide behind North Korea (Bouloc 2006; Carus et al. 2013; Amaducci et al. 2015).

Hemp is an ecologically and economically interesting plant for many reasons. It has a strong and rapid growth since it is harvested 4 months after seeding and produces a significant amount of biomass (Ash 1948). It does not need phytosanitary products because it has the ability to eradicate weeds, and is not affected by any pests or diseases. It is one of the few agricultural products to be grown without phytosanitary products in non-organic farms (Carus et al. 2013). It is used in rotation culture and increases the yield of the next culture by 10–15%. It has the ability to remove large quantities of heavy metals from the soil, and its long root system helps to prevent soil erosion. It also has low water and input requirements (Ranalli and Venturi 2004).

One hectare of hemp produces about 5 tons of straw and 1 ton of seed. All parts of hemp are recoverable. Fibers which represent about 30% of the straw are mainly used for pulp and paper, as well as for insulation. The woody core of the stems, that account for about 50% of the straw is mainly used as horse bedding or as garden mulch. The dusts, corresponding to about 15% of the straw are pelletized for incineration or used as compost. Flowers and leaves can also be used in pharmaceuticals or food supplements. Seeds are still undervalued, since they are mainly used for livestock feed, or consumed as such as human food (Carus et al. 2013). The large quantities of oils and proteins, of nutritional interests, contained in hemp seeds, are not yet valued at their true value in human food.

The purpose of this chapter is to synthesize scientific knowledge on hemp proteins in terms of composition, nutritional aspect, extraction, and physicochemical, functional and biological properties.

9.2 Hemp Seed Composition

The composition of hemp seed and its derivatives is reported Table 9.1 (Callaway 2004; Tang et al. 2006; House et al. 2010; Kim and Lee 2011b; Aluko 2017; Sarv 2017; Mattila et al. 2018; Balentić et al. 2019; Mamone et al. 2019). Hemp seeds have high contents in protein and fat. Whole seeds consist of approximately 26.2% protein and 36% lipid (all percentages in w/w dry basis). Hulled seeds have around 35.7% and 41.7% contents for proteins and lipids respectively. The seed hull contains about 69% carbohydrates, richer than the whole seeds with about 32% content, which are themselves richer in carbohydrates than the hulled seeds with about 16% content. Hemp carbohydrates are mostly fibers. Fibers account for about 93.2% of carbohydrates for the various hemp seed derivatives. Hemp seeds are usually cold-pressed to extract oil. The residual cakes or meals thus obtained have only around 11% lipids. The proteins are then concentrated at ~36%. Further delipidation by using organic solvent can be applied to the meal to remove almost all the lipids, increasing the protein content to ~43%. The minerals are mostly present in the seed pulp. The ash content of shelled seeds is indeed 6.7% against

Table 9.1 Composition of hemp seed and derived products

	Whole seeds				Hulled seeds		Seed hulls		Cold pressed hemp meal				Delipidated hemp meal			
g/100 g wet basis	Mean	±	SD	n	Mean	n	Mean	n	Mean	±	SD	n	Mean	±	SD	n
Proteins	24.5	±	0.4	5	35.9	1	12.7	1	33.4	±	5.2	4	39.2	±	9.7	3
					32.0	1	13.1	1								
Lipids	33.7	±	4.3	5	46.7	1	10.3	1	10.6	±	0.4	4	1.0			1
					32.5	1	11.5	1								
Moisture	6.4	±	0.4	4	4.9	1	5.1	1	6.4	±	1.7	4	8.6	±	1.6	3
							8.0	1								
Ashes	5.1	±	0.4	3	6.4	1	3.9	1	6.7	±	0.4	4	9.7			1
							3.2	1					3.2			1
Carbohydrates	27.6			1	15.1	1	64.1	1	45.3	±	3.5	3	57.3			1
	32.1			1												
Total fibers	21.6			1					42.6			1	45.4			1
	31.5			1					49.2			1				
Energy (kJ/100 g)	2248.9	±	133.5	3	2770.0	1	2020.0	1	2040.0			1				
							1738.8	1	1700.0			1				
References	Callaway (2004), House et al. (2010), Aluko (2017), (Sarv 2017), and Mattila et al. (2018)				House et al. (2010) and Kim and Lee (2011b)		House et al. (2010) and Mattila et al. (2018)		Callaway (2004), House et al. (2010), and Mamone et al. (2019)				Tang et al. (2006), Sarv (2017), and Balentić et al. (2019)			

SD: standard deviation, n: number of references

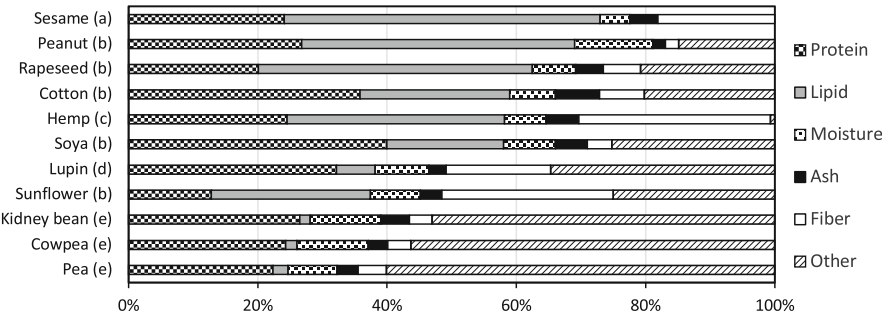

Fig. 9.1 Composition of different oleaginous and proteaginous seeds. a: Elleuch et al. (2007). b: Rodrigues et al. (2012). c: Callaway (2004), House et al. (2010), Aluko (2017), Sarv (2017), Mattila et al. (2018). d: Erbaş et al. (2005). e: Khattab et al. (2009)

3.8% for the seed hull. The energy value of hemp seed products ranges from 1997.9 kJ/100 g for meal to 2912.7 kJ/100 g for hulled seed.

The comparison of hemp seeds with other oilseeds and protein crops is presented Fig. 9.1 in terms of composition (Callaway 2004; Erbaş et al. 2005; Elleuch et al. 2007; Khattab et al. 2009; House et al. 2010; Rodrigues et al. 2012; Aluko 2017; Sarv 2017; Mattila et al. 2018). Hemp seeds have protein content in the range of other seeds and pulses. Their composition is similar to that of peas, cowpeas, red beans, peanuts, or sesame seeds. Their protein content is significantly higher compared to sunflower seeds or canola seeds, but still less rich than for their soybean, cotton and lupine counterparts. The amount of lipids contained in hemp seeds is higher than the average of seeds. Thus, hemp seeds contain more fat than peas, cowpeas, red beans, lupine seeds, sunflower seeds, soya beans and cotton seeds. They contain less fat than rapeseeds, sesame seeds and peanuts. Regarding protein and lipid contents, hemp seeds are then intermediate between oilseeds and proteaginous pulses.

9.3 Hemp Proteins

Hemp seed proteins are predominantly composed of about 65–80% of globulins. The remaining fraction corresponds to the albumins, amounting to about 20–30% (Tang et al. 2006; Park et al. 2012; Aluko 2017; Ponzoni et al. 2018).

9.3.1 Globulins

Hemp globulins are represented predominantly by 11S edestins representing about 65% of total proteins and 93% of globulins, and in a minority by 7S globulins

accounting for about 5% of total proteins and 7% of globulins (Osburn 1992; Tang et al. 2006; Ponzoni et al. 2018).

9.3.1.1 Edestin (11S)

Edestin is a homohexamer, like most 11S globulins, of about 320 kDa (Patel et al. 1994; Docimo et al. 2014; Aluko 2017). Each monomer of approximately 54 kDa is composed of an acid α subunit of ~34 kDa and a more heterogeneous basic β subunit, generally forming 2 characteristic bands at ~18 and ~21 kDa upon gel electrophoresis (Tang et al. 2009; Kim and Lee 2011a; Pihlanto et al. 2017). The acid α and basic β subunits are linked together by a disulfide bridge. No disulfide bridge is involved between the monomers, and hydrophobic bonds seem to be preferentially at the origin of the hexameric structure (Tang et al. 2006; Wang et al. 2008, 2018; Kim and Lee 2011b; Malomo and Aluko 2015a; Hadnađev et al. 2018; Mamone et al. 2019). Kim and Lee (2011b) detected edestin bands by gel electrophoresis after periodic acid-Schiff staining, suggesting that edestin could contain carbohydrate parts in its structure.

Three types of edestin have been identified: CsEde1, CsEde2 and CsEde3 (Docimo et al. 2014; Ponzoni et al. 2018). Each type is itself composed of two isoforms. There are then 6 genes encoding edestin. The 2 genes coding for type 1 edestin, CdEde1A and CdEde1B, and the 2 genes coding for type 3 edestin, CdEde3A and CdEde3B, are carried by a common DNA fragment of 16,071 bp while the 2 genes coding for type 2 edestin, CdEde2A and CdEde2B, are carried by another fragment of 8,232 bp. An edestin gene, composed of 4 exons and 3 introns, codes jointly for the acid α and basic β subunits. A cleavage site is present between these 2 sequences to form the acid α and basic β subunits after post-translational modifications. The edestin forms coded by the 6 genes have a common N-terminal sequence of 23 amino acids and contain 4 cysteine residues; 3 of these residues locate on the acid α subunit and the last one on the basic β subunit. These cysteine residues allow the inter-chain disulfide bond between the α and β subunits and an intra-chain disulfide bond within the acid α subunit.

9.3.1.2 7S Globulin

7S globulin consists of a subunit of about 48 kDa (Tang et al. 2006, 2009; Ponzoni et al. 2018; Mamone et al. 2019). A single gene encoding 7S globulin has been identified on a 1893 bp DNA fragment. This gene has 5 exons and 4 introns and encodes a 53.5 kDa precursor polypeptide. The 7S polypeptide has a N-terminal sequence of 21 amino acids, 4 N-glycosylation sites and contains 3 cysteine residues. Wang et al. (2008) observed that the 48 kDa subunit of 7S globulin appeared to be partially associated with the β basic subunit of edestin through disulphide bond.

9.3.2 Albumins

According to Malomo and Aluko (2015a), the albumins consist mainly of 7 polypeptides of 6–35 kDa, unaffected by the addition of reducing agent, thus meaning no inter-chain disulfide bond is formed in their structure. Odani and Odani (1998) and Ponzoni et al. (2018) have identified specifically a 2S albumin of 10 kDa. It consists of 2 subunits of 7 and 3 kDa respectively, linked by 2 disulfide bridges in contradiction with the previous authors. Two genes encode this protein: Cs2S-1 and Cs2S-2. These 2 genes are present on the same 13,738 bp DNA fragment, and encode the same 142 amino acid precursor polypeptide. The 2 subunits are coded by the same gene. The precursor polypeptide contains a N-terminal sequence of 23 amino acids and a cleavage site, which leads to the dissociation of the 2 subunits by post-translational modifications. Albumin 2S is very rich in sulfur amino acids representing 18% of the total amino acids of this protein. The small subunit contains 2 cysteine and 3 methionine residues, while the large subunit contains 6 cysteine and 5 methionine residues. The largest subunit forms 2 intra-chain disulfide bonds.

9.3.3 Amino Acid Composition and Nutritional Value

9.3.3.1 Amino Acids

The amino acid composition of hemp seed and its derivatives is presented Table 9.2. As usual presentation, sufficient and limiting/deficient amounts of amino acids have been established based on the recommendations of the FAO/World Health Organization and United Nations University (FAO/WHO and UNU 2007) for feeding children aged from 1 to 2 years or for adults over 18 years old. Hemp proteins from whole and hulled seeds, seed hull, press-cake and hemp protein isolates, contain all essential amino acids in sufficient quantities for adult nutrition, except lysine, which is the only limiting amino acid. According to Malomo and Aluko (2015a), only the albumins contain lysine in sufficient quantity, but they appear limiting in isoleucine, leucine, tryptophan and in aromatic amino acids (Phenylalanine + Tyrosine) for both adult and children nutrition, and in valine only for children nutrition. These authors also determined the amount of amino acids in hemp globulin and albumin isolates. According to the combined amino acid data for albumins and globulins, total hemp proteins should be limiting in isoleucine, leucine, tryptophan and valine, which is not the case according to all other authors. The amino acid composition of hemp protein isolates resulting from selective isoelectric point precipitation (HPI) is representative of globulins (Tang et al. 2006; Girgih et al. 2010, 2013a; Malomo and Aluko 2015b; Ren et al. 2016; Hadnađev et al. 2018; Wang et al. 2018). Tryptophan is present in insufficient amount in the seed hull proteins for children nutrition. On the other hand, it is sufficient for adult nutrition (House et al. 2010; Mattila et al. 2018). Finally, the 7S globulin fraction and the 11S globulin fraction have rather similar amino acid compositions. However, the 7S

Table 9.2 Amino acid composition of proteins from hemp seed and derived products

g/100 g of tot AA	Whole seed				Hulled seed				Hull				Hemp presscake				HPI				HMI	Glb	Alb	7S	11S	FAO recommandations	
																										1–2 Y/O	>18 Y/O
AA	Value	±	SD	n	Value	±	SD	n	Value	±	SD	n	Value	±	SD	n	Value	±	SD	n	Value	Value	Value	Value	Value	Value	
Alanine	4.7	±	0.8	3	4.6	±	0.3	2	4.7	±	0.1	2	4.4	±	0.2	2	4.4	±	0.3	8	4.5	2.8	4.1	5.1	4.9		
Arginine	11.9	±	1.6	3	12.6	±	0.8	2	10.3	±	1.2	2	12.4	±	2.8	2	12.9	±	2.0	8	10.9	16.1	13.4	9.2	10.7		
Asx	11.1	±	1.1	3	10.9	±	0.5	2	10.8	±	0.2	2	10.7	±	1.4	2	11.0	±	0.8	8	11.2	9.5	8.3	10.6	10.1		
Cysteine	1.7	±	0.2	3	1.1	±	1.1	2	1.9	±	0.3	2	1.8	±	0.2	2	0.9	±	0.6	8	1.6	3.3	3.4	0.2	0.3		
Glycine	4.8	±	0.3	3	4.7	±	0.1	2	4.9	±	0.0	2	4.8	±	0.5	2	4.2	±	0.2	8	4.1	4.1	8.7	4.6	4.4		
Glx	17.9	±	1.9	3	19.8	±	2.6	2	14.5	±	0.5	2	18.1	±	2.8	2	19.5	±	2.2	8	19.1	21.5	21.4	19.1	19.1		
Histidine	2.7	±	0.4	3	2.9	±	0.2	2	2.6	±	0.5	2	3.0	±	0.7	2	2.9	±	0.2	8	3.1	3.9	3.9	3.8	3.8	1.8	1.5
Isoleucine	3.9	±	0.4	3	3.9	±	0.2	2	4.3	±	0.4	2	3.9	±	0.0	2	4.0	±	0.3	8	5.1	2.9	2.1	3.7	3.8	3.1	3.0
Leucine	6.9	±	0.6	3	6.6	±	0.6	2	7.7	±	1.0	2	6.9	±	0.8	2	6.8	±	0.6	8	7.1	5.6	4.2	7.4	7.4	6.3	5.9
Lysine	4.0	±	0.5	3	3.6	±	0.0	2	3.9	±	0.0	2	3.9	±	0.5	2	3.5	±	0.6	8	3.1	3.7	7.7	4.0	3.8	5.2	4.5
Methionine	2.5	±	0.1	3	2.2	±	0.8	2	2.4	±	0.4	2	2.4	±	0.1	2	1.8	±	0.4	8	2.3	4.1	1.8	1.8	2.7		
Phenylalanine	4.7	±	0.4	3	4.4	±	0.4	2	5.7	±	0.8	2	4.7	±	0.5	2	4.6	±	0.4	8	4.6	3.3	1.4	4.9	4.6		
Proline	4.3	±	0.6	3	4.5	±	0.3	2	6.5	±	2.4	2	4.5	±	0.4	2	4.2	±	0.5	8	4.1	3.9	4.0	5.3	4.9		
Serine	5.3	±	0.4	3	5.2	±	0.4	2	4.9	±	0.1	2	5.4	±	1.1	2	5.1	±	0.5	8	5.6	5.7	5.4	6.7	6.1		
Threonine	4.0	±	0.6	3	3.8	±	0.1	2	4.1	±	0.3	2	3.8	±	0.3	2	3.9	±	0.6	8	3.6	2.6	4.9	4.8	4.6	2.7	2.3
Tryptophan	0.97	±	0.1	2	1.10	±	0.0	2	0.71	±	0.0	1	1.10	±	0.1	2	1.22	±	0.2	3	ND	0.34	0.17	ND	ND	0.74	0.60
Tyrosine	3.4	±	0.3	3	3.0	±	0.9	2	3.9	±	1.1	2	3.2	±	0.2	2	3.7	±	0.3	8	4.0	3.4	2.1	3.6	3.8		
Valine	5.2	±	0.4	3	5.1	±	0.1	2	6.3	±	1.2	2	5.1	±	0.0	2	5.3	±	1.0	8	6.2	3.4	3.0	5.2	4.9	4.2	3.9
Met+Cys	4.2	±	0.3	3	3.3	±	1.8	2	4.3	±	0.0	2	4.2	±	0.1	2	2.6	±	1.0	8	3.9	7.4	5.2	2.0	3.0	2.6	2.2
Phe+Tyr	8.1	±	0.7	3	7.5	±	0.5	2	9.6	±	2.0	2	7.9	±	0.7	2	8.3	±	0.7	8	8.6	6.7	3.5	8.4	8.4	4.6	3.8
Hydrophilic	62.0	±	7.0	3	63.0	±	6.7	2	56.9	±	4.3	2	62.3	±	9.9	2	63.4	±	7.7	8	62.1	69.7	70.4	61.9	62.3		
Hydrophobic	38.0	±	3.8	3	37.0	±	2.8	2	43.1	±	6.4	2	37.7	±	2.6	2	36.6	±	3.9	8	37.9	30.3	29.6	38.1	37.7		
Phobic/Philic	0.6	±	0.5	3	0.6	±	0.4	2	0.8	±	1.5	2	0.6	±	0.3	2	0.6	±	0.5	8	0.6	0.4	0.4	0.6	0.6		
Aromatic	9.1	±	0.9	3	8.6	±	1.4	2	10.4	±	2.0	2	9.0	±	0.8	2	9.6	±	0.9	8	8.6	7.0	3.7	8.4	8.4		
References	Callaway (2004), House et al. (2010), and Mattila et al. (2018)				House et al. (2010), and a Kim and Lee (2011b)				House et al. (2010), and Mattila et al. (2018)				House et al. (2010), and Malomo and Aluko (2015b)				Tang et al. (2006), Wang et al. (2008), Girgih et al. (2010, 2013), Malomo and Aluko (2015b), Ren et al. (2016), Hadnadev et al. (2018), and Wang et al. (2018)				Hadnađev et al. (2018)	Malomo and Aluko (2015a)	Malomo and Aluko (2015a)	Wang et al. (2008)	Wang et al. (2008)	FAO/WHO and UNU (2007)	

Tot AA: total amino acids. Y/O: years old. HPI: hemp protein isolate obtained by isolelectric precipitation. HMI: hemp protein isolate obtained by micellization. Glb: globulins. Alb: albumins. Asx = aspartic acid + asparagine. Glx = glutamic acid + glutamine. Met: methionine. Cys: cysteine. Phe: phenylalanine. Tyr: tyrosine. Hydrophilic = arginie + Asx + cysteine + Glx + histidine + lysine + serine + threonine + tyrosine. Hydrophobic = alanine + glycine + isoleucine + leucine + methionine + phenylalanine + proline + tryptophan + valine. Phobic/Philic = hydrophobic/hydrophilic. Aromatic = phenylalanine + tryptophan + tyrosine. Grey background, limiting amino acids according to FAO recommendations for children aged from 1 to 2 Y/O. Underlined AA, essential amino acids

globulin fraction is limiting in sulfur amino acids, unlike the 11S, explaining that the HPI, majorly represented by edestins are not deficient in sulfur amino acids (Callaway 2004; Wang et al. 2008; Kim and Lee 2011b).

9.3.3.2 Digestibility

Different in vitro and in vivo protein digestibility values available in the literature for hemp and other plant and animal protein sources are listed Table 9.3. The whole seed proteins of hemp and resulting press-cake have in vivo digestibility values of 85% and 87% respectively (House et al. 2010; Malomo and Aluko 2015b). Dehulling of hemp seeds increases in vivo protein digestibility by 10–95% (House et al. 2010). The hulled hemp seed proteins thus have digestibility values similar to those of animal proteins: 94% for meat and fish, 98% for beef, 94–95% for whole or skim milk, or 98% for eggs (Sarwar 1997; Schaafsma 2000; FAO/WHO and UNU 2007). In vivo digestibility values of hemp proteins are comparable to other vegetable proteins ranging from 71% for black beans to 99% for pea protein concentrates (PPC) (Sarwar 1997; Rutherfurd et al. 2015).

In vitro digestibility value of hemp meal proteins is close to that obtained in vivo: 85% vs. 87% respectively (House et al. 2010; Malomo and Aluko 2015b). This value is close to the in vitro protein digestibility of cottonseed meal measured at 81% (Hsu et al. 1977). Hemp protein isolate obtained by isoelectric point precipitation (HPI), has an in vitro digestibility of 88%, higher than that of soy protein isolate obtained by isoelectric point precipitation (SPI) evaluated at 80% (Hsu et al. 1977; FAO/WHO and UNU 2007; Wang et al. 2008; Malomo and Aluko 2015b).

9.4 Extraction of Hemp Proteins

In order to valorize all the components of the hemp seed, hemp proteins are generally extracted from cakes resulting from the mechanical pressing of whole seeds in hemp oil production.

Many authors have proposed the extraction of hemp proteins from hemp meal in order to characterize protein fraction properties and to produce hemp protein concentrates or isolates (Tang et al. 2006; Yin et al. 2007, 2008; Wang et al. 2008, 2009, 2018; Girgih et al. 2010, 2014; Kim and Lee 2011b; Malomo et al. 2014; Malomo and Aluko 2015b, 2016; Ren et al. 2016; Orio et al. 2017; Hadnađev et al. 2018; Mamone et al. 2019). The general extraction route of hemp proteins is presented Fig. 9.2. Hemp oil is extracted from whole or hulled seeds by cold mechanical pressing. The residual cake generally contains about 70% less oil than the original seeds. Further delipidation by solvent may be applied, generally with hexane, to substantially remove the lipids. The cakes are crushed and sometimes sifted before being hydrated. During extraction, the hydration is carried out typically by suspending 5% of cake (from 1% to 7.5%) in distilled water at pH 10 (from 8 to 10), 37 °C (from 20 °C to 37 °C), with 2 h stirring (from 1 h to 4 h). Successive

Table 9.3 In vivo and in vitro digestibility values of hemp proteins and other plant and animal proteins in percentage

Raw material		In vivo	In vitro
Hemp	Whole seed[a]	85	
	Hulled seed[a]	95	
	Meal[ab]	87	85
	HPI[bc]		88
	11S[c]		91
	7S[c]		88
Soy	Bean[de]	93	
	Flour[f]	86	
	Meal[g]	80	
	SPI[fghi]	95	80
Pea	Seed[ef]	86	
	PPC[i]	99	
Sunflower	Seed[e]	91	
	Flour[f]	90	
Cotton	Seed[f]	90	
	Meal[h]	85	81
Peanut[ef]		95	
Almond[j]			88
Faba bean[e]		88	
Black bean[g]		71	
Bean[f]		78	
Lentil[e]		84	
Rolled oat[e]		90	
Chickpea flour[k]		84	72
Rice PC[i]		88	
Wheat	Raw[d]	91	
	White flour[f]	96	
Milk	Whole[d]	95	
	Skim[g]	94	
	MPC[i]	98	
	Lactalbumin[g]	99	
Egg[d]		98	
Meat/fish[f]		94	
Beef[d]		98	

[a]House et al. (2010), [b]Malomo and Aluko (2015b), [c]Wang et al. (2008), [d]Schaafsma (2000), [e]Sarwar and Peace (1986), [f]FAO/WHO and UNU (2007), [g]Sarwar (1997), [h]Hsu et al. (1977), [i]Rutherfurd et al. (2015), [j]Ahrens et al. (2005), [k]Angulo-Bejarano et al. (2008). HPI and SPI, hemp and soy protein isolates respectively. PPC, rice PC and MPC, pea, rice and milk protein concentrates respectively

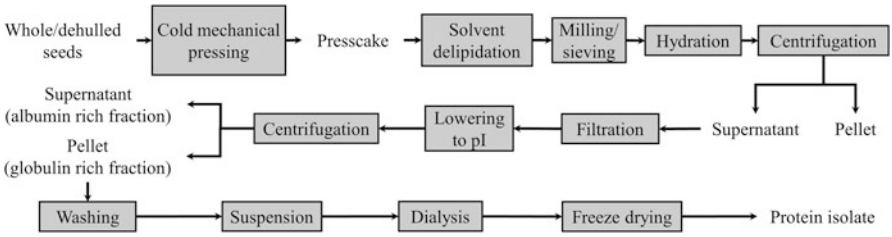

Fig. 9.2 General extraction procedure of proteins from hemp seed. pI: isoelectric point

centrifugation is carried out at 7000 g (from 6000 g to 10,000 g) at 20 °C (from 4 °C to 20 °C) during 30 min (from 15 min to 60 min). The pellet is discarded and the supernatant recovered. The pH of the supernatant is adjusted to pH 5 (from 4.5 to 5) and sometimes filtered on cheese-cloth or Whatman® paper. A new centrifugation step is applied at 8000 g (from 5000 g to 8000 g), at 20 °C for 10–60 min. The pellet is sometimes washed with distilled water and then resuspended at pH 7 (from pH 6.8 to 7). Dialysis is optionally applied, and the suspension is freeze-dried. The isolates obtained have protein content varying from 87% to 94% on a dry basis. The extraction yields are generally between 34 and 51% except in the work of Tang et al. (2006) where it reached 73%.

Alternative extraction methods based on micellization principle have been described in the literature. Malomo and Aluko (2015a) suspended the raw hemp meal at 10% in 0.5 M NaCl solution for 1 h at 24 °C. The supernatant obtained after centrifugation was filtered and dialyzed against distilled water. The content of the dialysis tubing was centrifuged. The supernatant was collected as the albumin fraction. After washing with distilled water and successive centrifugation, the resulting pellet was collected as the globulin fraction. After final freeze-drying, these authors obtained powders with protein contents (in w/w dry basis) and extraction yields of 80% and 6.33% for albumins and 100% and 6.07% for globulins, respectively. In a similar way, Hadnađev et al. (2018) proposed extraction by suspending 10% delipidated hemp cake in 0.8 M NaCl solution at pH 7 for 2 h at 35 °C. The supernatant obtained by centrifugation was diafiltered by ultrafiltration using a 12–14 kDa cut-off membrane with distilled water at pH 7. A new centrifugation step was performed on the retentate and the pellet was freeze-dried. The resulting protein isolate (HMI) had a high protein purity of ~99% with a protein yield of ~40%.

Otherwise, Malomo and Aluko (2015b) proposed an enzyme-assisted extraction method to facilitate the release of proteins from the plant material. A mixture of cellulase, hemicellulase, xylanase and phytase was applied to the aqueous suspension of hemp meal at pH 5. After incubation at 37 °C for 4 h, the solution was diafiltered by ultrafiltration at 10 kDa cut-off and then freeze-dried. The resulting powder (HEC) had at 74% protein content on a wet basis.

9.5 Physicochemical Properties and Functionality

9.5.1 Solubility

Protein solubility is a key-property for many liquid foods and influences basically other techno-functional properties such as gelling and emulsifying properties.

Protein solubility is generally determined by the balance between hydrophobic protein–protein and hydrophilic protein–water interactions. The common method to measure solubility evaluates the retention of proteins in the supernatant after centrifugation, which is influenced by the amount of aggregated proteins able to sediment. Protein solubility is sometimes referred to as "nitrogen solubility", since nitrogen from both protein and non-protein sources, such as nucleic acids, free amino acids, peptides, and phospholipids, is extracted in solubility tests. Nitrogen solubility can be defined as the ratio of solubilized nitrogen to total nitrogen in the sample, expressed as a percentage. Most of the authors who have investigated the solubility of hemp proteins have expressed the protein solubility, as relative nitrogen solubility. In this case, the amount of soluble protein, or soluble nitrogen, after hydration of the protein material in 0.1 M NaOH solution was considered to correspond to 100% solubility. The amount of soluble protein at the different pH of hydration is then divided by the amount of soluble protein in 0.1 M NaOH, to obtain the percentage of relative solubility. Thus, this method makes it possible to represent the solubility curve as a function of pH, but gives no indication of the actual solubility values of the protein material.

The extractability of hemp proteins from cake has been studied by Malomo et al. (2014) and Malomo and Aluko (2015b) by assessing their relative solubility from pH 3 to 9 (Fig. 9.3c). Relative solubility increased with increasing pH. From pH 3 to 5, it increased slightly from about 5 to 10%, respectively. Above pH 5, the increase in solubility is enhanced towards alkaline pH, reaching up about 25% at pH 9.

Many authors have studied the relative solubility of hemp protein isolates (HPI) produced by alkaline solubilization and isoelectric precipitation (Fig. 9.3c) (Tang et al. 2006; Kim and Lee 2011b; Malomo et al. 2014; Malomo and Aluko 2015b; Hadnađev et al. 2018; Wang et al. 2018; Dapčević-Hadnađev et al. 2019). Although the relative solubility values for a given pH varied considerably from one author to another, the profiles of solubility with pH were generally similar. The relative solubility is minimum at the isoelectric point of hemp globulins, around pH 5. It increases towards the alkaline pH to reach a maximum at pH 12. It seems that the maximum relative solubility is almost reached for pH 10. Starting from pH 5, relative solubility increases with decreasing pH up to pH 2. The maximum solubility encountered at acidic pH appears to be 2 times lower as the maximum solubility encountered at alkaline pH.

Malomo and Aluko (2015a) studied the relative solubility of hemp globulins and albumins separately from pH 3 to 9 (Fig. 9.3b). Hemp globulins presented the same solubility profile as HPI (Malomo and Aluko 2015b). This is consistent with the fact that HPI is obtained after selective isoelectric precipitation of globulins. The solubility profile of hemp albumins was totally different since their relative solubility

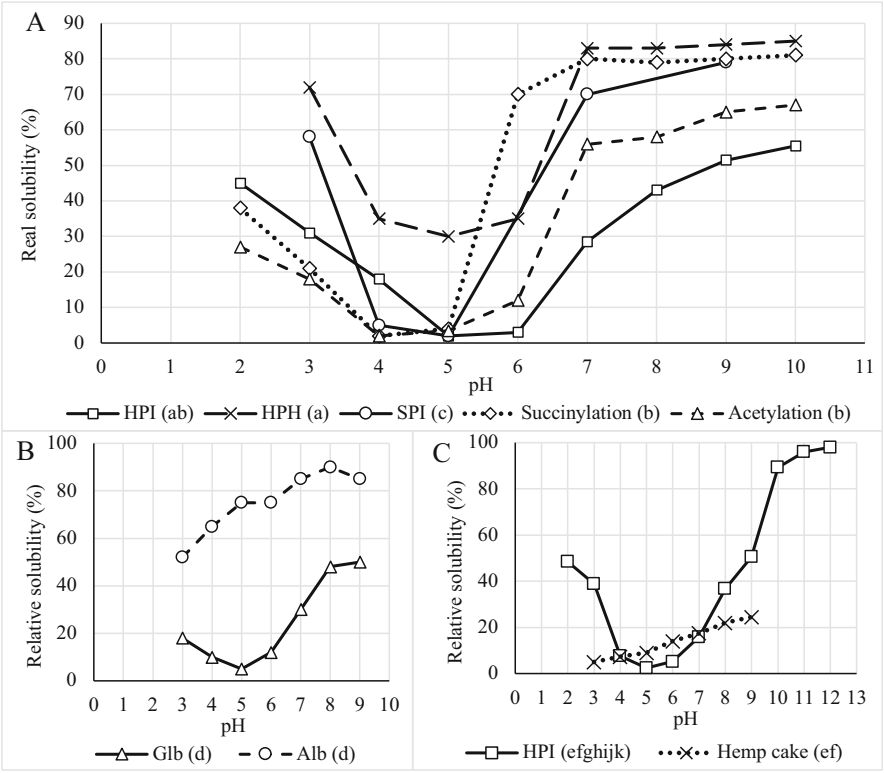

Fig. 9.3 Solubility of hemp proteins as a function of pH. (**a**) Real nitrogen solubility of hemp protein isolate obtained by isoelectric point precipitation (HPI), hemp protein hydrolysates (HPH), chemically modified HPI (succinylated or acetylated) and soy protein isolate obtained by isoelectric point precipitation (SPI). (**b**) Relative nitrogen solubility of isolated hemp globulins (Glb) and albumins (Alb). (**c**) Relative nitrogen solubility of hemp protein isolate obtained by isoelectric point precipitation (HPI) and hemp press cake. Results from a: Yin et al. (2008); b: Yin et al. (2009); c: Karki et al. (2009); d: Malomo and Aluko (2015a); e: Malomo et al. (2014); f: Malomo and Aluko (2015b); g: Kim and Lee (2011b); h: Hadnađev et al. (2018); i: Dapčević-Hadnađev et al. (2019); j: Tang et al. (2006); k: Wang et al. (2018)

tended to increase continuously with pH. The albumins showed significantly higher relative solubility values within the same pH range, varying from about 50 to 90%.

Yin et al. (2008, 2009) studied the real nitrogen solubility of HPI from pH 2 to 10 (Fig. 9.3a). They observed the same profile of solubility vs pH as the previous authors for relative solubility. Thus the solubility was minimal at pH 5 close to 0%, then increased with pH to reach the maximum value at pH 10 with about 60% of soluble protein. Solubility increased also from pH 5 to 2 up to about 45%. The effect of partial HPI hydrolysis by trypsin on the real protein solubility is shown in Fig. 9.3a (Yin et al. 2008). The hemp protein hydrolysates (HPH) having 2.3–6.7% degree of hydrolysis, generally showed the same solubility profile with pH as the initial HPI. On the other hand, the HPH solubility values were significantly higher within the entire pH range. The gain was 2–2.5 times at pH 3, more than

15 times at pH 5, about 3 times at pH 7 and about 1.5 times at pH 10. The authors also studied the impact of acetylation and succinylation of hemp proteins on HPI real solubility as reported in Fig. 9.3a (Yin et al. 2009). Both treatments did not change the profile of protein solubility curves. However, the chemical modifications of hemp proteins decreased their solubility at pH 2 and 3, but increased it from pH 6 to 10. Succinylation resulted in a more pronounced solubility increase from pH 6 to 10 than acetylation. The solubility of the succinylated hemp proteins reached a maximum value at pH 7 and remained constant up to pH 10, with similar values to those obtained for HPH in the same pH range (Yin et al. 2008).

In comparison, soy protein isolates obtained by isoelectric point precipitation (SPI) have a similar U-shaped solubility profile as that of HPI, with a minimum of solubility towards pH 4–5 (Karki et al. 2009) (Fig. 9.3a). Nevertheless, the actual solubility values are much higher for SPI than for HPI at acidic and basic pH. The solubility values of SPI and HPI are 60 and 30% at pH 3, 70 and 30% at pH 7 and 80 and 50% at pH 9, respectively.

The surface hydrophilic–hydrophobic balance of proteins and their ability to interact with water molecules are mainly related to their composition in amino acids and the net charge of these macromolecules. Therefore, the measure of surface hydrophobicity and net charge of proteins provide useful information regarding solubility behaviour of these compounds. The surface hydrophobicity of proteins can be measured through a fluorescent probe, mainly 8-anilino-1-naphthalene sulfonate or ANS, which fluorescence is amplified by binding to the hydrophobic pockets at the surface of the protein particle. The surface hydrophobicity values (Ho) are calculated by the slope of the fluorescence intensity as a function of protein concentration in mg/ml. Tang et al. (2009) and Wang et al. (2009, 2018) reported Ho values for hemp protein isolates obtained by isoelectric point precipitation (HPI) at pH 7 of 75, 89 and 179 respectively. These values were lower or close to the Ho values of 155 and 163 reported for soy protein isolates obtained by isoelectric point precipitation (SPI) by Sorgentini and Wagner (1999) and Jiang et al. (2009) respectively. Teh et al. (2016) measured the Ho values at neutral pH for acid soluble and alkali soluble hemp globulins respectively. Similar surface hydrophobicity values (Ho = 75) were obtained for these protein fractions. The same authors measured the zeta potential of hemp proteins at pH 7 from the same protein fractions. The 2 protein fractions had the same zeta potential value measured at −9.2 mV, significantly lower than those reported elsewhere for SPI at pH 7. Indeed Lam et al. (2007) and Liu and Tang (2015) reported zeta potential values of −25 mV and −33 mV for SPI, respectively. From these results, it can be assumed that the aqueous suspensions of hemp proteins are less stable than their soy counterparts at neutral pH.

9.5.2 Water Holding Capacity and Oil Holding Capacity

Water holding capacity (WHC) and oil holding capacity (OHC) are important properties for food products where soft texture is sought and limited water or lipid

release during and after cooking are expected. Water or oil retention capacity of oilcakes are generally little affected by their delipidation. Hemp, flax and canola meals presented similar OHC values (Teh et al. 2013). Hemp cake has WHC values close to those of canola and higher than those of flax. In addition, hemp cake had slightly higher WHC value and slightly lower OHC value as those of hemp protein isolates obtained by isoelectric point precipitation (HPI) respectively (Malomo et al. 2014). The WHC and OHC values obtained for acid soluble hemp globulins and alkali soluble hemp globulins were slightly higher for the later ones (Teh et al. 2013). OHC and WHC values for the acid or alkali soluble globulins are close to those of canola proteins, and higher than those of flax ones.

The hemp protein isolates obtained by micellization (HMI) by Hadnađev et al. (2018) presented the same OHC value as those produced by isoelectric precipitation (HPI), whereas the WHC value for HMI was 2 times lower compared to HPI. In the previous work of Malomo and Aluko (2015b), the authors showed that hemp protein concentrates obtained by enzymatic digestion of hemp cake by hemicellulase, cellulase, xylanase and phytase (HEC) and successive ultrafiltration, led to an OHC value similar to that of HPI and a slightly higher WHC value. Yin et al. (2008) observed that the digestion of HPI by trypsin resulted in a 3 to 4-fold decrease in WHC. OHC was also decreased by protein hydrolysis, except at the highest hydrolysis degree close to 7% where it was similar to that of HPI. A heat treatment at 95 °C for 10 min applied by the same authors to HPI did not affect the WHC, but slightly decreased OHC of the isolates.

9.5.3 Gelation

A protein gel is a continuous macromolecular network entrapping an aqueous phase, formed under physical stress, mainly by heating, or by applying specific physico-chemical conditions from stable protein suspensions. Scientific data on the gelation properties of hemp proteins are scarce and only concern thermal gelation (Table 9.4).

Hemp protein isolates obtained by isoelectric point precipitation (HPI), mainly containing globulins, begin to denature at 86 °C (Ton: onset temperature) as observed by differential scanning calorimetry (DSC) and have a thermo-denaturation temperature (Td) peak at 94 °C (Table 9.5). The average enthalpy of denaturation (ΔH) of this protein fraction was estimated at 11 J/g of protein (Tang et al. 2006; Yin et al. 2008; Wang et al. 2008; Hadnađev et al. 2018). This denaturation could be predominantly ascribed to 11S globulins. In fact, 7S hemp proteins have been reported to present no thermal denaturation peak during calorimetry measurement (Wang et al. 2008). Tang et al. (2006) indicated that the degree of ordered structure of 11S edestin was related to both hydrophobic interactions and disulfide bridges as revealed by the decrease in ΔH in presence of sodium dodecyl sulfate and dithiothreitol respectively. The presence of disulfide bridges seems to play an effective role in the thermal stability of hemp 11S globulins since a slight decrease in Ton and Td values was also noted in presence of dithiothreitol.

Table 9.4 Summary of the functional properties studied in the scientific literature

Functionality	Protein material	Operating conditions	Performed analyses	References
Water or oil holding capacity	HPM + HPI		OHC, WHC	Malomo et al. (2014)
	HPM + HPI + HEC		OHC, WHC	Malomo and Aluko (2015b)
	Hemp, canola or flax: raw or delipidated cake, alkali or acid soluble globulins		OHC, WHC	Teh et al. (2013)
	HPI + SPI		OHC, WHC	Tang et al. (2006)
	HPI + HMI		OHC, WHC	Hadnađev et al. (2018)
	HPI + HPI heated 95 °C 10 min + HPH by trypsin		OHC, WHC	Yin et al. (2008)
Geling	HPM + HPI	pH 7; 2–20%; 95 °C 1 h	LGC	Malomo et al. (2014)
	HPI + HMI	pH 7; 30% protein suspension with 0, 50 or 300 mM NaCl; 120 °C 15 min	Confocal microscopy, rheology	Dapčević-Hadnađev et al. (2018)
Emulsifying	HPM + HPI	pH 3, 5, 7 or 9; 1, 2.5 or 5% protein suspension with 83/17 protein solution to oil ratio	Droplet size, ES	Malomo et al. (2014)
	HPM + HPI + HEC	pH 3, 5, 7 or 9; 1, 2.5 or 5% protein suspension with 83/17 protein solution to oil ratio	Droplet size, ES	Malomo and Aluko (2015b)
	Hemp, canola or flax: raw or delipidated cake, alkali or acid soluble globulins	pH 7; 2% protein suspension with 50/50 protein solution to oil ratio	EA, droplet size, ES, CS	Teh et al. (2013)
	HPI + SPI	pH 3 to 8; 0.2% protein suspension with 75/25 protein solution to oil ratio	EAI, ESI	Tang et al. (2006)
	Glb + Alb	pH 3, 5, 7 or 9; 1, 2.5 or 5% protein suspension with 83/17 protein	Droplet size, ES	Malomo and Aluko (2015a)

(continued)

Table 9.4 (continued)

Functionality	Protein material	Operating conditions	Performed analyses	References
		solution to oil ratio		
	HPI + HMI	pH 3; 0.25, 0.75 or 1.5% protein suspension with 90/10 protein solution to oil ratio	Droplet size, FI, CI, CS, confocal microscopy, rheology	Dapčević-Hadnađev et al. (2019)
	HPI + HPI shifted at pH 12 for 5 min at 20, 30, 40, 50 or 60 °C	pH 7; 2% protein suspension with 75/25 protein solution to oil ratio	EAI, ES, confocal microscopy	Wang et al. (2018)
	HPI + HPI heated 95 °C 10 min + HPH by trypsin	pH 3, 5, 7 or 9; 0.2% protein suspension with 75/25 protein solution to oil ratio	EAI, ESI	Yin et al. (2008)
Foaming	HPM + HPI	pH 3, 5, 7 or 9; 2, 4 or 6% protein suspension	FC, FS	Malomo et al. (2014)
	HPM + HPI + HEC	pH 3, 5, 7 or 9; 2, 4 or 6% protein suspension	FC, FS	Malomo and Aluko (2015b)
	Glb + Alb	pH 3, 5, 7 or 9; 2, 4 or 6% protein suspension	FC, FS	Malomo and Aluko (2015a)
	HPI + HPI heated 95 °C 10 min + HPH by trypsin	pH 7; 1% protein suspension	FC, FS	Yin et al. (2008)

HPM: Hemp protein meal. HPI: Hemp protein isolate obtained by isoelectric precipitation. HEC: Hemp protein concentrate obtained by enzymatic digestion of press-cake by cellulase, hemicellulase, xylanase and phytase. SPI: Soy protein isolate obtained by isoelectric precipitation. HMI: Hemp isolate obtained by micellization. HPH: Hemp protein hydrolysates. Glb: Hemp globulins. Alb: Hemp albumins. OHC: Oil holding capacity. WHC: Water holding capacity. LGC: Least gelation concentration. ES(I): Emulsifying stability (index). EA(I): Emulsifying activity (index). CS: Creaming stability. FI: Floculation index. CI: Coalescence index. FC: Foaming capacity. FS: Foaming stability

Hadnađev et al. (2018) compared DSC data between hemp protein isolates obtained by isoelectric precipitation (HPI) and micellization (HMI) respectively. These authors found that micellization extraction led to increased Td and ΔH values. This result revealed the production of HMI with higher thermal stability, suggesting higher level of structural organization and a more preserved native state of proteins compared to HPI.

Table 9.5 Differential scanning calorimetry data for hemp proteins and other plant protein sources

		Hemp				Sun-flower[e]	Rape-seed[f]	Peanut[g]	Soy[h]	Pea[i]	Chick-pea[j]
		HPI[abcd]	HMI[c]	11S[b]	7S[b]	pI protein isolates					
Ton	(°C)	86		86	None						
Td		94	96	92	None	99	98	104	90	82	88
ΔH	(J/g)	11	22	7	None	8	10	12	6	7	4

Td: denaturation temperature, ΔH: denaturation enthalpy, Ton: onset temperature, HPI: hemp protein isolate obtained by isoelectric precipitation, HMI: hemp protein isolate obtained by micellization

[a]Tang et al. (2006), [b]Wang et al. (2008), [c]Hadnađev et al. (2018), [d]Yin et al. (2008), [e]Salgado et al. (2011), [f]He et al. (2013), [g]Colombo et al. (2010), [h]Shen and Tang (2012), [i]Liang and Tang (2013), [j]Paredes-López et al. (1991)

Otherwise, Yin et al. (2008) indicated that the denaturation enthalpy values for HPI decreased in proportion to their degree of hydrolysis by trypsin, traducing a decrease in the extent of ordered structures within hemp polypeptides.

In addition, the HPI had thermo-denaturation temperature and denaturation enthalpy of the same order of magnitude as those of other oilseed protein isolates, prepared from sunflower seed, rapeseed or peanut, having Td and ΔH values ranging from 98 to 104 °C and 8 to 12 J/g of protein respectively (Colombo et al. 2010; Salgado et al. 2011; He et al. 2013). Legume proteins extracted from soybeans, peas or chickpeas, had slightly lower Td and ΔH values than those of HPI, the former ranging from 82 to 99 °C and between 4 and 7 J/g of protein respectively (Paredes-López et al. 1991; Shen and Tang 2012; Liang and Tang 2013).

According to Malomo et al. (2014), hemp meal had better gelling properties than hemp protein isolates obtained by isoelectric point precipitation (HPI), probably due to the contribution of sugars and polysaccharides. The minimum gelation concentrations of hemp meal and HPI were measured at 12 and 22% (w/w) respectively. Dapčević-Hadnađev et al. (2018) investigated also the gelling properties of hemp protein isolates obtained by alkaline extraction/isoelectric precipitation (HPI) or by micellization (HMI). The gels were formed from 30% (w/w) protein suspension at pH 7, heated at 120 °C for 15 min. For the 2 types of isolates, true gels were formed with very close gel forces evaluated by dynamic rheology. However, in comparison to HMI gels, the HPI gels displayed a slightly higher elastic modulus and a higher breakdown limit during amplitude sweep test, revealing the formation of stronger and more cohesive HPI-based gels. The microstructures of the 2 gel types were very different. From confocal microscopic observations, the HPI gels showed extended interconnections between large protein granules forming a low porous network, while HMI formed a filamentous protein network with large pores. The addition of NaCl up to 300 mM had little impact on the structure of the HPI gels, while it resulted in a pore size increase for HMI gels. Finally, the two types of gel had a yellowish-red color probably due to the co-extraction and polymerization of phenolic compounds.

9.5.4 Emulsion

Proteins are commonly used to stabilize oil in water emulsion in food products. Emulsions are thermodynamically unstable because they increase the interfacial surface and consequently the free energy of the system. The presence of proteins as emulsifiers, able to adsorb at the oil/water interfaces, helps to reduce interfacial tension and contributes to the stability of the emulsion. The ability of protein to form emulsion is then generally evaluated through particle size measurement or emulsifying activity index (EAI) determination (Table 9.4). Over time, oil droplets in emulsion are subject to flocculation, coalescence and creaming phenomena, since the system tends to minimize its free energy. Flocculation is the reversible or irreversible aggregation of droplets. Coalescence occurs when the interfacial film

is broken, resulting in the irreversible fusion of dispersed droplets into larger ones. Finally, creaming is the migration of oily particles to the surface against gravity due to the difference in density between the two phases. The creaming kinetics is slowed down by the droplet size reduction and the viscosity increase of the continuous phase. Particle size change or phase separation in emulsion are evaluated as flocculation index (FI), coalescence index (CI) creaming stability (CS) and emulsion stability index (ESI).

In the literature, emulsifying properties of hemp proteins have been evaluated from hemp meals, hemp protein isolates and their separated fractions, i.e. globulins and albumins (Table 9.4).

9.5.4.1 Hemp Meal

Teh et al. (2013) have studied the emulsifying properties of hemp cakes at pH 7 compared to those obtained from rapeseed with or without delipidation. According to these authors, raw hemp meal had a lower emulsifying activity than canola meal. On the other hand, the delipidation of hemp cake increased its emulsifying activity, becoming higher than that of delipidated canola meal. Emulsions prepared from raw hemp cake were found more stable than those obtained from delipidated meal. Emulsion stability was higher for raw hemp meal than for raw canola meal. However, more stable emulsions were observed for delipidated canola meal than for their hemp counterpart.

9.5.4.2 Hemp Protein Isolate

Malomo et al. (2014) compared the emulsifying properties of hemp cake and hemp protein isolates obtained by isoelectric point precipitation (HPI) in the range of pH 3 to 9. Emulsions were prepared from 1% to 5% protein suspensions with a 83/17 protein solution to oil ratio. The authors found that the emulsifying properties of hemp meal were not significantly affected by pH and meal concentration. The droplet size measured by granulometry as the Sauter mean diameter (d_{32}) in the HPI emulsions was impacted by the pH, with some differences regarding the protein concentration used. In most cases, d_{32} values for HPI-based emulsions were higher than those prepared from hemp cake, except at 5% protein at pH 5, 7 and 9, where droplet size was slightly lower for the former emulsions. No difference in stability was observed between hemp cake-based and HPI-based emulsions.

Tang et al. (2006) investigated the emulsifying properties of HPI and soy protein isolate obtained by isoelectric point precipitation (SPI) from pH 3 to 8. Whatever the pH, the emulsifying activity (EAI) and emulsion stability (ESI) indexes were lower for HPI-based emulsions than for SPI-based emulsions. The EAI of both types of emulsion were maximum at pH 3, minimum around the isoelectric point, and

relatively constant from pH 5 to 8. The ESI measured for the SPI-based emulsions followed the same profile as EAI with pH, whereas the ESI has been relatively constant for HPI-based emulsions in the same pH range.

Some authors have applied different pretreatments to HPI to improve their emulsifying properties. Yin et al. (2008) produced hydrolysates from HPI digestion by trypsin. The hydrolysates led to lower EAI than HPI at pH 3, 5, 7 and 9. The ESI was increased by the hydrolysis treatment for emulsions at pH 3, 5 and 9, but decreased for emulsions at pH 7. EAI and ESI of HPI-based emulsions were the highest at pH 9 and pH 7 respectively and then decreased in the following order: pH 7 > pH 3 > pH 5 and pH 9 > pH 3 > pH 5 respectively. The same authors have demonstrated that a heat treatment at 95 °C for 10 min applied to HPI allowed to improve the emulsifying activity at all pH except at pH 5. The heat treatment only led to improve emulsion stability at pH 7 and impaired it at pH 5.

Recently Wang et al. (2018) showed that mild heat treatment (30–60 °C) applied to HPI suspensions in strong alkaline conditions at pH 12 enhanced the emulsifying properties of hemp proteins at pH 7, by decreasing droplet size and improving emulsions stability. The efficiency of the pretreatment was reinforced with the increase in temperature.

Yin et al. (2009) observed that both preliminary acetylation and succinylation applied to HPI improved the emulsifying activity of the modified hemp proteins at pH 7. In addition, the ESI for HPI appeared slightly increased after acetylation and decreased after succinylation.

Other authors have compared the emulsifying properties of hemp protein isolates obtained by different extraction processes. Recently, Dapčević-Hadnađev et al. (2019) compared the properties of emulsions prepared at pH 3 from hemp protein isolates obtained by isoelectric precipitation (HPI) or by micellization (HMI). The emulsions with a 90/10 protein solution to oil ratio presented the lowest oil droplet size for HMI regardless protein concentration varying from 0.25% to 1.25%. Droplet size decreased as a function of protein concentration in the case of HPI-based emulsions. The flocculation index (FI) was the lowest for HMI and unaffected by protein concentration. The coalescence index (CI) decreased with increased protein concentration for both types of hemp protein isolate. CI values were relatively close between HPI- and HMI-based emulsions. Earlier, Malomo and Aluko (2015b) have compared the emulsifying properties of HPI-type proteins with hemp protein isolates obtained after enzymatic digestion of cake by cellulase, hemicellulase, phytase and xylanase, and successive ultrafiltration (HEC). The emulsions were prepared at pH 3, 5, 7 or 9 from 2, 4 or 6% protein suspension with a 83/17 protein solution to oil ratio. In all conditions, the droplet size of emulsions prepared with HEC was higher than those prepared with HPI. The stability of the HEC-based emulsions was similar to lower depending on pH and protein concentration in comparison to HPI-based emulsions.

9.5.4.3 Globulins and Albumins

Malomo and Aluko (2015a) compared the emulsifying properties of isolated hemp globulins and albumins at pH 3, 5, 7 and 9 for 1, 2.5 or 5% protein suspensions applying a 83/17 protein solution to oil ratio. Overall within experimental conditions, droplet size and emulsion stability were relatively similar between globulins and albumins except under a few conditions. At 5% protein concentration and at pH 7 and 9, the globulins led to oil droplets of smaller diameter in emulsion than those obtained from albumins. At pH 7 and 1% protein concentration, the emulsion stability was significantly lower with globulins than with albumins.

Teh et al. (2013) have also compared the properties of oil in water emulsions based on alkali soluble hemp globulins or acid soluble ones respectively, to those of emulsions prepared from other hemp or oilseed protein materials. These authors reported similar emulsifying activity and emulsion stability between alkali and acid soluble globulins-based systems at pH 7. Moreover, the emulsifying activity of delipidated hemp cake was higher than that of hemp alkali and acid soluble globulins, itself higher than raw hemp cake. Nevertheless, the raw hemp cake led to more stable emulsions than those formed with delipidated hemp cake and even more than those prepared from acid or alkali soluble hemp globulins. In addition, the emulsifying activity of alkali and acid soluble hemp globulins were similar to their rapeseed counterparts, whereas the emulsifying stability was significantly higher for rapeseed protein-based emulsions.

9.5.5 Foam

Food foam results from the stable dispersion of air bubbles in a liquid phase. Generally, protein suspensions have good foamability. The efficiency of proteins to form foam structure is evaluated by foaming capacity (FC) and foaming stability (FS) tests, based on the determination of the foam volume initially generated in the system and its conservation with time respectively.

Several authors have evaluated the foaming properties of hemp protein materials (Table 9.4). Malomo et al. (2014) reported that hemp cakes had lower foaming properties (FC and FS) evaluated at pH 3, 5, 7 and 9 and at 2, 4 or 6% protein concentration than hemp protein isolate produced by isoelectric precipitation (HPI). The FC of the hemp cake increased slightly with pH, whereas a maximum and a minimum were assessed at pH 3 and 5 respectively for HPI. The FS value for HPI was measured close to 100% and was affected nor by pH, nor by protein concentration except at 6% protein concentration-pH 5 condition, where it decreased. The FS value was lower for hemp cakes and depended on pH and protein concentration.

Malomo and Aluko (2015b) compared foaming ability of the same hemp protein isolates obtained by isoelectric point precipitation (HPI) or enzymatic digestion of hemp meal (HEC), as those used to evaluate emulsifying properties as reported in

Sect. 9.5.4. Compared to HPI, HEC led to significantly higher FC values of about three-fold within experimental pH (3–9) and protein concentration (2–6%) ranges. Foams formed from HEC at 2 or 4% protein concentration were generally less stable than those formed from HPI. At 6% protein concentration, similar values of FS were measured for HPI- and HEC-based foams except at pH 5 where the FS value was significantly higher for HEC-based foam.

The enzymatic digestion of HPI by trypsin degraded its foaming properties (FC and FS) at pH 7 (Yin et al. 2008). However, heat treatment of HPI at 95 °C for 10 min increased FS and did not impact FC.

9.6 Biological Activity

9.6.1 Antioxidant Property

According to Pojić et al. (2014), hemp cakes have antioxidant activity evaluated by DDPH (α, α-diphenyl-β-picrylhydrazyl) free radical scavenging method, mainly ascribed to the presence of phenolic compounds. Kim and Lee (2011b) found that hemp protein isolates obtained by isoelectric point precipitation (HPI) had also antioxidant activity. The antioxidant activity of HPI was proportional to protein concentration, with DDPH activity values ranging from 20% at 0.5 mg protein/ml to 80% at 3 mg protein/ml. This antioxidant activity would be partly due to the 11S edestins. Indeed, Kim and Lee (2011a) reported DDPH activity values of edestin ranging from 1% at 0.5 mg protein/ml, to over 20% at 3.5 mg protein/ml.

HPI hydrolysates (HPH) prepared by pancreatin and pepsin had antioxidant activity values measured by the DDPH method, ranging from about 15–65%, and metal chelation rates ranging from about 45–95% depending on the peptide composition (Girgih et al. 2014). Girgih et al. (2010) separated similar HPH into several fractions of <1, 1–3, 3–5 and 5–10 kDa molecular weight. HPH and all peptide fractions had antioxidant activity according to DDPH measurements and displayed effective hydroxyl radical scavenging, metal chelation, ferric reduction and inhibition of linoleic acid oxidation. HPH had the highest metal chelation activity, whereas small and large size peptide fractions had the highest DDPH antioxidant activity and ferric reduction ability respectively. All peptide fractions, regardless of molecular weight, displayed higher hydroxyl radical scavenging activity than HPH. Elsewhere, Girgih et al. (2013b) demonstrated that oxygen radical absorbance capacity (ORAC) and DDPH antioxidant activity of peptides tended to increase with their content in hydrophobic amino acids, whereas hydroxyl radical scavenging decreased.

Tang et al. (2009) generated different hemp protein hydrolysates (HPH) from enzymatic digestions with alkalase, Flavourzyme®, Neutrase®, pepsin, Protamex® or trypsin. By evaluating DDPH antioxidant activity, Fe^{2+} chelation and reducing power for all hydrolysates, the authors highlighted more or less antioxidant efficiency depending on the enzymes used. Wang et al. (2009) also revealed that HPH

produced by Neutrase® had significant antioxidant activity determined from similar analytical characterizations.

Both alkali and acid soluble hemp globulins have antioxidant properties (Teh et al. 2016). According to the last authors, the ORAC activity of alkali and acid soluble hemp globulins remained unchanged after their hydrolysis by different proteases, namely AFP 4000 (AFP), HT proteolytic concentrate (HT) or protease G (ProG) from Enzyme Solutions (Australia), actinidin or zingibain. In contrast, DDPH antioxidant activities could increase nearly 5-fold after proteolysis. Proteolysis by HT led to hydrolysates with the highest DDPH antioxidant activity rate reaching up to 73%.

9.6.2 Renal Disease Prevention

Aukema et al. (2011) have studied the impact of the diet of rats having renal polycystic insufficiency, on the symptoms of this pathology including cardiovascular hypertrophy. The rats had different diets based on various protein isolates from hemp (HPI), soybean (SPI) or pea (PPI), or casein (CPI), the last chosen as a reference. SPI and HPI had the same effects for reducing the symptoms of the pathology. Indeed, in comparison with the CPI-based diet, a diet based on HPI or SPI improved the growth of rats, caused a decrease in kidney weight and liquid content, a reduction in the volume of cysts and fibrosis, and led to healthy levels of creatinine and chemokine receptors 2 (CCR2), and heart weights of healthy subjects. On the other hand, the diet based on PPI, compared to that based on CPI, only improved the growth of rats and led to lower creatinine and CRR2 levels. It did not change the volume of fibrosis, and increased kidney weight, renal liquid content and cyst volume.

9.6.3 Antihypertensive Activity

Girgih et al. (2013a) investigated the effect of diets based on hemp meal protein hydrolysates produced by pepsin and pancreatin (HPH), or hemp protein isolates obtained by isoelectric point precipitation (HPI), on rats having spontaneous hypertension. The reference diet was based on casein protein isolates. The HPH- or HPI-based diet reduced systolic blood pressure, through mainly decreases in angiotensin-converting enzyme (ACE) activity and quantitative level of plasma renin. ACE is an enzyme that converts angiotensin 1 to angiotensin 2, which has a strong vasoconstrictor effect. These positive effects were more pronounced for the HPH-based diet than for the HPI-based diet and have been observed during tests in the prevention or treatment phase of hypertension. Contrariwise Girgih et al. (2011) found an increase in systolic blood pressure following hemp-based feeding of

rats having spontaneous hypertension. This difference may be due to the different amounts of hemp protein isolates ingested by the rats.

Hemp protein hydrolysates produced by pepsin and pancreatin (HPH) also exhibited antihypertensive abilities, by reducing ACE and renin activity, and systolic blood pressure (Girgih et al. 2014). The involved peptides enabling simultaneously the highest antioxidant (DDPH test), metal chelation and ACE inhibition activities, and the highest systemic blood pressure reduction, have been identified: Proline-Serine-Leucine-Proline-Alanine and Tryptophan-Valine-Tyrosine-Tyrosine. The peptides having the highest combined ACE and renin inhibition rates, and the highest reduction in systolic blood pressure were: Serine-Valine-Tyrosine-Threonine and Isoleucine-Proline-Alanine-Glycine-Valine. According to Girgih et al. (2011), peptides having <1 kDa and 1–3 kDa molecular weights had higher ACE and renin inhibitions than overall HPH. On the other hand, the HPH potential to reduce the systolic blood pressure was 2 times higher than that of <1 kDa and 1–3 kDa peptide fractions.

Orio et al. (2017) performed acid hydrolysis of HPI at 110 °C. The authors obtained protein hydrolysates inhibiting almost 45% of ACE activity and isolated 3 peptides exhibiting ACE inhibition rates higher than 93%: Glycine-Valine-Leucine-Tyrosine, Leucine-Glycine-Valine and Arginine-Valine-Arginine. These 3 peptides were originated from the hydrolysis of the acid subunit of edestin 1 and/or edestin 2.

According to Teh et al. (2016) alkali and acid soluble hemp globulins have limited ACE inhibition properties. These properties can be significantly improved, by nearly 30 times, by the production of enzymatic hydrolysates with AFP 4000 (AFP), HT proteolytic concentrate (HT) or protease G (ProG) (Enzyme Solutions, Australia), actinidin or zingibain. HT hydrolysates showed the highest ACE inhibition rates reaching up to about 77% inhibition.

9.6.4 Hyperglycemia and Inhibition of α-Glucosidase

α-glucosidase is an enzyme that hydrolyses starch and disaccharides to glucose. Enzymatic hydrolysates (HPH) of hemp protein isolates obtained by isoelectric point precipitation (HPI) have been reported to have α-glucosidase inhibition property of interest for the treatment of patients having hyperglycemic diseases (Ren et al. 2016). The last authors found that the HPH obtained by alkalase treatment had much higher α-glucosidase inhibitory activity, reaching almost 60% inhibition rate, than those obtained after enzymatic digestion of HPI with Flavourzyme, Protamex®, neutrase, trypsin or papain, having from 5% to 22% inhibition. From about 10% hydrolysis, the inhibition of α-glucosidase increased in proportion to the increases in hemp protein concentration from 0.1 to 100 mg/ml and hydrolysis degree up to 27%. Inhibition of α-glucosidase was mainly ascribed to 2 peptides: Leucine-Arginine and Proline-Leucine-Methionine-Leucine-Proline.

9.6.5 *Degenerative Brain Disease and Inhibition of Acetylcholinesterase*

Acetycholinesterase (AChE) is an enzyme that converts the neurotransmitter acetylcholine (Ach) into inactive metabolites, i.e. choline and acetate. With aging and dietary changes of humans, less acetylcholine is synthesized, while AChE continues normal activity. Thus, a clear decrease in ACh leads to dysfunctions of the central nervous system, which can in the long run contribute to memory disorders such as dementia or Alzheimer's disease. Malomo and Aluko (2016) showed that hemp protein hydrolysates (HPH) from hemp protein isolate obtained by isoelectric point precipitation (HPI) had AChE inhibition activities that would prevent progression to such troubles. This inhibition did not seem directly related to the degree of hydrolysis of hemp proteins. HPH obtained with 1% pepsin had the greatest AChE inhibition effect, compared with those obtained at other enzyme concentrations varying from 0.5% to 4% or with other types of enzyme such as alkalase, papain, pepsin + pancreatin, thermoase or Flavourzyme®. The authors hypothesized that the higher inhibition for this condition could result from increased synergistic effects between peptides of a wider size range present in the sample.

9.7 Conclusion

Hemp seeds are a good source of plant proteins with a protein content comparable to that of most of legume seeds. The hemp proteins have interesting nutritional value since they are rich in essential amino acids and were demonstrated highly digestible. They present also interesting techno-functional abilities such as emulsifying, foaming and gelling properties that could be exploited in the food industry. In addition, they have revealed a wide range of biological activities, enhanced after partial hydrolysis by various proteases. The most limiting factors in the valorization of hemp proteins in food industry seemed to be their low extraction yield and low solubility.

Previous works have shown that some post-extraction treatments of hemp proteins improved their solubility in the food pH range. However, few have evaluated the impact of these treatments on the general functional properties of hemp proteins.

The challenges for developing hemp protein ingredients are to obtain high extraction yields and highly soluble proteins, while maintaining their nutritional and functional properties. To meet the issue, further studies exploring extraction conditions and post-extraction functionalization treatments of hemp proteins are required.

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