Chapter 7
Hempseed Protein: Processing and Functional Properties

Anne Pihlanto, Markus Nurmi, and Sari Mäkinen

Abstract Increasing the utilization of plant proteins is needed to support the production of protein-rich foods that could replace animal proteins in the human diet so to reduce the strain that intensive animal husbandry poses to the environment. The seeds of non-drug *Cannabis sativa* L. commonly referred to as hemp, are an important source of nutrition. Hempseed typically contains over 30% oil and about 25% protein, with considerable amounts of dietary fiber, vitamins, minerals, and specific phenolic compounds. However, the utilization of hempseed in food products is still at the very beginning. Previously, few research groups have reported trials on extracting and purifying proteins from hempseed. However, most of these methods have focused on protein isolates using different precipitation techniques, but these methods may not be suitable for commercial scale production, due to their intensive costs. Also, precipitation techniques may adversely affect the functional properties of hempseed proteins. This review aims to provide an overview of the current knowledge of the hempseed protein extractions and the functional properties of the enriched protein fractions.

Keywords Hemp seed protein · Extraction · Isolation · Functional properties · Digestibility

Abbreviations

EA	emulsifying activity
EC	emulsifying capacity
ES	emulsion stability
HPC	Hemp protein concentrate
HPI	Hemp protein isolate
HSM	Hempseed meal or cake
PDCAAS	protein digestibility corrected amino acid score

A. Pihlanto (✉) · M. Nurmi · S. Mäkinen
Natural Resources Institute Finland, Jokioinen, Finland
e-mail: anne.pihlanto@luke.fi; markus.nurmi@luke.fi; sari.makinen@luke.fi

G. Crini, E. Lichtfouse (eds.), *Sustainable Agriculture Reviews 42*,
Sustainable Agriculture Reviews 42, https://doi.org/10.1007/978-3-030-41384-2_7

7.1 Introduction

There is an accruing body of evidence showing the need to shift toward a more plant-based diet for both environmental and health reasons. The global population is rapidly growing and simultaneously the demand for dietary protein, mainly of animal origin, is projected to increase by more than 50% by 2030 compared to the year 2000. The traditional Western dietary pattern focuses predominantly on animal-based products to satisfy protein requirements. This diet is environmentally detrimental, as it relies on intensive livestock farming, which contributes to the depletion of natural resources. Besides, the high intake of meat, especially red and processed meat, is associated with a higher incidence of coronary heart diseases, type 2 diabetes mellitus and several forms of cancer (Wolk 2017).

The development and market of the new meat and dairy analogue has accelerated in recent years, with some of the most promising alternatives based on plant sources such as soybeans and peas. Plant protein-based meat and dairy substitutes can deliver high nutritional and sensory quality, and at the same time fulfill the World's priority challenge of reducing greenhouse gas emissions and limiting the destruction of forestland (Dijkstra et al. 2003).

The production and consumption of food that includes high-protein crops may contribute toward achieving a sustainable diet. As reviewed by Callaway (2004) hemp has been an important source of food, fiber, and medicine for thousands of years. Hemp grows well in a variety of climates and soil types and has a short maturity period of 3–4 months. It also absorbs carbon dioxide up to five times more efficiently (thus slow global warming) than the same acreage of forest trees.

Hemp is a multipurpose crop due to its considerable amounts of over 30% oil and about 25% of easily digested protein containing significant amounts of all essential amino acids (Callaway 2004; Kriese et al. 2004; Teh and Birch 2013). After the oil is extracted from the food-grade hempseeds by cold pressing technology, the remaining hempseed meal contains high protein, fibre and carbohydrate amount as illustrated in Table 7.1. Milled hempseed meal is nowadays offered commercially as a source of vegetable protein and dietary fibre in the form of different products such as protein powders, flours, shake drinks, snacks, etc. Over the past few years, the availability of non-drug varieties with low δ-9-tetrahydrocannabinol (THC) contents has increased its industrial utilization for food product manufacture.

This review describes the properties of hemp seed as a food source and the current knowledge on producing functional protein concentrates from hemp seed.

7.2 Hempseed as Food

According to House et al. (2010), the crude protein concentration ranged between 21.3% and 27.5% in fresh whole hempseed products, 30.3–38.7% in dehulled hempseeds and 31–53.3% in hemp seed meal. The protein is concentrated in the

Table 7.1 Typical nutritional content (%) of hempseed

	Whole seed[a]	Seed meal[a]	Seed cake[b]	Whole seed[c]
Oil (%)	35.5	11.1	14.0	34.6
Protein	24.8	33.5	33.5	25.6
Carbohydrates	27.6	42.6	22.1	34.4
Moisture	6.5	5.6	7.1	6.7
Ash	5.6	7.2	5.9	5.4
Energy (kJ/100 g)	2200	1700	ND	2301
Total dietary fiber (%)	27.6	42.6	14.4	33.8
Soluble fiber	5.4	16.4	ND	2.9
Non-soluble fiber	22.2	26.2	ND	30.9

[a]Callaway 2004; [b]Teh et al. 2014; [c]Mattila et al. 2018 ND not determined

inner parts of the seed because the hulls contain lower levels of protein, 8.8–16.3% (Mattila et al. 2018). The crude protein content of commercial hemp seed flour was also analyzed by Multari et al. (2016) and their result was 38.6%. There was only a minor variation in the protein content between the ten industrial cultivars grown in southern Quebec (23.8–28.0% fresh weight) (Vonapartis et al. 2015). The quality of the hempseed protein is high and comparable to high-quality proteins sources such as egg white and soybean (Callaway 2004).

Besides the protein content, also the total carbohydrates content of whole hempseed (cv Finola) and cold-pressed hempseed meal has been reported to be high, 27.6% and 42.6%, respectively (Table 7.1). Hempseed is also a rich source of dietary fibre, especially non-digestible.

The mean results for oil content by House et al. (2010) for 11 hempseed products, by Vonapartis et al. (2015) for 10 industrial hemp cultivars and by Galasso et al. (2016) for seeds of 20 hemp traits and cultivars were 30.4%, 29.2%, and 31.9%, respectively. The hemp seed oil has a unique fatty acid composition. The seed oil typically contains over 90% unsaturated fatty acids which have large amounts of essential fatty acids (EFAs) which cannot be synthesized by humans.

7.2.1 Hempseed Proteins

Hempseed protein consists mainly of globulin (edestin) and albumin. Edestin accounts for approximately 60–80% of the total protein content, while albumin constitutes majority of the rest (House et al. 2010).

7.2.1.1 Globulins

The globular edestin is located inside the aleurone grains as large crystalloidal substructures and is composed of six identical subunits and each subunit consists

of acidic and basic subunits linked by one disulphide bond (Patel et al. 1994). Hemp seed legumin consists of mainly the 11S and 7S protein types, which can be separated using the pH shifting technique as described by Wang et al. (2008). The molecular weight (MW) of edestin is estimated to be approximately 300 kDa. The acid subunit is approximately 34.0 kDa and is relatively homogeneous, while basic subunit consists mainly of two subunits of about 20.0 and 18.0 kDa (Wang et al. 2008). Kim and Lee (2011) isolated and characterized the edestin protein from Korean variety. The first seven and six amino acid residues of the acid subunit had a sequence of Ile-SerArg-Ser-Ala-Val-Tyr in the N-terminus, while two constituents of basic subunit showed an identical N-terminus of Gly-Leu-Glu-Glu-Thr-Phe. Wang et al. (2008) isolated the 7S and 11S fractions using a similar extraction method than for 7S and 11S fractions of soy protein isolate (SPI). The main component in hemp 11S is edestin, basic subunit and a subunit of about 4.8 kDa, makes up the 7S. Further analysis showed that the 7S polypeptide has no thermal transition, while the 11S protein exhibits a similar denaturation temperature as hemp protein isolate (HPI) at 91.9 °C, indicating that the HPI thermal property was due mainly to the 11S component (Wang et al. 2008).

7.2.1.2 Albumins

The albumin fraction constitutes about 25% of hempseed storage protein. Malomo and Aluko (2015a) found that the albumin fraction contains few disulfide-bonded proteins and hence a less compact structure with greater flexibility than the globulin fraction. This was further confirmed by intrinsic fluorescence and circular dichroism analysis which illustrated greater exposures of tyrosine residues when compared with globulin. On the other hand, albumins had highly ordered secondary structure and very little tertiary conformation at pH 3.0 but the tertiary conformation increased at higher pH values. The high degree of flexibility and ordered secondary structure are probably structural factors that contribute to the high solubility and foaming capacity of albumin in comparison to the more compact or aggregated globulin.

7.2.1.3 Other Proteins

Additionally, a methionine- and cysteine-rich seed protein (10 kDa protein, 2S albumin) has been isolated from hempseed. The protein consisted of two polypeptide chains (small and large) with 27 and 61 amino acid residues, respectively (Odani and Odani 1998). The two polypeptide chains contain 18% by weight of sulfur-containing amino acids (cysteine and methionine) and are held together by two disulfide bonds. This protein had no trypsin inhibitory activity and could serve as a rich thiol source to improve the nutritional quality of plant-based foods since various plant food proteins, especially legumin proteins from soybean, pea, and beans, are deficient in sulfur. The gene families encoding the precursor polypeptides of 2S albumin have recently been identified by Ponzoni et al. (2018), and two genomic

isoforms for 2S albumin were obtained, namely, Cs2S-1 and Cs2S-2. The alignment of the deduced gene with the mature 2S protein sequence published in the literature (Odani and Odani 1998) showed that Cs2S is 97% identical to the mature 2S protein.

There is not much information available concerning protease inhibitors in hemp. Pojić et al. (2014) and Mattila et al. (2018) were able to measure trypsin inhibitory activity from HSM, seed hull and whole hemp seeds.

7.2.1.4 Protein Quality for Food

In human nutrition, the quality of a protein is defined by (1) the relative contribution that the amino acids contained in the protein make to an individual's amino acid requirement and (2) the digestibility of the protein. Hempseed and products derived from it contain all essential amino acids required by humans. The respective amino acid scores are presented in Fig. 7.1. The amino acid score of a protein reflects the extent to which a dietary protein meets the needs of an individual for a particular amino acid. The essential amino acids of hempseed are comparable to other high-quality proteins, such as casein and soy protein (Tang et al. 2006), and are sufficient for the Food and Agriculture Organization (FAO)/World Health Organization (WHO) suggested requirements for 2- to 5-year-old children. Hemp protein contains an exceptionally high amount of arginine and glutamine (Lu et al. 2010). Arginine accounts for approximately 12% of hempseed protein when compared with less than 7% for most other food proteins, including the proteins from potato, wheat, maize, rice, soy, rapeseed, egg white, and whey (Callaway 2004). Furthermore, whole hempseed and hemp protein products contain excellent amounts of the sulfur

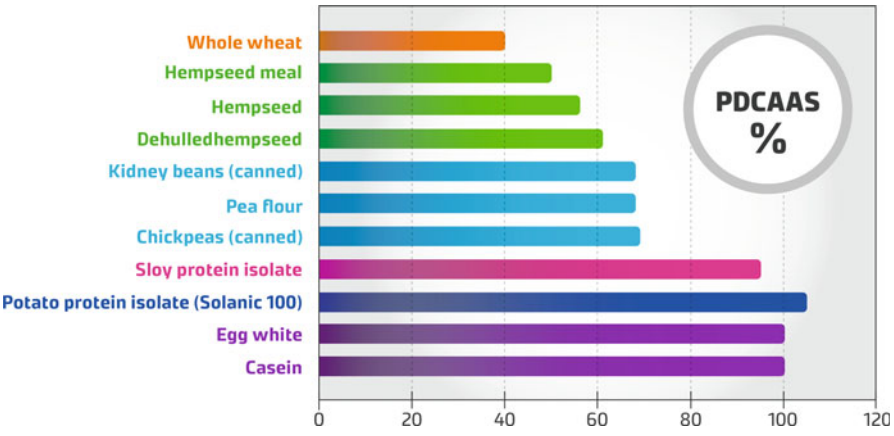

Fig. 7.1 Protein digestibility-corrected amino acid scores (PDCAAS) of hempseed protein sources in comparison to other food proteins. The values are from FAO/WHO (2011), House et al. 2010 and https://www.avebe.com/nutritional-value-of-potato-protein/

amino acids cysteine and methionine (Callaway 2004; Russo and Reggiani 2013; Mattila et al. 2018). Total sulfur-containing amino acids are in the range of 3.5–5.9%, which is close to the reference protein profiles established by United Nations University (UNU) as requirements for infants and preschool children 2- to 5-year-old. House et al. (2010) calculated the respective amino acid scores of hemp protein, identifying that lysine (score 0.5–0.62) was the first-limiting amino acid in hemp protein, followed by leucine and tryptophan.

Digestibility of dietary proteins affects the bioavailability of amino acids and thus, is a critical factor for the nutritional quality of the proteins. The digestion of dietary proteins depends on enzyme accessibility, which is affected by the molecular structure as well as other components associated with proteins. House et al. (2010) measured the protein digestibility and corrected amino acid score (PDCAAS) of whole hempseed, dehulled hempseed and HSM using a rat bioassay for protein digestibility and the FAO/WHO amino acid requirement of children (2–5 years of age) as reference. The protein digestibility of dehulled hempseed, depending on the sources, was 90.8–97.5%, almost comparable to 97.6% for casein. The PDCAAS values for hemp protein sources varied between 0.48 and 0.61. These values are within the range of major pulse proteins and are above the values of cereal grain products. Lysine as the main limiting amino acid in hempseeds and rather low level of tryptophan presumably contribute to the relatively low PDCAAS score.

Wang et al. (2008) compared the digestibility of hempseed protein isolates (7S, and 11S) and soy protein isolate using an *in vitro* digestion model. During the pepsin digestion, edestin was rapidly degraded similarly to the digestion of the soy protein isolates, and oligo-peptides with MWs less than 10 kDa were released. In addition, the total digestibility (pepsin plus trypsin digestion) of hempseed protein isolates (88–91%) was distinctly higher than that of soy protein isolate (71%). The most recent studies by Mamone et al. (2019) showed that hemp flour and protein isolate had a high degree of digestibility on a molecular basis. These findings hint that HPI is an efficient source of protein nutrition for human consumption.

7.3 Processing, Isolation, and Concentration of Protein from Hemp Seed

Hempseed proteins must be extracted or otherwise enriched from seeds, press cake or meal before application as food ingredient due to their limited functional properties, color and antinutritive compounds present in the raw materials. Oil has been identified as one of the major components limiting the extraction of plant protein, particularly in alkaline extraction in which formation of lipid-protein complexes hinder the recovery of protein (Manamperi et al. 2011). Processing options for producing hempseed protein products are illustrated in Fig. 7.2.

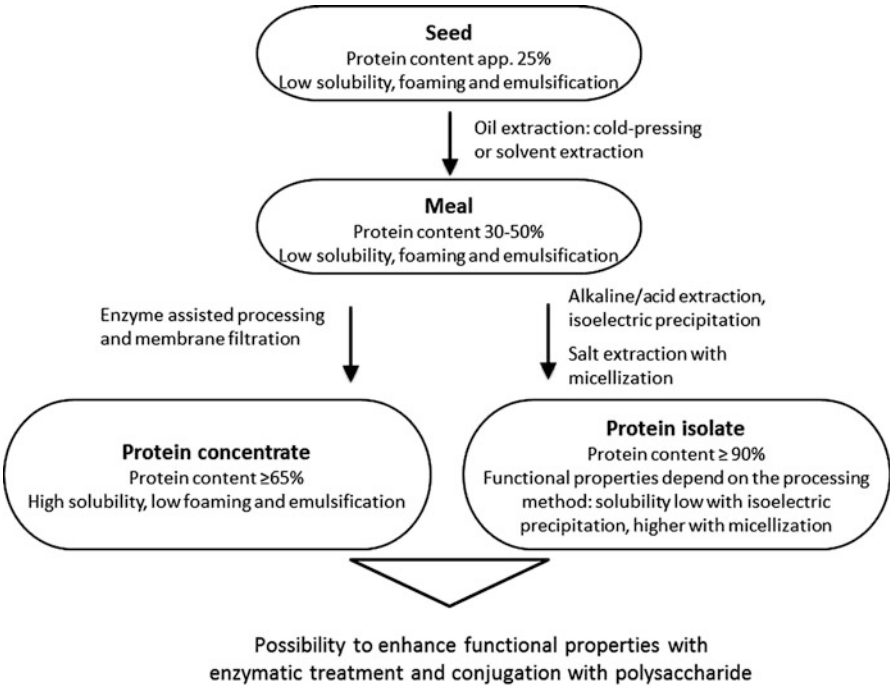

Fig. 7.2 Processing options to produce hempseed protein concentrates and isolates from hempseed

7.3.1 Hempseed Meal

The oil extraction by-product of crushed hempseeds is commonly referred to as hempseed meal or cake. The protein content in hempseed meal ranges from 30% to 50% in the dry matter depending on the used hempseed variety, the oil extraction method (cold-pressing or solvent extraction) and efficiency (Malomo et al. 2014).

7.3.2 Hemp Protein Concentrate

Hemp protein concentrate is prepared from dehulled and defatted hempseed or hempseed meal by removing most of the water-soluble, nonprotein constituents. The protein concentrates contains at least 65% protein (N × 6.25) on a dry weight basis. Malomo and Aluko (2015b) obtained protein concentrate by enzymatic digestion of fiber using carbohydrase and phytase coupled with membrane ultrafiltration that enriched protein content up to 70%. Protein digestibility of the protein concentrate was significantly higher than that of meal and traditional isoelectric protein isolate.

7.3.3 *Hemp Protein Isolate*

The most purified and enriched form of the commercial protein product, protein isolate (>90% protein), is prepared to meet food processing needs with minimal influence of unwanted non-protein components. The applied extraction method will affect the final protein content of the protein isolate, composition, and functionality. Alkaline extraction followed by isoelectric precipitation is the most common method to prepare hemp protein isolates (Lu et al. 2010; Malomo et al. 2014; Wang et al. 2008). Depending on specific extraction conditions (e.g pH, temperature, time), a purity up to 94% can be obtained. At the alkaline extraction, pH is generally 9–10, since the native hempseed proteins are tightly compacted, and may be integrated with other components, like phenolic compounds and phytic acid.

Several studies have reported that elevated extraction temperatures can improve protein solubility. However, an adverse chemical reaction such as the formation of lysinoalanine compounds from cysteine and serine residues can occur at highly alkaline conditions during heating. For example, protein isolates extracted at pH 10 and room temperature had a low level of lysinoalanine (0.8 mg/100 g protein) but at pH 12 at 40 °C for 5 min, the lysinoalanine content increased to 4 mg/100 g protein (Wang et al. 2018).

Teh et al. (2014) used acid extraction to prepare protein isolates. The yield of protein extracted at acidic pH was lower than that extracted at alkaline pH. In addition, a method, known as "salt extraction with micellization", has been described by Dapčević-Hadnađev et al. (2018). Protein isolate obtained by this method has a very high purity (98.9% protein, on a dry basis).

However, the limited functional properties, especially protein solubility, reduce the application of this protein in food formulations (Tang et al. 2006). The poor functional properties have been linked to the formation of covalent disulfide bonds between individual proteins and subsequent aggregation at neutral or acidic pH, due to its high free sulfhydryl content from sulfur-containing amino acids. The effects of limited or extensive enzymatic protein hydrolysis as a means of improving functional properties of hemp seed proteins have been reported (Yin et al. 2008).

7.4 Functional Properties of Hemp Seed Products

Besides nutritional value, the quality of food proteins is often determined by their techno-functional properties. The value and usefulness of a protein ingredient depend on its functionality, namely, its behavior and performance in food systems during preparation, processing, storage, and consumption. The functional properties of proteins depend on the nature and extent of interactions among protein molecules and with the presence of other components (for example, water and oil) in the food

system. Since many formulated foods exist as beverages, emulsions, foams or solids, the ability of proteins to bind water or fat and their solubility, foaming, gelation, emulsification and film formation properties are essential to the quality of food products. Although the functional properties of hemp seed proteins have not yet been extensively studied, some properties have been reported. Below is summarized the most studied functional properties (solubility, foaming, and emulsification) of hempseed products.

7.4.1 Solubility

Solubility and dispersion stability of proteins are important factors that influence several other functional properties. Proteins are generally most soluble at their native state, whereas denaturation by heat, shear or chemicals may promote aggregation due to changed surface properties of proteins in the unfolded state. Hemp seed protein products have shown a typical U-shaped pH-solubility profile, with the minimum between pH 4.0 and 7.0 (Malomo et al. 2014; Tang et al. 2006; Dapčević-Hadnađev et al. 2018). In general, hemp seed protein exhibits low solubility in comparison to other plant proteins, such as soy. The low solubility is attributed to edestin aggregation at pH below 7 (Tang et al. 2006; Malomo et al. 2014). Albumin fraction has minimum solubility at pH 3.0 and globulin fraction at pH 5.0 (Malomo and Aluko 2015a, b). Albumin fraction has a higher solubility than globulin fraction due to the reduced level of aromatic and hydrophobic amino acids or a high isoelectric point when compared to the globulin fraction. Protein isolate made by isoelectric precipitation showed low solubility across the pH 3 to 9 (Malomo et al. 2014; Malomo and Aluko 2015a) or pH 4 to 7 (Dapčević-Hadnađev et al. 2018). The hemp seed protein product made by micellization had higher solubility across the pH range from 3.0 to 5.0 when compared to protein isolates made by isoelectric precipitation. In general, micelle protein isolates were found to have significantly higher solubility compared to those prepared by isoelectric precipitation. This was ascribed to the more preserved native state of protein isolates extracted using the micellization technique, while the isoelectric precipitation method led to protein denaturation and thereby resulted in hydrophobic interactions among protein molecules and formation of insoluble aggregates. HSM proteins are also insoluble, mainly because crosslinking by phytate and high levels of insoluble fiber that reduce the protein-water interactions. On the other hand, protein concentrates have shown higher solubility, indicating that less denaturation has occurred during processing. Especially using membrane ultrafiltration to prepare protein concentrates, up to 74% protein solubility was seen at pH 4.0–5.0 (Malomo and Aluko 2015a, b). The results indicated differences in polypeptide types, especially the absence of protein aggregation.

7.4.2 Emulsification

Emulsifying properties of proteins are related to many factors, for example, the rate of protein adsorption at the oil-water interface, the amount of protein adsorbed (loading), the conformational rearrangement at the interface, the extent of interfacial tension reduction, and the rheology of the cohesive film. A number of quality indexes, such as emulsifying activity (EA), emulsifying capacity (EC), emulsion stability (ES), and droplet size, are commonly used to evaluate the emulsifying properties of proteins. Even when proteins are not fully soluble, they may form stable dispersions eg. gels, foams and emulsions.

Reports on the EA of hemp seed protein products are highly variable (Tang et al. 2006; Malomo and Aluko 2015a, b), most probably due to different protein extraction procedures causing variation in the edestin and albumin ration and state of the proteins. Malomo and Aluko (2015b) showed that protein concentrates had generally a low EC, and the size of oil droplets was typically 6–15 μm while protein isolates formed emulsions with oil droplet sizes less than 1 μm. The results show, that the technique used to prepare the product influences on the emulsifying properties. Protein isolates preserved the native state in the isolation process and thus, were able to form emulsions with small droplets with sufficient droplet-droplet static repulsion properties. The protein isolates stabilized emulsions possessed low viscosity which enabled fast droplet movement and led to increased creaming and coalescence at lower protein concentrations (0.25–0.75% w/w). The isolation technique favored pH-induced structural unfolding of protein molecules and exposure of hydrophobic sites and sulfhydryl groups. Subsequently, protein connected droplet aggregates were formed during emulsification.

7.4.3 Foaming Properties

Foaming properties of protein isolates are mainly determined by their molecular flexibility and ability to reduce surface tension. Factors such as protein concentration, solubility, and hydrophobicity/hydrophilicity ratio can also influence on the foaming properties (Damodaran 2006; Malomo et al. 2014). The differences in structural conformation of hemp seed protein products at different pH values were reflected as different foaming capacities. Products made from protein isolates had better foaming capacity at pH 3.0 when compared to pH 5.0, 7.0 and 9.0 (Malomo et al. 2014). Albumin fraction had significantly higher foaming capacity than globulin fraction at pH range from 3.0 to 9.0 (Malomo and Aluko 2015a). The higher foaming capacity of the albumin is consistent with the observed higher solubility and suggests that greater interactions with the aqueous phase enhance the ability of the protein molecules to encapsulate air particles. This is because interactions with the hydrophilic aqueous phase will enhance protein unfolding and hence increase the foam forming ability (Sai-Ut et al. 2009). In hemp seed meals,

foaming capacity improved as the environment changed from pH 3.0 to 9.0, which suggests increased interaction with water and protein unfolding at neutral and alkaline pH values. The foaming capacity and stability of the meal were inferior when compared to the purer protein isolates (Malomo and Aluko 2015a). Interference from nonprotein materials may have reduced the ability of meal proteins to form and stabilize foams.

7.5 Processing to Improve Functional Properties

As described above, due to the compact structure, hemp seed protein generally has limited functionality and industrial application when compared to many other food proteins. Therefore, structural modifications to improve the functionality are needed. Several physical, chemical, and enzymatic treatments have been applied to hemp protein, of which heat treatment, enzymatic hydrolysis, acylation, and pH shift have demonstrated promising efficacies.

Raikos et al. (2015) heated protein isolates from 40 to 100 °C for 10 min to modify the protein structure. Heating at 80 °C or higher temperatures produced insoluble large molecular aggregates via covalent linkages. Approximately 60–80% of the native protein was converted to an aggregated state. A similar observation was reported by Yin et al. (2008), who heated protein isolates at 95 °C for 10 min, noting that the thermally treated isolates had a significantly lower protein solubility than untreated isolates at pH 3.0 to 10.0. However, Wang et al. (2018) found that heating at 80 °C up to 60 min actually slightly improved the solubility of protein isolate. This might be due to different testing methods, as well as the protein composition. Heat treatment increased EA of protein isolates at pH values away from the isoelectric point, water holding capacity and the foam stability but did not affect the foaming capacity (Yin et al. 2008).

Several studies have been published on applying enzymes to improve the functionality of protein isolates (Tang et al. 2009; Wang et al. 2009; Yin et al. 2008). The studies are mainly focused on enhancing the antioxidant properties with commercial proteases, such as alcalase, flavourzyme, neutrase, protamex, pepsin, and trypsin (Tang et al. 2009; Wang et al. 2009), however, trypsin has also been applied to modify the protein solubility, water holding, emulsifying and foaming properties (Yin et al. 2008). Trypsin treatment to hydrolysis degree of 2–6% increased remarkably protein solubility but decreased emulsifying properties, water holding and fat absorption capacity (Yin et al. 2008). The results suggest, that mild protease treatment to enhance protein solubility could be combined to further treatment with transglutaminase or Maillard reaction with polysaccharide to improve the other functional properties (e.g. Martineza et al. 2005). This approach might be useful on producing hemp protein ingredients with diverse functional properties.

For plant proteins in general, conjugation with polysaccharides is suggested to be a feasible option for improving functional properties, reviewed recently by Akhtar and Ding (2017). Linking of proteins with polysaccharides has been reported to

enhance the functional properties of native proteins in different circumstances, such as low and neutral pH conditions and with coloring agents (Liu et al. 2012). Alternative processing technologies, such as a spinning disc reactor, have also been applied for producing the protein-polysaccharide conjugates. In the spinning disc reactor, there is no need for additives and freeze drying, the benefits include decreased processing time and lower energy need (Burns and Jachuck 2003). Although the benefits of conjugating proteins with polysaccharides have been reported widely, more research is needed to achieve a better understanding on the conjugate structure-function relationship and also, the possible effects of the conjugates on digestion and gut health.

7.6 Conclusions

Due to hemp's well-recognized nutritional value, food manufacturers have developed a wide range of retail products from hemp, such as nuts, oil, protein flour, energy bars, granola, hemp nut butter, pasta, and ice cream (Leson 2006). A recent emphasis has been on hemp seed protein, which is used not only as a nutritive additive but also as a functional ingredient in formulated foods to enhance the product quality attributes. The low allergenicity of hemp protein when compared with most of the other plant proteins also permits it as a substitute for other proteins in some food product. The use of hemp seed protein products as an alternative to the commonly used casein, whey, wheat, and soy protein is on a rise. For instance, some studies have shown that hemp seed protein products can be used as value-added ingredients in the production of bread with increased protein and macro- and microelement contents, and lower baking loss and baking time (Korus et al. 2017; Lukin and Bitiutskikh 2017; Pojić et al. 2015). The key to keeping hemp protein competitive in the plant protein market is to assure its nutritional value, functionality, safety, and acceptable sensory characteristics.

Hemp seeds, an emerging protein-rich plant material, is becoming an important alternative protein source in the food due to consumers worldwide interest and demand of plant proteins are expected to grow rapidly. Although research has made progress in recent years in understanding the chemical composition, nutritional and health benefits, processing properties, and functional behavior of hemp seed proteins in food processing, much remains unknown about it. Therefore, it is clear that more systematic research is required to explore the structure–functionality relationship of hemp protein, technologies to modify functionalities must be vigorously explored through scientific research to convert hemp protein into a more suitable form. Additional research is also needed to investigate the health benefits. The research is essential to develop this valuable protein source and broadening its market potential in the food industry.

Acknowledgements We are grateful for funding from the Strategic Research Program (SRC) Academy of Finland (grant numbers 293045 and 314243 ScenoProt, Novel protein Sources of Food Security).

References

Akhtar M, Ding R (2017) Covalently cross-linked proteins & polysaccharides: formation, characterisation and potential applications. Curr Opin Colloid Interface Sci 28:31–36. https://doi.org/10.1016/j.cocis.2017.01.002

Burns JR, Jachuck RJJ (2003) Recent advances on spinning disc reactor technology: better processes for bigger profits. 5th international conference on process intensification. BHR Group, Maastricht

Callaway J (2004) Hemp seed as a nutritional resource: an overview. Euphytica 140:65–72. https://doi.org/10.1007/s10681-004-4811-6

Damodaran S (2006) Protein stabilization of emulsions and foams. J Food Sci 70:R54–R66. https://doi.org/10.1111/j.1365-2621.2005.tb07150.x

Dapčević-Hadnađev T, Hadnađev M, Lazaridour A, Moschakis T, Biliaderis CG (2018) Hemp seed meal protein isolates prepared by different isolation techniques. Part II. Gelation properties at different ionic strengths. Food Hydrocoll 81:481–489. https://doi.org/10.1016/j.foodhyd.2018.03.022

Dijkstra DS, Linnemann AR, van Boekel TA (2003) Towards sustainable production of protein-rich foods: appraisal of eight crops for western Europe. PART II: analysis of the technological aspects of the production chain. Crit Rev Food Sci Nutr 43:481–506. https://doi.org/10.1080/10408690390246332

FAO/WHO Consultation, FAO Expert (2011) Dietary protein quality evaluation in human nutrition. FAO Food Nutr Pap 92:1–66

Galasso I, Russo R, Mapelli S, Ponzoni E, Brambilla IM, Battelli G, Reggiani R (2016) Variability in seed traits in a collection of *Cannabis sativa L.* genotypes. Front Plant Sci 7:688. https://doi.org/10.3389/fpls.2016.00688

House JD, Neufeld J, Leson G (2010) Evaluating the quality of protein from hemp seed (*Cannabis sativa L.*) products through the use of the protein digestibility-corrected amino acid score method. J Agric Food Chem 58:11801–11807. https://doi.org/10.1021/jf102636b

Kim JJ, Lee MY (2011) Isolation and characterization of edestin from Cheungsam hemp seed. J Appl Biol Chem 54:84–88. https://doi.org/10.3839/jabc.2011.015

Korus J, Witczak M, Ziobro R, Juszczak L (2017) Hemp (*Cannabis sativa* subsp. *sativa*) flour and protein preparation as natural nutrients and structure forming agents in starch based gluten-free bread. LWT-Food Sci Technol 84:143–150. https://doi.org/10.1016/j.lwt.2017.05.046

Kriese U, Schumann E, Weber W, Beyer M, Brühl L (2004) Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa L.* genotypes. Euphytica 137:339–351. https://doi.org/10.1023/b:euph.0000040473.23941.76

Leson G (2006) Hemp foods in North America: status and joint industry research. J Ind Hemp 11:87–93. https://doi.org/10.1300/j237v11n01_08

Liu J, Ru Q, Ding Y (2012) Glycation a promising method for food protein modification: physicochemical properties and structure, a review. Food Res Int 49:170–183. https://doi.org/10.1016/j.foodres.2012.07.034

Lu R-R, Qian P, Sun Z, Zhou X-H, Chen T-P, He J-F, Zhang H, Wu J (2010) Hemp seed protein derived antioxidative peptides: purification, identification and protection from hydrogen peroxide-induced apoptosis in PC12 cells. Food Chem 123:1210–1218. https://doi.org/10.1016/j.foodchem.2010.05.089

Lukin A, Bitiutskikh K (2017) On potential use of hemp flour in bread production. Bull Transilvania University of Brasov For Wood Ind Agric Food Eng Series II 10:113–118

Malomo SA, Aluko RE (2015a) A comparative study of the structural and functional properties of isolated hemp seed (*Cannabis sativa L.*) albumin and globulin fractions. Food Hydrocoll 43:743–752. https://doi.org/10.1016/j.foodhyd.2014.08.001

Malomo SA, Aluko RE (2015b) Conversion of a low protein hemp seed meal into a functional protein concentrate through enzymatic digestion of fibre coupled with membrane ultrafiltration. Innov Food Sci Emerg Technol 31:151–159. https://doi.org/10.1016/j.ifset.2015.08.004

Malomo SA, He R, Aluko RE (2014) Structural and functional properties of hemp seed protein products. J Food Sci 79:C1512–C1521. https://doi.org/10.1111/1750-3841.12537

Mamone G, Picariello G, Ramondo A, Nicolai MA, Ferranti P (2019) Production, digestibility and allergenicity of hemp (*Cannabis sativa L.*) protein isolates. Food Res Int 115:562–571. https://doi.org/10.1016/j.foodres.2018.09.017

Manamperi WA, Wiesenborn DP, Chang SK, Pryor SW (2011) Effects of protein separation conditions on the functional and thermal properties of canola protein isolates. J Food Sci 76: E266–E273. https://doi.org/10.1111/j.1750-3841.2011.02087.x

Martineza KD, Baeza RI, Millán F, Pilosof AMR (2005) Effect of limited hydrolysis of sunflower protein on the interactions with polysaccharides in foams. Food Hydrocoll 19:361–369. https://doi.org/10.1016/j.foodhyd.2004.10.002

Mattila P, Mäkinen S, Eurola M, Jalava T, Pihlava JM, Hellström J, Pihlanto A (2018) Nutritional value of commercial protein-rich plant products. Plant Food Hum Nutr 73:108–115. https://doi.org/10.1007/s11130-018-0660-7

Multari S, Neacsu M, Scobbie L, Cantlay L, Duncan G, Vaughan N, Stewart D, Russell WR (2016) Nutritional and phytochemical content of high-protein crops. J Agric Food Chem 64:7800–7811. https://doi.org/10.1021/acs.jafc.6b00926

Odani S, Odani S (1998) Isolation and primary structure of a methionine-and cystine-rich seed protein of Cannabis sativa. Biosci Biotechnol Biochem 62:650–654. https://doi.org/10.1271/bbb.62.650

Patel S, Cudney R, McPherson A (1994) Crystallographic characterization and molecular symmetry of edestin, a legumin from hemp. J Mol Biol 235:361–363. https://doi.org/10.1016/s0022-2836(05)80040-3

Pojić M, Mišan A, Sakač M, Dapčević Hadnađev T, Šarić B, Milovanović I, Hadnađev M (2014) Characterization of byproducts originating from hemp oil processing. J Agric Food Chem 62:12436–12442. https://doi.org/10.1021/jf5044426

Pojić M, Dapcevic Hadnađev T, Hadnađev M, Rakita S, Brlek T (2015) Bread supplementation with hemp seed cake: a by-product of hemp oil processing. J Food Qual 38:431–440. https://doi.org/10.1111/jfq.12159

Ponzoni E, Brambilla I, Galasso I (2018) Genome-wide identification and organization of seed storage protein genes of Cannabis sativa. Biol Plant 62:693–702. https://doi.org/10.1007/s10535-018-0810-7

Raikos V, Duthie G, Ranawana V (2015) Denaturation and oxidative stability of hemp seed (*Cannabis sativa L.*) protein isolate as affected by heat treatment. Plant Food Hum Nutr 70:304–309. https://doi.org/10.1007/s11130-015-0494-5

Russo R, Reggiani R (2013) Variability in antinutritional compounds in hemp seed meal of Italian and French varieties. Plant 1:25–29. https://doi.org/10.11648/j.plant.20130102.13

Sai-Ut S, Ketnawa S, Chaiwut P, Rawdkuen S (2009) Biochemical and functional properties of proteins from red kidney, navy and adzuki beans. Asian J Food Agro-Ind 2:493–504

Tang C, Ten Z, Wang X, Yang X (2006) Physicochemical and functional properties of hemp (*Cannabis sativa L.*) protein isolate. J Agric Food Chem 54:8945–8950. https://doi.org/10.1021/jf0619176

Tang CH, Wang X-S, Yang X-Q (2009) Enzymatic hydrolysis of hemp (*Cannabis sativa L.*) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. Food Chem 114:1484–1490. https://doi.org/10.1016/j.foodchem.2008.11.049

Teh S, Birch J (2013) Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. J Food Compos Anal 30:26–31. https://doi.org/10.1016/j.jfca.2013.01.004

Teh SS, Bekhit ADE, Carne A, Birch J (2014) Effect of the defatting process, acid and alkali extraction on the physicochemical and functional properties of hemp, flax and canola seed cake protein isolates. J Food Meas Charact 8:92–104. https://doi.org/10.1007/s11694-013-9168-x

Vonapartis E, Aubin M, Seguin P, Mustafa AF, Charron J (2015) Seed composition of ten industrial hemp cultivars approved for production in Canada. J Food Compos Anal 39:8–12. https://doi.org/10.1016/j.jfca.2014.11.004

Wang XS, Tang CH, Yang XQ, Gao WR (2008) Characterization, amino acid composition and in vitro digestibility of hemp (*Cannabis sativa L.*) proteins. Food Chem 107:11–18. https://doi.org/10.1016/j.foodchem.2007.06.064

Wang XS, Tang CH, Chen L, Yang X-Q (2009) Characterization and antioxidant properties of hemp protein hydrolysates obtained with Neutrase®. Food Technol Biotechnol 31:428–434

Wang Q, Jin Y, Xiong YL (2018) Heating-aided pH shifting modifies hemp seed protein structure, cross-linking, and emulsifying properties. J Agric Food Chem 66:10827–10834. https://doi.org/10.1021/acs.jafc.8b03901

Wolk A (2017) Potential health hazards of eating red meat. J Intern Med 281:106–122. https://doi.org/10.1111/joim.12543

Yin S, Tang C, Cao J, Hu E, Wen Q, Yang X (2008) Effects of limited enzymatic hydrolysis with trypsin on the functional properties of hemp (*Cannabis sativa L.*) protein isolate. Food Chem 106:1004–1013. https://doi.org/10.1016/j.foodchem.2007.07.030