Chapter

Modification of Legume Proteins for Improved Functionality

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Abstract

Recent studies have indicated that legume proteins can be potentially used as an alternative to animal-derived protein ingredients for many food and biomaterial applications, however some modifications may be first required to improve their functionality since they show relatively lower solubility and functional properties compared to commonly used animal-based proteins. A variety of physical, chemical or biological processes can be used to achieve these modifications in structural, physicochemical, and functional properties of legume proteins. The aim of this chapter was to review the most recent studies focusing on modification of structural properties and improvement of functionality of legume proteins. Effects of processing conditions on protein functionality were discussed. Special emphasis was given to the structure–function mechanisms behind these changes. Since the performance of modified legume proteins has been shown to depend on a variety of factors; parameters used in the modification process have to be optimized to achieve the desired level of improvement in legume protein functionality. Each modification method has been indicated to have its own advantages and limitations in terms of performance and applicability in different food matrices. Further studies are required to investigate the interactions of modified legume proteins with other food components during food processing and storage. Furthermore, additional research on the effects of modification treatments on flavor profile and nutritional properties of legume proteins is needed as well.

Keywords: legume protein, modification, structure, functional properties, physicochemical properties

1. Introduction

Legumes belong to the *Fabaceae* family plants and involve different species including peas, beans, lentils, and chickpeas [1]. Legume proteins have gained the interest of food industry due to their low cost, low risk of allergy, good functional and nutritional properties [2]. Their potential use as ingredients in a variety of food applications has been widely investigated recently. However, due to their relatively lower solubility and performance compared to animal-derived proteins, some modifications may be required to obtain optimum functionality. There are many different techniques available for altering the structural properties and improvement of functionality of legume proteins. Each method has its unique advantages and limitations while selection of the most suitable method of modification depends on various factors including protein source and composition, processing conditions, feasibility, cost, and the application area of the modified protein.

Recently, Sharif et al. [3] reviewed the modification methods applied to legume proteins for improvement of emulsifying properties for encapsulation applications. Ge et al. [4] discussed the modifications applied specifically to pea protein for improved functionality. This chapter presents an overview from a broader perspective of the most commonly used methods for modification of functional properties of legume proteins, with a major focus on the most recent studies.

2. Physical modifications

Functional properties of legume proteins can be modified by application of a variety of physical methods including thermal treatment, ultrasonication, and high pressure. A brief summary of the findings of recent studies focusing on investigation of the effects of physical methods on functionality of legume proteins is presented in **Table 1**.

2.1 Thermal treatment

Thermal treatment is commonly applied to legume proteins for modification of structure and functionality. Tang et al. [5] applied heat treatment to kidney, red bean and mung bean protein isolates at 95 °C for 30 min and studied its effects on structural properties and functionality of the proteins. The authors observed heat-induced protein denaturation which was evident by the modifications in the secondary and tertiary protein structures. Heat treatment was indicated to increase the surface hydrophobicity of bean proteins due to unraveling of previously buried hydrophobic moieties. Although the authors observed protein denaturation and significant increase in hydrophobicity, solubility of heat-treated bean proteins was reported to increase as well. This finding was attributed to the possible increase in charged residues on protein surface due to denaturation and partial unfolding of the molecule. Emulsifying activity of heat-treated bean proteins was also reported to increase compared to the native proteins due to increased hydrophobicity and solubility after heat treatment. Peng et al. [8] investigated the effect of heat treatment (95 °C, 30 min) on surface hydrophobicity, interfacial properties, emulsifying activity and stability of pea proteins. It was reported that heat treatment increased surface hydrophobicity due to unraveling of protein structure and exposure of hydrophobic groups buried inside the molecule. Application of heat treatment was reported to result in slightly reduced interfacial tension and significantly higher percentage of adsorbed proteins at the interface compared to the native protein. Heat-treated pea protein was able to form emulsions with significantly smaller droplets and higher stability against creaming due to higher adsorption of proteins at the oil–water interface. Chao and Aluko [9] applied heat treatment to pea protein isolate at a wider temperature range (50–100 °C, 30 min) for modification of emulsifying and foaming properties. The authors observed changes in protein conformation and increased hydrophobicity which were attributed to protein unfolding. Those changes in protein structure were reported to lead to protein aggregation induced by thermal treatment up to 100 °C. Emulsion formation and stabilization by pea proteins were reported to improve at pH 7.0, which was indicated by reduced oil droplet size compared to the native protein. However, average oil droplet size of heat treated pea protein-stabilized emulsions were reported to be larger than that of the native protein-stabilized emulsions at pH 5.0. Foaming properties of pea protein were observed to be negatively affected by heat treatment regardless of the pH applied. In another recent study, Bühler et al. [7] applied dry heating to faba bean protein concentrate in an air oven (75–175 °C, 1 h) for modification of water holding capacity. The authors used

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Thermal treatment	Red kidney bean, red bean and mung bean proteins	95 °C, 30 min	Improved solubility and emulsifying activity	[5]
	Red kidney bean protein	95 °C, 15–120 min	Improved solubility, emulsifying and foaming activities with moderate heating (15–30 min)	[6]
	Faba bean protein	75–175 °C, 60 min	Increased water-holding capacity, decreased solubility	[7]
	Pea protein	95 °C, 30 min	Higher adsorption at the oil–water interface, improved creaming stability, increased viscosity	[8]
	Pea protein	50–100 °C, 30 min, pH 3.0–7.0	Improved emulsifying activity at pH 7.0, decreased foaming properties regardless of pH	[9]
Ultrasonication	Black bean protein	20 kHz, 150–450 W, 12–24 min	Improved solubility	[10]
	Soybean β-conglycinin and glycinin	20 kHz, 400 W, 5–40 min	Improved solubility, emulsifying activity, and stability	[11]
	Faba bean protein	20 kHz, 50–75% amplitude, 15–30 min	Higher adsorption at the oil–water interface, improved foaming properties	[12]
	Pea protein	20 kHz, 30–90% amplitude, 30 min	Improved foaming properties	[13]
	Chickpea protein	20 kHz, 300 W, 5–20 min	Improved solubility, emulsifying, foaming, water holding and gelling properties	[14]
High pressure	Kidney bean protein	200–600 MPa, 15 min	Improved water holding capacity, foaming, and emulsifying properties, increased viscosity	[15]
	Yellow field pea protein	200–600 MPa, 5 min	Improved emulsifying and foaming properties	[16]
	Lentil protein	25–150 MPa	Improved solubility, emulsifying and foaming properties with increasing pressure up to 100 MPa, improved gelling properties at 50–150 MPa	[17]
Other techniques	Pea protein	Controlled shear at 100–1500 s^{-1}	Improved solubility and heat stability	[18]
	Pea protein	Cold atmospheric pressure plasma	Increased water and fat binding capacities, improved solubility	[19]
	Grass pea protein	Cold atmospheric pressure plasma	Higher adsorption at the oil–water interface, improved emulsifying properties	[20]

Table 1.
Summary of recent studies on physical modification of legume proteins for improved functionality.

soy protein concentrate as a reference. Heat treatment was reported to induce partial denaturation, which in turn increased hydrophobicity and resulted in decreased protein solubility. On the contrary, water holding capacity of faba bean protein was observed to increase and became comparable to soy protein.

2.2 Ultrasonication

Ultrasonication has been recently investigated as a novel tool for modifying the structure and improving functionality of legume proteins. Gharibzahedi and Smith [21] recently reviewed the current applications and challenges of this technology for legume proteins. Jiang et al. [10] applied low-frequency (20 kHz) ultrasonication to black bean protein isolate. Primary structure of black bean protein was reported to remain unchanged after the ultrasonication treatment indicated by the electrophoresis profile. On the other hand, secondary structure of black bean protein was observed to change after ultrasonication where the proportion of α-helix decreased and β-sheet increased. Ultrasonication-related alterations in tertiary structure were monitored by the changes in fluorescence spectra. Average particle size of ultrasound-treated black bean protein was reported to be larger compared to that of native protein which was attributed to formation of aggregates. Net surface charge of ultrasonicated black bean protein was observed to change depending on the power applied. Surface hydrophobicity of black bean protein was also reported to increase after the ultrasonication treatment. Similar to heat treatment applications, ultrasonication is indicated to result in unfolding of the protein molecule up to a certain degree and increase hydrophobicity. However, surface hydrophobicity was reported to decrease with a further increase in the ultrasonication power due to aggregation of protein molecules which in turn enclose the hydrophobic sections of the protein. Ultrasonication treatment was reported to result in an increase in solubility of black bean protein, directly related to ultrasonication power and treatment time which was based on increased interaction between protein molecules and water. The authors concluded that the modifications on protein functionality could be optimized based on the ultrasonication conditions. Martinez-Velasco et al. [12] studied the effect of ultrasound treatment on surface characteristics and foaming properties of faba bean protein isolate. The authors optimized the parameters of ultrasonication treatment and reported that an amplitude of 73% and duration of 17 min resulted in lower interfacial tension, higher solubility, higher adsorption at the interface, smaller bubble diameter, and higher foam stability. In a similar study, Xiong et al. [13] investigated the effect of ultrasound treatment on structural characteristics and foaming ability and stability of pea protein isolate. Ultrasonication was reported to induce unfolding of the protein molecule up to a certain degree and hence increased hydrophobicity. These changes combined with decreased particle size were reported to result in decreased surface tension, improved foaming ability and stability after the treatment. In a recent study, Wang et al. [14] applied ultrasound treatment to chickpea protein isolate and investigated the changes in interfacial, physicochemical, and gelling properties. The authors observed increase in solubility, foaming capacity, emulsifying activity, and gel strength as a result of the ultrasonication treatment. Improved gelling properties of the ultrasonicated chickpea protein were attributed to the changes observed in hydrophobicity and particle size as a result of the ultrasonication treatment.

2.3 High pressure

Potential of high pressure application to modify the structure and functionality of legume proteins has been recently investigated by various researchers. Chao et al. [16]

applied high hydrostatic pressure (200–600 MPa) to yellow field pea protein isolate and determined its effects on physicochemical characteristics and functional properties of the protein. Gel electrophoresis patterns were observed to indicate aggregation due to increased interaction between protein molecules after high pressure application. High pressure treatment was reported to induce changes in the tertiary structure identified by fluorescence spectroscopy. The extent of structural changes depended on the level of pressure applied. The highest level of denaturation and unfolding of the molecule was observed at 600 MPa pressure. Despite those changes, protein solubility was found to remain unchanged. At the same time, emulsifying properties of yellow field pea protein were reported to be improved after the high hydrostatic pressure treatment. Mean droplet size of emulsions stabilized by high pressure-treated yellow field pea protein was reported to be significantly smaller compared to that of emulsions stabilized by untreated protein. Formation of smaller oil droplets was attributed to increased interactions between oil droplets and unraveled hydrophobic groups of high pressure-treated protein. Effect of high pressure treatment on emulsion stability was found to change depending on protein concentration and pH. Specifically, at relatively lower protein concentrations, pH 3.0 and 5.0, emulsion stability was reported to decrease as a result of high pressure treatment which was attributed to protein aggregation decreasing the ability of the protein to form strong interfacial membranes. On the other hand, at pH 7.0, stability of pressure-treated protein-stabilized emulsions was reported to be higher compared to that of untreated protein due to increased surface charge. Foaming properties of yellow field pea protein isolate were found to be improved with high pressure treatment at all protein concentrations and pH values studied. Sim et al. [22] compared the efficacy of high pressure treatment (250–550 MPa) with heat treatment (95 °C, 15 min) with respect to gel forming properties of pea protein concentrate. The authors indicated that high pressure treatment changed the tertiary and quaternary structure of pea protein which resulted in protein denaturation, aggregation, and network formation. The extent of these changes increased with increasing pressure level. Pea protein subjected to heat treatment formed a gel at a lower concentration (12 g/100 g) compared to the pea protein subjected to high pressure at 250 MPa for 15 min (16 g/100 g). Moreover, heat-treated pea protein was reported to show greater gel strength compared to high pressure-treated protein. Ahmed et al. [15] applied high pressure treatment (200–600 MPa) to kidney bean protein isolate and determined the changes in protein structure and functionality. Investigation of thermal properties of kidney bean protein revealed that high pressure treatment resulted in unraveling of protein structure. Protein unfolding and changes in tertiary structure due to high pressure application were also verified by the shifts observed in Fourier transfer infrared (FTIR) spectroscopy profile. The magnitude of those changes increased with increasing pressure level. High pressure-treated pea protein was reported to show higher thermal denaturation temperature, improved water holding capacity, foaming, and emulsifying properties when the treatment was applied at above 600 MPa. In a recent study, Saricaoglu [17] applied high pressure (25–150 MPa) homogenization for improving functional properties of lentil protein isolate. The author reported that an increase in pressure from 50 to 150 MPa resulted in unraveling of the protein structure and improved solubility, emulsifying, and foaming properties of lentil protein. However, high pressure-treated lentil protein was observed to form weak gel-like structures.

2.4 Other techniques

Other physical techniques including cold plasma and controlled shear have also been applied to legume proteins for modification of their structural and functional properties. In a recent study, Mehr and Koocheki [20] applied atmospheric cold

plasma to Grass pea protein isolate and monitored the changes in protein structure and emulsifying properties. The authors reported that the extent of changes in protein structure depended on the level of voltage applied and duration of application. An initial increase in voltage and treatment time was indicated to result in an increase in the content of carbonyl groups which was determined to monitor the interaction between the amino acid side chains and reactive chemical species of plasma. On the other hand, the amount of free sulfhydryl groups was observed to decrease with the plasma treatment which indicated the structural modification of Grass pea protein. At the optimized treatment conditions globulins were dissociated, increasing the absorption rate of protein into the oil–water interface. Cold plasma treatment applied was indicated to alter the protein structure on secondary and tertiary levels. Ability of the plasma treated protein to decrease the interfacial tension was reported to be affected by the treatment conditions in such a way that lower voltage values resulted in lower interfacial tension. On the contrary, emulsion stabilized with the protein treated with higher voltage was observed to have smaller oil droplets and show higher stability against creaming. Bussler et al. [19] compared the efficiency of thermal treatment with cold plasma treatment for improvement of functionality of grain pea proteins. Plasma treatment was reported to induce changes in the tertiary or quaternary structure which in turn resulted in increased solubility, water and fat binding capacities. It was concluded that the plasma treatment applied could potentially be used as an alternative non-thermal method for improving protein functionality. In a recent study, Bogahawaththa et al. [18] applied controlled shear (100 or 1500 s^{-1}) to pea protein isolate and determined the changes in protein solubility and heat stability during heating at 90 °C for 5 min. It was reported that pea protein subjected to shear at 1500 s^{-1} showed significantly higher solubility and heat stability compared to the protein subjected to shear at 100 s^{-1} which was based on the changes in secondary structure and formation of soluble hydrophobic aggregates at high shear.

3. Chemical modifications

Structure and functionality of legume proteins are also modified by application of a variety of chemical methods including attachment of various molecules to protein structure via different pathways. Some recent reports on chemical methods applied to legume proteins for improved functionality are summarized in **Table 2**.

3.1 Attachment of low molecular weight molecules

One of the most commonly applied methods for improving protein functionality is attachment of low molecular weight molecules to protein structure via different chemical pathways [3]. Yin et al. [33] reported that acetylation and succinylation treatments induced changes in the secondary and/or tertiary structures of kidney bean protein isolate and resulted in a decrease in isoelectric point and net surface charge at pH 7.0. Succinylation was reported to decrease surface hydrophobicity of kidney bean protein while acetylation increased hydrophobicity. Charoensuk et al. [25] indicated that the effect of succinylation treatment on mung bean protein isolate depended on the ratio of succinic anhydride to protein. A decrease in isoelectric point and net surface charge at pH 7.0 was observed as a result of succinylation whereas the amount of free sulfhydryl groups was not affected. Improved emulsifying activity after succinylation treatment was attributed to increased flexibility of succinylated protein. Shah et al. [27] hydrophobically modified pea

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Attachment of low molecular weight molecules	Chickpea protein	Acetylation (6–49%)	Higher solubility at pH > 8.0. Lower solubility at pH 2.0–7.0. Improved water and oil absorption capacities, higher emulsion capacity, lower emulsion stability	[23]
	Lentil protein	Succinylation (58–90%)	Higher solubility at pH > 4.0. Lower solubility at lower pH. Increased water absorption capacity, viscosity, emulsifying activity and stability. Decreased oil absorption capacity, foaming capacity and stability.	[24]
	Mung bean protein	Succinylation (9–40%)	Improved solubility and emulsifying activity	[25]
	Black kidney bean protein	PEGylation	Higher gelling strength and shorter gelling time	[26]
	Pea protein	Hydrophobical modifications by *N*-substitutions	Improved solubility, foaming capacity, stability, emulsion stability, and water holding capacity	[27]
Attachment of high molecular weight molecules	Mung bean protein	Ultrasound pre-treatment and Maillard reaction with glucose	Improved solubility, emulsifying activity and stability	[28]
	Mung bean protein	Maillard reaction with dextran	Improved solubility, emulsifying activity and stability	[29]
	Black bean protein isolate	Ultrasound pre-treatment and Maillard reaction with glucose	Improved solubility and emulsifying properties	[30]
	Pea protein	Maillard reaction with gum Arabic	Improved solubility and emulsifying properties	[31]
	Soya bean protein	Maillard reaction with maltodextrin	Improved solubility, decreased gel strength and water holding capacity	[32]

Table 2.
Summary of recent studies on chemical modification of legume proteins for improved functionality.

protein by *N*-substitutions using different reactants. Hydrophobical modifications applied induced changes in the secondary structure of pea protein which were observed with FTIR spectroscopy. Alterations in secondary structure were attributed to increased negative charge. Changes in thermal profile were based on partial denaturation of protein and aggregation. Solubility and water holding capacity of modified pea protein were observed to increase due to increased negative charge. Emulsion stability index was reported to increase as a result of increased charge, solubility and addition of hydrophobic groups to the protein molecule. Hydrophobically modified pea protein with improved functionality was indicated to have a potential to be used as egg replacers in cake formulations.

3.2 Maillard reaction

Forming protein-polysaccharide based conjugates through glycation or Maillard reaction has been indicated as a promising method for modification of protein functionality [34]. Zhou et al. [29] formed conjugates between mung bean protein isolate and dextran at 80–90 °C for changing durations of 1–6 h. Electrophoretic profile of mung bean protein showed that both vicilin and legumin subunits participated in the Maillard reaction. Conjugation was indicated to alter the secondary structure, decreasing the α-helix content due to heat-induced unfolding of the molecule and attachment of dextran. Fluorescence spectra were used as an indicator to monitor the changes in tertiary structure. The authors proposed that conjugation induced unfolding of mung bean protein up to a certain extent and increased the flexibility of protein structure. Solubility of the conjugates formed at 2–3 h was reported to be increased compared to mung bean protein due to the increased number of hydrophilic moieties introduced by the grafted dextran. However, a slight decrease in solubility was observed with increasing graft time due to formation of insoluble protein aggregates. Emulsifying activity and stability indices of conjugates followed a similar trend and first increased compared to the native mung bean protein and then decreased with increasing graft time. Initial increase in emulsifying activity and stability indices was attributed to improved solubility and flexibility due to conjugation. The possible mechanism behind impaired emulsifying properties observed with increasing graft time was explained by heat induced protein aggregation and reduction of interfacial activity of mung bean protein with increased attachment of dextran. The authors reported that conjugates formed at 80 °C with lower glycosylation degrees and browning showed better functionality compared to the conjugates formed at 90 °C.

Wang et al. [28] investigated the effect of ultrasound treatment on conjugation of mung bean protein isolate and glucose. Similar to the study of Zhou et al. [29], the authors reported that Maillard reaction resulted in changes in the secondary structure of mung bean protein. Furthermore, ultrasound-treated conjugates were reported to have a less compact tertiary structure compared to the heat-treated conjugates and the native protein. Application of ultrasound treatment in Maillard reaction was indicated to form conjugates with a higher degree of glycosylation and improved solubility. Higher solubility observed in ultrasound-treated conjugates was attributed to two factors: breaking of insoluble aggregates and addition of more hydrophilic groups due to enhanced conjugation with the ultrasonication treatment. Similarly, ultrasound-treated conjugates showed better emulsifying activity and stability compared to heat-treated conjugates due to dispersion of aggregates and improved mobility of the protein molecule. Jin et al. [30] also investigated the effect of ultrasound treatment on conjugation of black bean protein isolate and glucose via Maillard reaction. Ultrasound treatment was reported the increase the reaction rate indicated with a higher degree of glycation at a shorter time. FTIR profile of the samples indicated that ultrasound-treated conjugates lost more ordered secondary structure (α-helix and β-sheet content) compared to the heat-treated conjugates. Alterations in protein structure resulting in increased unordered structure content were reported to improve the flexibility of the molecule and hence, emulsifying properties. Changes in fluorescence spectra indicated that the Maillard reaction resulted in alterations in the tertiary structure and ultrasonication treatment further increased the extent of these changes. Moreover, ultrasound-treated conjugates were reported to show higher surface hydrophobicity, improved solubility, emulsifying activity and stability indices compared to the heat-treated conjugates and the native protein. In another recent study, Zha et al. [31] formed conjugates between pea protein and gum Arabic and monitored the changes in functional properties

and flavor profile of pea protein. Pea protein-gum Arabic conjugates were reported to show improved solubility compared to the native pea protein. Incubation time was indicated as an important factor affecting the solubility of conjugates. Emulsion forming and stabilizing ability was determined by measuring droplet size. Emulsions stabilized by pea protein-gum Arabic conjugates were reported to have smaller droplets compared to emulsions stabilized by pea protein. Conjugate-stabilized emulsions showed the highest stability against environmental factors including temperature, pH and ionic strength when incubation time was kept at 3 days. The undesired (beany or grassy) flavor markers in pea protein were reported to decrease significantly after 1 day of incubation. Increasing the incubation time was reported to improve the flavor profile of the conjugates further.

4. Biological modifications

Functional properties of legume proteins can be improved via various biological methods including enzymatic hydrolysis, cross linking and fermentation. A brief summary of the findings of recent studies focusing on biological modification of legume proteins is presented in **Table 3**.

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Hydrolysis	Lentil protein	Hydrolysis with trypsin (4–20% DH[1])	Decreased interfacial tension. Decreased emulsifying activity and stability	[35]
	Chickpea protein	Hydrolysis with Flavourzyme (1–10% DH)	Improved solubility, oil absorption and foaming capacities. Decreased emulsifying activity	[36]
	Chickpea protein	Hydrolysis with Alcalase (4–15% DH)	Improved solubility. Decreased interfacial tension. Decreased emulsifying activity and stability	[37]
	Chickpea protein	Hydrolysis with Alcalase and Flavourzyme (12–50% DH)	Improved solubility. Hydrolysates with lower DH showed better emulsion properties	[38]
	Bambara bean protein	Hydrolysis with pancreatin (5–34% DH)	Improved solubility and oil-holding capacity. Decreased emulsifying activity	[39]
	Black bean protein	Hydrolysis with pepsin and Alcalase (24–28% DH)	Alcalase hydrolysates showed higher emulsion stability	[40]
	Faba bean protein	Hydrolysis with different proteases (2–16% DH)	Improved solubility, foaming capacity, oil holding capacity	[41]
	Pea protein	Hydrolysis with trypsin (2–4% DH)	Improved solubility. Samples at 2% DH showed better emulsion properties	[42]
	Pea protein	Hydrolysis with different proteases (2–10% DH)	Improved solubility. Hydrolysates obtained with trypsin showed the highest foaming and emulsifying capacities	[43]

	Legume protein studied	Treatment conditions	Effects observed on protein functionality	Ref.
Cross-linking with transglutaminase	Kidney bean protein	5 U enzyme/g protein, 37 °C, 0–240 min	Decreased solubility, emulsifying activity and stability	[44]
	Pea protein	10.5% protein, 0.3 M NaCl, 10 U enzyme, 40 °C, 30 min	Higher gel strength and more elastic gel formation	[45]
	Pea protein	17–23% protein, 5–7 U enzyme/g protein, 40–90 °C, 30–60 min	Higher gel strength and flexibility of the gel network	[46]
	Chickpea protein	200 U enzyme/g of protein, 37 °C, 60 min	Formation of a gel-like emulsion, improved emulsion stability	[47]
Fermentation	Protein-enriched pea flour	*Lactobacillus plantarum* NRRL B-4496, 7 log CFU/g flour, 32 °C, 11 h	Improved foaming capacity, emulsion stability, and oil-holding capacity. Decreased foam stability and emulsifying activity	[48]
	Protein-enriched pea flour	*Aspergillus niger* NRRL 334, *Aspergillus oryzae* NRRL 5590, 7 log CFU/g flour, 40 °C, 6 h	Decreased solubility and foaming properties. No significant changes in emulsifying properties. Improved water and oil binding properties	[49]
	Lupin protein	Fermentation with eight different microorganisms, 7 log CFU/g protein, 24 h	No significant changes in foaming activity and emulsifying capacity. Decreased solubility at pH 7.0	[50]

[1]*DH: degree of hydrolysis.*

Table 3.
Summary of recent studies on biological modification of legume proteins for improved functionality.

4.1 Enzymatic hydrolysis

Application of enzymatic hydrolysis for modification of structural properties and improving functionality of legume proteins has been widely studied recently. Klost and Drusch [42] applied enzymatic hydrolysis for modification of solubility and interfacial properties of pea protein. Hydrolysis with trypsin up to 4% degree was reported to increase solubility from 30% to 60% at pH 4.0–6.0 due to the increased amount of terminal COO^- and $NH3^+$ groups. On the other hand, net surface charge and solubility were reported to decrease at pH 3.0 and 7.0 due to exposure of previously buried hydrophobic moieties leading to aggregation. Pea protein hydrolysates were observed to form emulsions with wider oil droplet size distributions which were not stable against creaming as a result of decreased net charge and lack of repulsion. Enzymatic hydrolysis was reported to positively affect the strength of interfacial films formed. In a recent study 11 different proteolytic enzymes were used for hydrolyzing pea protein isolate for improved functional and sensory properties [43]. Solubility of most of the hydrolysates at pH 4.5 was reported to be improved with decreasing peptide size and release of hydrophilic amino acids with hydrolysis. Among the hydrolysates studied, Esperase hydrolysates were reported to show the highest protein solubility whereas the highest foaming and emulsifying capacities were observed in Trypsin hydrolysates.

All hydrolysates were observed to have improved foaming capacity and stability compared to the native protein which was based on decreased peptide size and modification of surface hydrophobicity with hydrolysis. Among the sensory attributes evaluated, only bitterness was reported to change significantly after hydrolysis. Some of the hydrolysates were reported to have lower bitterness scores than that of the native protein. However, increased degree of hydrolysis resulted in increased bitterness. In another recent study, four different proteases were for used for investigation of combined effects of enzymatic hydrolysis and ultrafiltration treatment on faba bean protein functionality [41]. It was reported that hydrolysis with pepsin resulted in significant increases in solubility, foaming and oil holding capacities of faba bean protein. Fractionation with ultrafiltration was observed to allow for further improvements in foaming, oil holding and emulsifying capacities of the peptides obtained.

4.2 Cross-linking

Enzymatic cross-linking with transglutaminase is another biological approach used for improving protein functionality. Tang et al. [44] studied the effect of cross-linking on kidney bean protein isolate. The authors observed unfolding of the vicilin units and formation of higher molecular weight oligomers. Thermal stability of vicilin-rich kidney bean protein was reported to increase after the cross-linking treatment. However, the authors observed gradual decrease in solubility and emulsifying properties with increasing incubation time. Moreno et al. [46] compared the efficiency of cross-linking and thermal processing in gelation of pea protein. The main aim of the cross-linking treatment was to obtain improved gelling properties for various meat and seafood applications. It was reported that cross-linking resulted in polymerization of vicilin and legumin subunits forming new intermolecular protein complexes indicated by alterations in protein structure. Transglutaminase treatment was reported to improve conformational stability and flexibility of the gel network. Cross-linking with transglutaminase is generally applied to legume proteins for improving gelling properties. However, Glusac et al. [47] studied its effects on characteristics of emulsions stabilized by chickpea protein. Cross-linking treatment was reported to increase mean droplet size of the emulsion compared to the native protein and formed a gel-like structure. Emulsions were observed over a month and cross-linked chickpea protein-stabilized emulsions were indicated to show higher stability against phase separation compared to the emulsions stabilized by native protein.

4.3 Fermentation

Although fermentation is a traditional process, its application for improving functional, nutritional and sensory properties of legume proteins and protein-rich flours has gained interest in the recent years. Cabuk et al. [48] investigated the effect of fermentation on properties of pea protein-enriched flour (~40 g/100 g protein). Fermentation was conducted for 11 h where the degree of hydrolysis was reported to reach 13.5%. Net surface charge at pH 4.0 was reported to increase at 1 h of fermentation and then decreased. Net surface charge at pH 7.0 and surface hydrophobicity at pH 4.0 were reported to increase. Solubility at pH 7.0 was reported to decrease from ~43% to 36% after 11 h of fermentation. The highest foaming capacity was reported for pea protein-enriched flour fermented for 5 h at pH 4.0. Emulsifying activity of pea protein-enriched flour was observed to decrease after 5 h whereas emulsion stability increased. It was concluded that functionality of fermented protein-enriched flours can be optimized depending on fermentation

conditions. In a follow-up study, fermentation was conducted with two different *Aspergillus* strains for 6 h and the degree of hydrolysis was reported to reach 10–11% [49]. The authors observed an increase in surface charge with increasing fermentation time whereas surface hydrophobicity was reported to decrease. Fermentation was indicated to result in negative effects on solubility and foaming properties where emulsifying properties were reported to remain unchanged. Decrease in solubility was attributed to increased protein–protein interactions and aggregation. On the other hand, water and oil binding properties were observed to be improved after fermentation. In another recent study, Schlegel et al. [50] used eight different microorganisms for fermenting lupin protein isolate. Fermentation was reported to result in no significant difference in solubility of lupin protein at pH 4.0; however, solubility at pH 7.0 decreased from ~64% to <42%. Emulsifying capacity of lupin protein was not affected by fermentation. On the other hand, foaming activity of fermented lupin protein was reported to be higher than that of native protein. Among the microorganisms studied, only two of them resulted in improved emulsifying capacity in the fermented lupin protein compared to the native protein. All microorganisms used for fermentation were found to decrease the bitterness score of lupin protein. *Lactobacillus brevis* was reported to be the most effective microorganism for improving the sensory profile as it was noted to decrease the intensity of undesired flavor markers.

5. Conclusion

A variety of physical, chemical and biological methods can be applied to legume proteins for modification of their structural and functional properties. Each method has its own advantages and limitations in terms of performance and applicability in food systems. Many factors affect the performance of the modified legume proteins: protein structure and composition, modification method and processing conditions applied, and extrinsic factors such as pH, ionic strength, and temperature. Modification method has to be optimized in terms of processing parameters to obtain the desired level of functionality for the legume protein studied. Complexity of the food matrix is also an important factor affecting the end product performance of modified legume proteins. Further research has to focus on interactions of modified legume proteins with other food components during processing and storage. More studies are required to investigate the effects of modification treatments on flavor profile and nutritional properties of legume proteins.

Conflict of interest

The author declares no conflicts of interest.

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