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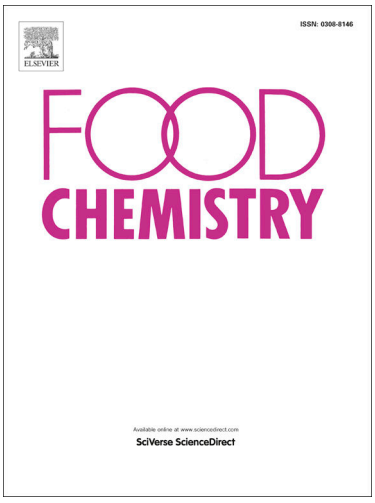

Review

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Faba bean protein: a promising plant-based emulsifier for improving physical and oxidative stabilities of oil-in-water emulsions

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Abstract

Faba bean is a protein-rich, sustainable, but understudied legume. Faba bean protein isolates (FBPIs) can serve as promising emulsifiers. This review aims to summarize the research on FBPIs as emulsifiers and various modification methods to improve the emulsifying functionalities. The emulsifying activities of FBPIs depend on several physiochemical characteristics (e.g. solubility, surface hydrophobicity, surface charge, interfacial activity). Physical modifications, especially via linking FBPIs electrostatically to polysaccharides can effectively increase the interfacial layer thickness/compactness and maintain the interfacial protein adsorption. Chemical modifications of FBPIs (e.g. acetylation and Maillard reaction) could improve the interfacial activity and affect the droplet-size distribution. Enzymatic modifications, usually either via hydrolysis or cross-linking, help to optimize the molecular size, solubility, and surface hydrophobicity of FBPIs. It is critical to consider the lipid/protein oxidative stability and physical stability when optimizing the emulsifying functionality of FBPIs. With suitable modifications, FBPI can serve as a promising emulsifier in food production.

Keywords:

Faba bean protein; emulsion; physical stability; oxidative stability; protein modifications

1 Introduction

The oil-in-water (O/W) emulsion commonly exists in food products. The formation of emulsions is associated with increased positive free energy in the interface, which makes the emulsions thermodynamically unstable (i.e. natural tendency to separate into oil and water phases over time) (Sharma & Shah, 1985). In addition, emulsions have greater interfacial area; this could increase the contacts between unsaturated lipids in the oil phase and prooxidant compounds (e.g. metal ions) and oxygen dissolved in the aqueous phase, making emulsions highly susceptible to oxidation (Berton-Carabin, Ropers, & Genot, 2014). Instability of food emulsions causes undesirable sensory outcomes (e.g. appearance, texture, mouthfeel, and flavor) and nutrition loss; consequently, various emulsifiers are routinely added to food emulsions (D. J. McClements, Bai, & Chung, 2017).

With increasing awareness of food safety and growing demands for natural ingredients, proteins are receiving more attention. Proteins can serve as natural emulsifiers, because the amphiphilic properties of proteins allow them to self-aggregate, and to form continuous and homogeneous layer around oil droplets (Dickinson, 1986). Considering the economic cost and sustainability issues, more research interests are focused on plant proteins as substitutes for animal proteins (Multari, Stewart, & Russell, 2015). The application of different plant proteins as emulsifiers has been studied and reviewed. For example, Burger and Zhang (2019) discussed the impacts of origin, processing, and environmental conditions (e.g. temperature, pH, and ionic strength) on emulsifying properties of pea proteins. Based on the stability behavior of model emulsions prepared by soy protein isolate and sodium caseinate, Dickinson (2019) summarized the critical factors to control and inhibit the flocculation of O/W emulsions; these factors included protein/oil ratio, emulsification conditions, protein solubility, protein denaturation, pH conditions, ionic strength, and calcium ion content; the author further emphasized covalent protein-polysaccharide conjugate as a consistently

successful strategy. Kim et al. (2020) compared the emulsifying properties of several plant proteins (soy, peas, cereal, and oilseed) with dairy proteins and highlighted that emulsifying properties were highly dependent on protein sources, structure, molecular weight, and adsorption behavior. Compared to the plant proteins mentioned above, faba bean protein is considered economical but underutilized.

Faba bean contains high level of proteins (ranging from 27% to 34% of dry weight) (Samaei et al., 2020). It not only has excellent adaptability in a wide range of climates and environmental conditions, but also is considered as a sustainable crop in that it offers ecosystem services via biological nitrogen fixation and diversifications of cropping systems (Lizarazo, Lampi, Liu, Sontag-Strohm, Piironen, & Stoddard, 2015). Like many plant proteins mentioned above, faba bean proteins can serve as emulsifiers. However, the unmodified plant proteins usually exhibit unfavorable emulsifying activities, which could be attributed to the suboptimal intrinsic physiochemical properties (e.g. surface charge, hydrophobicity, and molecular weight) that are decisive factors for emulsifying properties (Schwenke, 2001). Various efforts have been devoted to improving the emulsifying properties by physical, chemical, and enzymatic modifications. The majority of these efforts are focused on modifying the protein configuration, changing the surface charge or hydrophobicity, increasing the interfacial layer thickness/compactness, as well as obtaining desirable molecular weight and solubility (Chen, Chen, Ren, & Zhao, 2011; Luisa, Gaspar, & de Goes-Favoni, 2015). Different modifications on FBPIs for improving the emulsifying property and the stability of corresponding emulsions have been reported, making FBPI a promising plant-based emulsifier in O/W emulsions. The focus and objective of this review is to provide an update on applications of unmodified and modified FBPIs as emulsifiers in O/W emulsions, and to give insights into the possibilities to enhance faba beans utilization.

2 O/W emulsions and emulsion stability

Emulsions are colloidal systems that consist of two immiscible liquids, where one of the liquids is dispersed as spherical droplets in the other liquid (Lam & Nickerson, 2013). In most emulsion-type foods, these two liquids usually refer to oil droplets dispersed in a water phase, hence classified as O/W emulsions (Bakry et al., 2016).

2.1 Physical stability

Because the contact between oil and water is thermodynamically unstable, O/W emulsions suffer from various physical instabilities evidenced by changes in the spatial distribution or structural organization (Elwell, Roberts, & Coupland, 2004). Creaming, flocculation, and coalescence are the major examples of physical instabilities (**Figure 1**). Creaming in O/W emulsions occurs when oil droplets move upward and accumulate at the top of the aqueous phase due to density difference; over time, a creamed layer of oil droplets is floating on the top, leaving an aqueous phase at the bottom (Robins, 2000). Flocculation is generally defined as the aggregation of droplets when two or more oil droplets come in close proximity but still maintain individual integrity (David Julian McClements, 2015). Coalescence happens when two or more oil droplets nearby merge into a single larger oil droplet with the partitioning layer disrupted; as a result of the merging, the average droplet size increases over time while the interfacial area is reduced (Tcholakova, Denkov, & Lips, 2008).

2.2 Oxidative stability of protein-emulsified O/W (P-O/W) emulsions

P-O/W emulsions are not only physically unstable, but also are prone to various adverse oxidative deterioration due to the lipid and protein contents. Lipid oxidation can generate off flavor, undesirable color, and potentially toxic by-products such as malondialdehyde and 4-hydroxynonenal, while protein oxidation might induce loss in sensory quality and nutrition (Esterbauer, 1993; Lund, Heinonen, Baron, & Estevez, 2011). Moreover, the oxidative

stability has a strong interplay with physical stability. Some lipid oxidation products such as lipid hydroperoxides are surface active and can interact with protein interfacial layer, consequently inducing coalescence of oil droplets and protein oxidation; surface active antioxidants such as *p*-hydroxyphenylacetic acid and its conjugates with a butyl or dodecyl group were able to effectively adsorb at the interface and scavenge free radicals (Yuji, Weiss, Villeneuve, Lopez Giraldo, Figueroa-Espinoza, & Decker, 2007). Thus, it is critical to improve both oxidative stability and physical stability.

Lipid oxidation is usually elucidated as pathways of autoxidation, enzymatic oxidation, and photo-oxidation (Bartosz, 2013; Dey & Neogi, 2019; Frankel, 2014). In food products, oxygen scavengers are commonly used to remove or decrease the level of oxygen in package; the light that can accelerate lipid oxidation is usually blocked by glass or plastic containers while lipoxygenases and other enzymes that catalyze the oxidation of free polyunsaturated fatty acids are mostly deactivated during thermal processing (Bartosz, 2013; Dey et al., 2019; Frankel, 2014). Autoxidation via free radical chain reactions are particularly important; the chain reactions generally consists of three well-established steps: initiation, propagation, and termination (**Figure 1**) (Bartosz, 2013; Frankel, 2014). In emulsions, hydroperoxides can act as surface-active compounds, in that they often accumulate at the interfacial layer around oil droplets. The decomposition of lipid hydroperoxides at the oil droplet surface into highly reactive radicals is proposed to be the most likely mechanism for initiating and accelerating lipid oxidation in O/W emulsions (Decker & McClements, 2001).

In P-O/W emulsions, the physical instability and lipid oxidation can be perceived readily due to visible structural alterations, rancidity, or color changes; on the other hand, protein oxidation is usually underestimated. In fact, protein oxidation not only leads to depletion of essential amino acids, but also causes undesirable changes in protein functionalities such as emulsifying properties (Lund et al., 2011). More importantly, protein oxidation *in vivo* contributes to a variety of diseases such as Alzheimer's disease, Parkinson's syndrome,

rheumatoid arthritis, muscular dystrophy, cataractogenesis, inflammatory bowel's disease (IBD), and cataractogenesis (Estevez & Luna, 2017). Similar to lipid autoxidation, protein oxidation is usually initiated by reactive oxygen species that originate from irradiation, oxygen, metal-catalyzed systems (e.g. Fe(II), Mn (II)), peroxides, as well as non-protein radicals and free radicals including hydroxyl radicals (HO·), peroxyl radicals (HO_2·), and superoxide anion radicals (O_2·$^-$); these radicals then effectively initiate formations of protein radicals; oxidative modifications of protein not only lead to changes in amino acid side chain such as thioloxidation, aromatic hydroxylation, and formation of carbonyl groups, but also produce peptide backbone cleavage and cross-linking (Stadtman & Levine, 2003).

2.3 Interplay between lipid oxidation and protein oxidation in P-O/W emulsions

In P-O/W emulsions, lipid oxidation and protein oxidation and closely interrelated. Proteins can either be freely distributed in the aqueous phase as unadsorbed, or adsorbed at the interfacial layer. The unadsorbed proteins can affect lipid oxidation by several mechanisms. Firstly, aqueous proteins can chelate or bind metals by forming metal complexes, altering the physical or redox state of metal ions, or changing the physical location of metal ions (Berton-Carabin et al., 2014). Secondly, proteins in the aqueous phase can reduce hydroperoxides; for example, sulfur-containing amino acids can reduce hydroperoxides into the inactive hydroxylic derivatives (Pokorny, Yanishlieva, & Gordon, 2001). Thirdly, unadsorbed proteins can trap or quench free radicals (Kong & Xiong, 2006; Wang & Xiong, 2005). Tryptophan and cysteine (rich in electrons and therefore nucleophilic) are preferentially oxidized prior to lipid oxidation in an O/W emulsion system, so that the radical transfer reactions for lipid oxidation could be partially inhibited (Elias, McClements, & Decker, 2005).

Apart from the aqueous phase in a P-O/W emulsion, proteins are the major components for constructing the interfacial layer where lipids and prooxidants such oxygen, transition metals, and free radicals contact. Thus, the interfacial properties of proteins could strongly influence lipid oxidations (Berton-Carabin et al., 2014). Firstly, interfacial proteins determine the surface charges of oil droplets. As transition metals are positively charged, a positively charged protein-interfacial layer of a P-O/W emulsion could repel the prooxidant metals, thus slowing down the lipid oxidation (Hu, McClements, & Decker, 2003). Secondly, increased thickness and packing density of the protein-interfacial layer also decrease lipid oxidation by preventing free radicals, oxygen, and other prooxidants in the aqueous phase from reaching the fatty acids in the oil droplets (Gürbüz, Kauntola, Diaz, Jouppila, & Heinonen, 2018). Lastly, proteins at the interface may protect lipid from oxidation by acting as antioxidants as they do in the aqueous phase (Berton, Ropers, Guibert, Sole, & Genot, 2012). It needs to be pointed out that neither of the above mentioned factors is the single determining factor, as lipid oxidation is the collective result of various internal and external factors.

Lipid oxidation also plays an important role in protein oxidation. Under oxidative conditions, the onset of lipid oxidation normally takes place faster than the oxidation of protein (Lund, Hviid, & Skibsted, 2007). It is likely that protein oxidation is initiated and promoted by lipid oxidation products (Lund et al., 2011). Various protein oxidative products are formed and accumulated after reactions of amino acids/peptides/proteins with lipid-derived oxidative products such as hydroperoxides and aldehydes. Pre-oxidized reactive lipid species serve as sources of radicals and create protein radicals; for example, protein oxidation is initiated via abstracting hydrogen atoms from protein molecules by peroxyl radicals formed during lipid oxidation (W. Wu, Zhang, Kong, & Hua, 2009).

2.4 Emulsifiers

In food industry, emulsifiers are widely used to facilitate the homogenization process and maintain emulsion stability (Padial-Dominguez, Espejo-Carpio, Garcia-Moreno, Jacobsen, & Guadix, 2020). Emulsifiers can reduce the interfacial tension between oil and water phase, and consequently decrease the energy needed during homogenization; in addition, emulsifiers can readily adsorb to the surface of freshly formed oil droplets due to the amphiphilic nature, and consequently form a protective layer that prevents oil droplets from aggregation (Kim et al., 2020). Considering the role of protein in lipid oxidation in emulsion, as well as the consumer needs for more sustainable and environmentally friendly food products, increasing research are directed to investigating plant proteins as emulsifiers (Burger et al., 2019). Recent years, the potentials of faba bean proteins as emulsifiers have been explored and reported.

3 Faba bean proteins

3.1 Protein composition

Faba bean storage proteins mainly consist of legumin-like globulins (11S) and vicilin-like globulins (7S), as well as less amount of albumins (2S), glutelins, and polyamines (El Fiel, El Tinay, & Elsheikh, 2002). Legumin-like globulins (11S) are hexameric proteins containing six intermediary subunits with a molecular weight (MW) of 300-400 kDa (Bailey & Boulter, 1970). Each intermediary subunit comprises of an acidic α subunit (~ 36 kDa) and a basic β subunit (~ 22 kDa) (Wright & Boulter, 1974). The α subunits are hydrophilic and situated at the surface, whereas β subunits contain more hydrophobic amino acids (e.g. leucine, valine, and phenylalanine) hidden in the core. The α and β subunits are mainly connected by a single disulfide bond and closely packed via electrostatic and hydrophobic interactions to form a stabilized rigid structure (Wright et al., 1974). Vicilin-like globulin

(7S) is a trimeric protein containing three subunits, with an average MW of 150 ± 2.5 kDa; vicilin fraction accounts for ~ 20% of the total globulins (Wright et al., 1974).

The thermal denaturation midpoint temperatures of 11S and 7S globulins in faba bean are higher than those in soybean (95.4 °C *vs.* 93.5 °C for 11S, and 83.8 °C *vs.* 78.5 °C for 7S) at ionic strength of 0.5; this indicates faba bean protein has comparable or better thermal stability than soybean protein and is suitable for the production of foods requiring high thermal stabilities (Kimura, Fukuda, Zhang, Motoyama, Maruyama, & Utsumi, 2008). Despite the rich protein content in faba bean, its use is relatively limited, not only because of the poor understanding of its functional properties, but also due to the presence of anti-nutritional and negative factors (Rahate, Madhumita, & Prabhakar, 2021).

3.2 Emulsifying property of FBPIs

Our recent research interests have been devoted to exploring the use of FBPIs in food production, with a focus on its emulsifying functionalities (Liu, Bhattarai, Mikkonen, & Heinonen, 2019; Liu, Damodaran, & Heinonen, 2019; Liu, Pei, Peltonen, & Heinonen, 2020); meanwhile, the potentials of FBPIs as emulsifiers have been studied and reported by other groups (**Table 1**). The emulsifying property of FBPIs mainly depends on several physiochemical characteristics.

3.2.1 Solubility

Solubility is essential for the emulsification by facilitating the migration of proteins to and spreading at the oil-water interface (W. U. Wu, Hettiarachchy, & Qi, 1998). With an isoelectric point (pI) of ~ 4.5, the solubility of FBPIs markedly decreases at near pI (~ 4 to 6) and mostly solubilizes at pH of 8-9 (Otegui, Fernandez-Quintela, De Diego, Cid, Macarulla, & Partearroyo, 1997). pH is a decisive factor for solubility, as it affects hydrophobicity and surface charge of proteins which in turn influence the equilibrium between the protein-solvent (hydrophilic) and the protein-protein (hydrophobic) interaction,

and electrostatic repulsion, respectively (Karaca, Low, & Nickerson, 2015). For example, heating at pH of 11.0 significantly increased the solubility of FBPIs (from 43% at pH 3.0, 14% at pH 6.0, and 35% at 7.0 to more than 90%); correspondingly, the FBPIs with greater solubility produced more stable emulsions with smaller particle size (Farhad Alavi, Chen, Wang, & Emam-Djomeh, 2021). Similarly, Felix et al. (2019) reported that FBPIs were 19% and 71% more soluble at pH 3.0 and 8.0 than at pH 5.0, respectively; meanwhile, emulsions prepared at pH 3.0 and 8.0 demonstrated smaller droplet sizes and better rheological properties than that at pH 5.0. MWs influence protein solubility as well. FBPI hydrolysates with smaller MWs exhibited higher solubility because smaller peptides produced by hydrolysis can form stronger hydrogen bonds with water and become more soluble. Hydrolysis of FBPIs by alcalase improved the solubility by 6-10% at pH 8; accordingly, moderately hydrolyzed FBPIs (degree of hydrolysis at 4%) showed increased surface charge and surface hydrophobicity which favored the emulsifying activity (Liu, Bhattarai, et al., 2019). Similarly, Xu et al. (2016) reported that hydrolysis of rice glutelin by trypsin changed the MW, increased flexibility, altered surface hydrophobicity, and greatly increased the solubility (from < 10% to > 60%), which in turn increased the stability of emulsions.

3.2.2 Surface hydrophobicity and surface charge

Surface hydrophobicity and surface charge of proteins remarkably influence the emulsification (Schwenke, 2001). The amphiphilic nature of proteins allows them to remain in the aqueous phase while simultaneously adsorb at the surface of oil droplets and generate stabilizing electrostatic forces and steric hindrance (Claesson, Blomberg, & Poptoshev, 2001). The hydrophobic patches of proteins, which relate to the hydrophobic amino acid residues exposed at the surface, are the precondition for protein adsorption at the oil-water interface (Jiang, Cheng, & Sun, 2020). Johnston et al. (2015) reported the hydrophobicity in decreasing order with: chickpea (~137 arbitrary units, AU) > lentil = soya (~70 AU) > FBPI (~24 AU), which might partly explain the relatively poorer emulsifying capacity of

unmodified FBPI. Compared to the unmodified FBPI, moderate hydrolysis of FBPIs by alcalase increased the surface hydrophobicity by 18%, which could be attributed to exposing more buried hydrophobic groups; correspondingly, the emulsions prepared by hydrolyzed FBPI demonstrated greater physical and oxidative stability (Liu, Bhattarai, et al., 2019). Heat treatment increased the surface hydrophobicity from 181 to 504 relative fluorescence units, which could partly explain the increase in emulsion activity index (EAI) from 25.1 to 27.4 m^2/g (Nivala, Nordlund, Kruus, & Ercili-Cura, 2020). However, it needs to be stressed that the balance between hydrophobicity and hydrophilicity is the key factor that determines accessible surface area, unfolding, and re-orientation at the interface (T. Xu et al., 2020).

Surface net charge is another critical factor contributing to the surface characteristic. A high protein surface charge not only promotes greater hydration of proteins, but also induces high repulsive interfacial charges (Schwenke, 2001). In FBPIs, the net negative charge, for example at pH 7.0, is mainly due to the negatively charged side chains of aspartic acid (pKa = 3.65) and glutamic acid (pKa = 4.25) residues spatially located on the protein surface (Lide, 2004). Thus, the net charge of FBPIs in emulsions is primarily dependent on pH and other components at the interface. For example, the surface charges were changed drastically when adjusting the pH of FBPI solutions, with ~ 30, 10, -10, -20, and -30 mV at pH of 3.0, 4.0, 5.0, 6.0, and 7.0, respectively; the emulsions prepared under different pH displayed diverse physical stability as determined by changes in particle size, which was proposed to be partly explained by the surface charge effects (Farhad Alavi et al., 2021).

3.2.3 Interfacial tension

Last but not least, interfacial tension is another important factor for emulsifications. In the preparation of P-O/W emulsions, proteins migrate to the oil-water interfaces where they re-align themselves to position the surface hydrophobic amino acids toward the oil phase and hydrophilic moieties within the aqueous phase. These migrated proteins form an interfacial

film surrounding oil droplets that maintains stability via electrostatic repulsive forces, steric stabilization, and decreased interfacial tension (Schwenke, 2001; Singhal, Stone, Vandenberg, Tyler, & Nickerson, 2016). It has been demonstrated that FBPIs, as well as protein isolates from chickpeas, lentils, and soybeans, can efficiently decrease the interfacial tension by ~50% in canola oil-water mixtures (Johnston et al., 2015).

4 Modifications to enhance the FBPIs' emulsifying properties

Most plant proteins, including FBPIs, do not exhibit desired emulsifying properties for food industries, due to suboptimal physiochemical attributes (Gumus, Decker, & McClements, 2017a, 2017b, 2017c; Karaca, Low, & Nickerson, 2011). Based on the key physiochemical characteristics for emulsification discussed above, various modification methods are reported to improve the emulsifying activity of FBPIs, as well as to increase the stability of FBPI-stabilized emulsions (**Figure 2**). Generally, the modifications of proteins are categorized into physical, chemical, and enzymatic methods (**Table 1**).

4.1 Physical modifications

Physical association with polysaccharides via electrostatic interaction can effectively improve the emulsifying activity of proteins. In general, a protein-polysaccharide complex could be formed first via electrostatic interaction and then introduced as emulsifiers (i.e. complex model); or the polysaccharides could be added to existing protein-emulsified O/W emulsions to form an extra outer layer around the droplets (i.e. layer-by-layer/LBL model) (Berton-Carabin et al., 2014). The two different preparation methods (complex *vs.* LBL model) are expected to have a major impact on the interfacial properties, especially the thickness and compactness (Berton-Carabin et al., 2014). We recently investigated how different combinations of FBPIs and chitosan (complex *vs.* LBL model) affected O/W emulsion stability (**Table 1**) (Liu et al., 2020). Compared to the complex model, the

interfacial structure in the LBL model appeared to be more compact and denser; one likely explanation was that the FBPI formed a denser inner layer around the oil droplet which facilitated a more uniformed secondary layer by chitosan (Liu et al., 2020). As a result, the LBL model produced both better physical stability and greater oxidative stability (Liu et al., 2020).

The combination of other polysaccharides and plant-based proteins, as well as different preparation methods in the O/W emulsion systems have been reported. For example, adding xanthan gum to wheat protein-emulsified emulsions improved emulsion stability to high ionic strengths (Qiu, Zhao, & McClements, 2015). Emulsions emulsified with soluble glycinin-chitosan complex displayed improved stability at certain ratio due to synergistic effect of the two molecules (Yuan, Wan, Yang, & Yin, 2014). Extra pectin coating enhanced spray-dry stability of pea protein-stabilized O/W emulsions, which was proposed to be attributed to increased steric effects (Gharsallaoui, Saurel, Chambin, Cases, Voilley, & Cayot, 2010). Conversely, some studies showed that additions of cationic polyelectrolytes to protein-emulsified emulsions promoted droplet aggregation, creaming instability, and viscosity enhancement (Nylander, Arnebrant, Cárdenas, Bos, & Wilde, 2019). The inconsistent conclusions might be mainly attributed to the different experiment conditions such as the type and the ratio of polysaccharides and proteins, environmental pH and ionic strength, and preparing methods, etc. The potential application of combing FBPIs and these polysaccharides and different preparation procedures need further investigation.

Another means of physical modification is by modulating the pH. Felix, et al. (2019) investigated the effects of pH on the interfacial viscoelastic properties of FBPI layer in O/W emulsions; FBPI dispersions were subjected to pH of 3.0, 5.0, and 8.0 and the interfacial properties were determined by dilatational measurement and interfacial small amplitude oscillatory shear measurements. They found that the strength of interfacial FBPI film at O/W interface depended on the pH values; the measurement of adsorption kinetics for penetration

and unfolding of the protein at the interface showed that protein adsorption took place significantly faster at pH 3.0 and 5.0 than at pH 8.0 (Felix, Romero, et al., 2019). This group further examined the microstructural characteristics of emulsion stabilized by FBPIs at different pH, as well as the *in vitro* antioxidant activities; the emulsions were prepared with 250 mL of 30 mg/mL FBPIs adjusted to different pH values (3.0, 5.0, and 8.0) and 250 mL of sunflower oil; smaller droplet sizes and better rheological properties were obtained at pH 3.0 and 8.0; in addition, the emulsions at pH 3.0 displayed the lowest extent of lipid oxidation (Felix, Cermeño, et al., 2019). Both studies demonstrated that interfacial properties are important targets of modification for improving the emulsifying functionalities of FBPIs.

Treatments by heating and high-pressure are other conventional modification methods of proteins (Tcholakova, Denkov, Ivanov, & Campbell, 2006). Our group showed that heating faba beans with an air oven at 170°C for 30 min prior to emulsification could modified the surface hydrophobicity of proteins via exposing or burying more hydrophobic amino acid groups (Gürbüz, Liu, et al., 2018). High pressure homogenization could dramatically improve FBPI solubility by dissociating large insoluble protein aggregates; high pressure homogenization also increased surface hydrophobicity by promoting certain level of protein unfolding (Yang, Liu, Zeng, & Chen, 2018). However, the improved solubility and surface hydrophobicity did not translate into better emulsifying property. The authors proposed that the negative effects of high pressure homogenization on emulsifying capacity could be attributed to the reduced viscoelasticity of the interfacial film and the flocculation effect caused by the soluble supramolecular aggregates; the unadsorbed supramolecular aggregates might also be responsible for the formation of flocs and destabilization of the emulsion (Yang et al., 2018).

4.2 Chemical modifications

Many studies have reported that chemical modifications could improve the functionality of proteins (e.g. solubility, foaming, and emulsifying) and the rheological behavior and stability of P-O/W emulsions (Oliver, Melton, & Stanley, 2006).

In the early 1990s, chemical modification focused on acetylation of FBPIs. Schmandke et al. (1990) compared the emulsifying properties of unmodified and acetylated FBPI (degree ~97%); they found that acetylation improved the flow, gelifying, surface and emulsifying properties, as well as the physical stability of emulsions. This group later examined the association of low methoxylated pectin and acetylated FBPI as emulsifiers in O/W emulsions. They found that the combination could increase the protein content in the protein film around the oil droplets; as a result, the apparent viscosity and flow behavior index of the emulsions were increased and creaming was inhibited accordingly (Schultz, Schmidt, Krause, Seifert, & Schmandke, 1991). Another group compared the emulsifying activities of unmodified and acetylated FBPIs (~96%) with a focus on the ultrastructure of formed emulsions. They reported that acetylation could improve the emulsifying activity, which was associated with tangible difference in different droplet-size distribution and ultrastructure (Krause & Buchheim, 1994). This group also proposed that the increased emulsifying activity by acetylation was due to increased interfacial activity indicated by changes in Gibbs' adsorption isotherms and the inflection point of the adsorption isotherms (Krause & Schwenke, 1996).

Maillard reaction is another common chemical modification for improving emulsifying properties; it starts between the non-protonated amine group from proteins and the electrophilic carbonyl carbon of a reducing sugar in polysaccharides (Martins, Jongen, & van Boekel, 2000). One group investigated the effects of heating under alkaline pH alone or in the presence of maltodextrin on emulsifying properties of FBPIs (Farhad Alavi et al.,

2021). The FBPI-maltodextrin conjugates were obtained by heating the FBPIs and maltodextrin (w/w ratio of 2:1) at 90 °C for 30 min at pH of 7.0 or 11.0; then, emulsions were prepared with 10% (v/v) sunflower oil and 10 mg/mL unmodified and modified FBPIs; they found that FBPI-maltodextrin samples heated at pH 11.0 produced emulsions that were stable against flocculation and phase separation for 15-day storage at pH 6-7 or in the presence of salt; the improved emulsifying functionality was partly attributed to increased surface hydrophobicity, increased solubility, and steric hindrance (Farhad Alavi et al., 2021). Similarly, covalent attachment of polysaccharides to β-lactoglobulin can increase steric layer thickness, and consequently improve stability of emulsions against calcium induced flocculation (Wooster & Augustin, 2006). The Maillard type complex of wheat protein and dextrans could form a thicker interfacial layer and provide enhanced steric stabilization of the O/W emulsions (Wong, Day, & Augustin, 2011). Conjugation of soy protein isolates (SPI) with citrus pectin and apple pectin under controlled dry-heating conditions was shown to improve emulsifying properties of SPI by increasing the solubility and modifying the surface hydrophobicity (Ma et al., 2020). Other chemical modification involves attaching specific groups. For example, covalent binding between rice protein hydrolysates and chlorogenic acid was shown to improve the emulsifying activity of rice protein hydrolysates and resulted in more physically and chemically stable emulsions; the authors proposed that the complex could form a thicker interfacial layer around the oil droplets (Pan et al., 2019). By optimizing the different aspects of physiochemical characteristics, these chemical modifications of plant proteins can improve the emulsifying activities. However, in the light of consumer awareness of food safety and demands for eco-friendly foods, chemical modification might not be favored. To address these concerns, enzymatic modifications on proteins have been pursued.

4.3 Enzymatic modifications

Enzymatic modifications of proteins are favored by commercial manufacturers because of food safety, lower costs, easier control of the reactions, and acceptability by consumers and regulatory agencies. In order to improve the emulsifying properties, enzymatic modifications of proteins applied in P-O/W emulsions are generally either via decreasing the molar mass of proteins by proteolytic enzymes, or increasing the molar mass by cross-linking enzymes (Chen et al., 2011; Luisa et al., 2015).

We recently investigated the effects of different degrees of hydrolysis (DH) by alcalase on the emulsifying activity of FBPIs (**Table 1**) (Liu, Bhattarai, et al., 2019). DH of 4% produced suitable molecular weight and surface net charge that in turn promoted better interfacial layer stability, as well as increased repulsive electrostatic force; DH of 4% also increased the surface hydrophobicity and expose more buried hydrophobic groups, thus improving the adsorption of proteins at the interface and emulsifying capability (Liu, Bhattarai, et al., 2019). Another advantage of hydrolysis treatment is that protein hydrolysates exhibit higher capacity to inhibit lipid oxidation, indicated by higher metal-chelating activities and radical-scavenging activity; in particular, alcalase is a commonly used protease which could produce hydrolysates which have higher antioxidant activities and are more resistant to digestive enzymes (Bucko, Katona, Popovic, Petrovic, & Milinkovic, 2016; Shen, Zhou, Zhang, Yuan, Zhao, & Zhao, 2020; Thaiphanit, Schleining, & Anprung, 2016). Similarly, hydrolysis of FBPIs by alcalase could produce emulsions with significantly less oxidation (i.e., less conjugated dienes and hexanal) while maintaining protein oxidative stability compared to the unmodified FBPI (Liu, Bhattarai, et al., 2019).

It has been underlined that enzyme specificity, protein confirmation, enzyme dose, and environmental conditions (e.g. pH, ionic strength, temperature, other components such as inhibitory substances, etc.) all together influence the emulsifying activities of hydrolysates

(Tavano, 2013). Our work showed that the hydrolysates with higher DHs (9 and 15%) had decreased emulsifying activity which might be caused by unduly decreased surface hydrophobicity and increased surface load (Liu, Bhattarai, et al., 2019). Similarly, another hydrolysis study reported that excessive hydrolysis of whey proteins may accelerate the desorption of proteins/peptides at interfacial layer, and thereby decrease stability of emulsions (Schroder, Berton-Carabin, Venema, & Cornacchia, 2017). Therefore, it is critical to determine the optimum DH for the best stable P-O/W emulsion. Choosing optimal reaction conditions such as pH and temperature based on the type of enzyme is always the prerequisite (Tavano, 2013).

Another major enzymatic modification is through inter- or intramolecular cross-linking reactions of proteins. One commonly used enzyme is microbial transglutaminase (MTG). MTG catalyzes acyl transfer reactions between γ-carboxyamide of peptide or protein-bound glutamyl residue (acyl donor) and primary amino group (acyl acceptor). When lysine residues act as acyl acceptor, ε-(γ-glutamyl)- lysine "isopeptide" covalent bonds are formed, resulting in polymerization or amine incorporation (Luisa et al., 2015). We have investigated the effects of MTG treatment on the emulsifying activity of FBPIs in O/W emulsion (**Table 1**). Apparent cross-linking in FBPIs was induced by MTG treatments, which led to 5%-8% increases in protein net surface charges and 19%-135% increases in emulsion particle size; moreover, MTG-treated FBPIs showed inhibiting effects against lipid oxidation (e.g. less conjugated dienes and hexanal production) (Liu, Damodaran, et al., 2019). This may be attributed to thicker interfacial layer around oil droplets, larger emulsion droplet size, and protective effect of proteins (Azuma, Kimura, Hosokawa, & Miyashita, 2009; Berton-Carabin et al., 2014). However, MTG-FBPIs moderately promoted protein oxidation (120 min > 240 min ≈ 0 min). Moreover, MTG treatment for 120 and 240 min but not 60 min showed decreased emulsifying activity and physical stability of emulsion, which could be

explained by excessive surface hydrophobicity by cross-linking (Liu, Damodaran, et al., 2019). Another group examined the combined effect of heating and MTG on emulsifying properties of FBPI; FBPIs were treated with heat (90 °C, 5 or 30 min) and MTG (0.6, 6 or 60 U); then, emulsions were made with 20% sunflower oil and 8.0 mg/mL unmodified or modified FBPIs (Nivala et al., 2020). They found that cross-linking by MTG was facilitated by heat treatment; heat/MTG treatment caused an increase in EAI from 25.1 m^2/g to 28.9 m^2/g (Nivala et al., 2020).

On the other hand, cross-linking of proteins may induce undesirable results. For example, one study showed that emulsions with more cohesive MTG cross-linked casein at the interface of oil-water did not show better lipid oxidative stability compared to untreated emulsions (Kellerby, Gu, McClements, & Decker, 2006). This might be attributed to the ability of prooxidants in the aqueous phase to diffuse through the interfacial layer where they might have longer time to contact with lipid hydroperoxides and promote the decomposition into free radicals; consequently, more free radicals would induce lipid oxidation in the droplet core (Kellerby et al., 2006). Similar to hydrolysis, it should be emphasized that choosing an optimum level of enzymatic treatment for proteins is important for achieving desired functionalities. Apart from the major two types of enzymes discussed above, a few other enzymes have been reported. For example, enzymatic deamidation by protein-glutaminase was shown to improve the emulsifying activities of evening primrose seed cake protein by increasing the solubility of protein and enhancing protein's ability to form a layer around fat globules (Hadidi, Ibarz, & Pouramin, 2021). The effects of deamidation of FBPIs needs further investigation.

Besides the modifications of FBPIs discussed above, there are other factors that need be considered. FBPIs were commonly extracted via the wet methods (alkaline extraction and isoelectric precipitation) followed by lyophilization or spray drying, which might change the native protein structure and influence the functional properties. Recently, Keivaninahr et al.

(2021) compared the emulsifying properties of faba bean protein extracted by the conventional wet method and by air classification. They found that the latter method produced proteins with lower protein content but higher solubility; the proteins obtained by air classification also produced emulsions with better or comparable characters in terms of particle size, zeta potential, and microstructure. Another factor to consider was the role of other ingredients in plants. Karefyllakis et al. (2019) suggested milder and simpler extraction processes which could not only yield proteinaceous fractions, but also preserve other native multicomponent (e.g. lipid, phenols, and carbohydrates). In particular, the multicomponent from sunflower seeds exhibited comparable or even better emulsifying properties than the pure proteins. Moreover, this approach is more sustainable and efficient in that it reduces the energy and chemicals that are required for isolating pure proteins. The combined effects of different modifications and other extraction approaches (e.g. air classification, mild methods for preserving other native multicomponent) on emulsifying properties of FBPIs warrant future investigation.

5 Conclusions

To meet the increasing consumer demands for natural, healthy, and sustainable food ingredients, the plant protein can serve as an alternative emulsifier in O/W emulsions to replace synthetic emulsifiers. Faba bean is a promising source of plant protein due to its high protein content, easy cultivation, and high yields. However, the emulsifying functionality of unmodified FBPIs is relatively inadequate due to undesirable physiochemical characteristics (e.g. low solubility, suboptimal surface hydrophobicity and surface charge). Various physical, chemical, and enzymatic modifications have been examined to produce desired physiochemical characteristics for emulsifying activities via different mechanisms (**Figure 2**). After suitable modifications, the emulsifying activity of FBPIs and emulsion stability could be remarkably improved, making modified FBPIs as promising plant-based

emulsifiers in food production. It is important to optimize the modification conditions to achieve favorable outcomes. However, more research is needed on the effects of other types of safe-for-consumption modifications on FBPIs and the underlying working mechanisms need to be elucidated.

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Table 1. Applications of unmodified and modified FBPIs as emulsifiers in O/W emulsions since 1990.

	Methods	Key findings	Reference
Unmodified	Protein solutions were obtained by acid precipitations, alkaline dissolutions, centrifugation, and reconstitution from commercial protein concentrates. Emulsions were prepared with 10% fish oil and 20 mg/mL of pea, lentil, or faba bean protein solutions with whey protein as control.	Whey protein produced smaller initial particle size and better stability; initial oxidative stability: pea < lentil < faba bean < whey in whole emulsions; whey < PI = FBPI in washed emulsions.	(Gumus et al., 2017c)
	Protein isolates were obtained by acid precipitations, alkaline dissolutions, and lyophilization from commercial protein concentrates. Emulsions were prepared with 10% (w/w) algae oil and 5% (w/w) protein isolates from pea (PPI), lentil (LPI), faba bean protein (FBPI).	The LPI stabilized emulsions showed better stability to environmental stresses (pH, salt, and temperature) than FBPI and PPI.	(Gumus et al., 2017a)
	Protein isolates were obtained by acid precipitations, alkaline dissolutions, and lyophilization from defatted legume flours. The physicochemical and emulsifying properties of protein isolates from chickpea (CPI), faba bean (FBPI), lentil (LPI) and soy (SPI) were compared.	Solubility: CPI (94 %) > LPI (90 %) > FBPI (85 %) > SPI (50 %); surface charge: CPI ≈ LPI ≈ FPI (-47 mV) < SPI (-44 mV); all could effectively decrease interfacial tension (from 16.73 to 8.42 mN/m).	(Johnston et al., 2015)
	Protein isolates were obtained by either isoelectric precipitation or salt extraction, and lyophilization from defatted legume flours. The physicochemical and emulsifying properties of protein isolates from chickpea (CPI), faba bean (FBPI), lentil (LPI), and pea (PPI) were compared.	Surface hydrophobicity (isoelectric precipitation): FBPI ≈ SPI < LPI < CPI ≈ PPI; surface charge (salt-extracted): FBPI (-18.3 mV) > CPI ≈ LPI ≈ PPI (-20.4 mV) > SPI (-21.7 mV); emulsion capacity: PPI ≈ CPI < FBPI < SPI ≈ LPI; emulsion stability (ESI): PPI < FBPI < CPI ≈ SPI ≈ LPI.	(Karaca et al., 2011)
Physical	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 0.5% (w/v) FBPIs electrostatically associated with chitosan via either a complex model (by a soluble FBPI-chitosan complex) or LBL model (by FBPI first and thereafter a secondary layer of chitosan added via electrostatic attraction).	Both LPL and complex model increased interfacial layer thickness; but only with the LBL model produced increased compactness and protein adsorption, resulting better physical and oxidative stability.	(Liu et al., 2020)

	FBPIs were prepared from a commercial protein concentrate produced via direct milling of faba beans followed by dry densification. FBPI dispersions were subjected to three pH values (3.0, 5.0, and 8.0); interfacial properties were determined by dilatational measurement and interfacial small amplitude oscillatory shear measurements.	The strength of interfacial FBPI film at O/W interface depended on the pH values; protein adsorption took place significantly faster at pH 3.0 and 5.0 than at pH 8.0.	(Felix, Romero, et al., 2019)
	FBPIs were prepared from a commercial protein concentrate produced via direct milling of faba beans followed by dry densification. Emulsions were prepared with 250 mL of 30 mg/mL FBPIs adjusted to different pH values (3.0, 5.0, and 8.0) and 250 mL of sunflower oil.	Smaller droplet sizes and better rheological properties were obtained at pH 3.0 and 8.0; the emulsions at pH 3.0 displayed the lowest extent of lipid oxidation during storage.	(Felix, Cermeño, et al., 2019)
	FBPIs were obtained by filtration of aqueous extraction from raw or heat-treated faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 3% (w/v) FBPIs that were heated with either oven at 170 °C for 30 min or microwave at 950 watts for 1.5 min.	Oven heating showed better ability to increase surface hydrophobicity while decreasing native lipoxygenase and peroxidase, resulting decreased lipid oxidation.	(Gürbüz, Liu, et al., 2018)
	Faba bean proteins were extracted by alkaline solution, isoelectric precipitation, and lyophilization from dehulled faba bean flours. FBPIs were treated with high pressure (15 kpsi) homogenization.	Homogenization increased protein solubility, surface hydrophobicity by inducing certain level of protein unfolding; but did not improve emulsifying property due to competition of supramolecular aggregates with protein molecules at the interface.	(Yang et al., 2018)
	FBPIs were obtained by alkaline extraction, isoelectric precipitation, and spray drying from dehulled faba bean flours. FBPIs were subjected to alkaline shifting or ultrasound treatment alone, or their combination. Emulsions were prepared with 10% (w/w) canola oil and 10 mg/mL modified FBPIs.	The combined treatment increased protein surface hydrophobicity and decreased free sulfhydryl groups; FBPI solubility was significantly increased (>95% at pH 3 and 7; >80% at pH 6.0); meanwhile, FBPIs modified by the combined treatment produced emulsions with smaller particle size and improved stability at pH values 3 and 7.	(F. Alavi, Chen, & Emam-Djomeh, 2021)
	FBPIs were obtained by alkaline extraction, isoelectric precipitation, and spray drying from dehulled faba bean flours. FBPIs were heated under alkaline pH alone or in the presence of maltodextrin; emulsions were prepared with 10% (v/v) sunflower oil and 10 mg/mL unmodified and modified FBPIs.	The heating under alkaline pH improved the protein solubility (from 43% at pH 3.0, 14% at pH 6.0, and 35% at 7.0 to more than 90%). FBPI-maltodextrin samples heated at pH 11.0 produced emulsions that were stable against flocculation and phase separation.	(Farhad Alavi et al., 2021)

Chemical	FBPIs were obtained by neutral aqueous extraction, acidic precipitation, and spray drying from dehulled faba bean flours. Various degree of acetylation was induced by treating FBPI with acetic anhydride.	Acetylation increased the interfacial activity, Gibbs' adsorption isotherms, and the inflection point of the adsorption isotherms.	(Krause et al., 1996)
	FBPIs were obtained by neutral aqueous extraction, acidic precipitation, and spray drying from dehulled faba bean flours. The ultrastructure of unmodified and acetylated FBPI(~96%) were compared.	Acetylation improve emulsifying activity, which was associated with tangible difference in different droplet-size distribution and ultrastructure.	(Krause et al., 1994)
	FBPIs were obtained by alkaline extraction from faba bean flours. Acetylation (~97%) was induced by treating FBPI with acetic anhydride.	Acetylation increased the flow, gelifying, surface and emulsifying properties, as well as the physical stability.	(Schmandke et al., 1990)
	FBPIs were obtained by aqueous extraction at pH 7.5 followed by isoelectric precipitation from faba bean flours. Acetylation was in combination with methoxy pectin.	The combination increased the protein adsorption at interfacial phase and viscosity but decreased the creaming capacity.	(Schultz et al., 1991)
Enzymatic	FBPIs were obtained by alkaline extraction, acid precipitations, and lyophilization from defatted faba bean flours. FBPIs were treated with heat (90 °C, 5 or 30 min) and MTG (0.6, 6 or 60 U); emulsions were made with 20% sunflower oil and 8.0 mg/mL unmodified or modified FBPI.	Heat treatment increased surface hydrophobicity; heat treatment facilitated cross-linking by MTG; heat/MTG treatment caused a minor increase in EAI.	(Nivala et al., 2020)
	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 1% (w/v) FBPIs that were hydrolyzed by alcalase to different degree of hydrolysis (DH of 4%, 9%, and 15%).	DH of 4% produce increased physical and oxidative stability due to suitable molecular weight, surface net charge and hydrophobicity; higher DHs (9% and 15%) induced decreased surface hydrophobicity and decreased emulsifying activity.	(Liu, Bhattarai, et al., 2019)
	FBPIs were obtained by acid precipitations, alkaline dissolutions, dialysis, and lyophilization from defatted faba bean flours. Emulsions were prepared with 5% (w/v) rapeseed oil and 3% (w/v) FBPIs that were cross-linked by microbial transglutaminase (MTG) for 60, 120, or 240 mins.	MTG induced significant cross-linking, increased protein surface charge and particle size, and decreased lipid oxidation; longer MTG treatment (120 and 240 min) decreased emulsifying activity and physical stability of emulsion.	(Liu, Damodaran, et al., 2019)

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758 **Figure 1.** Emulsion stability. Emulsion is thermodynamically unstable and highly
759 susceptible to oxidation. Physically, emulsion instability usually includes creaming,
760 flocculation, and coalescence. Lipid oxidation in emulsion mainly involves autoxidation via
761 free radical chain reactions. In protein-containing emulsions, protein oxidation is also
762 initiated by metal ions and oxygen radical and is closely related to lipid oxidation, producing
763 peptide backbone cleavage, side-chain modification, and cross-linking. Protein oxidation can
764 also be initiated and prompted by lipid oxidation products.

765 **Figures 2**. The mechanisms by which physical, chemical, and enzymatic modifications of
766 FBPIs affect the emulsifying functionalities. The emulsifying activities depend on several
767 physiochemical characteristics such as solubility, surface hydrophobicity, surface charge,
768 interfacial activity, etc. These characteristics of FBPIs can be optimized by various
769 modifications via: increasing the interfacial layer thickness and compactness (1); increasing
770 steric hindrance by either electrostatically or covalently linked to other polysaccharides or
771 proteins (3, 4, 7); improving surface hydrophobicity by inducing protein unfolding and
772 changing configuration (2), acetylation (3), Maillard reaction (4), hydrolysis (6), or cross-
773 linking to other proteins (7); inducing greater electrostatic repulsion by increasing surface
774 charge (5); increasing protein solubility by reducing molecular size and increasing surface
775 charge (5, 6).