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Review Article

Author(s):
Nikbakht Nasrabadi, Maryam; Sedaghat Doost, Ali; Mezzenga, Raffaele

Publication date:
2021-09

Permanent link:
https://doi.org/10.3929/ethz-b-000480008

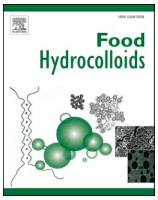
Originally published in:
Food Hydrocolloids 118, https://doi.org/10.1016/j.foodhyd.2021.106789

Food Hydrocolloids 118 (2021) 106789

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: http://www.elsevier.com/locate/foodhyd



Review

Modification approaches of plant-based proteins to improve their techno-functionality and use in food products

Maryam Nikbakht Nasrabadi [a], Ali Sedaghat Doost [a], Raffaele Mezzenga [b,*]

[a] Particle and Interfacial Technology Group (PaInT), Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent, 9000, Belgium

[b] Food and Soft Materials, Institute of Food, Nutrition and Health, ETH Zurich, Schmelzbergstrasse 9, Zurich, 8092, Switzerland

ARTICLE INFO

Keywords:
Plant-based proteins
Modulation
Modification
Food applications
Challenges

ABSTRACT

Plant-based proteins have recently attracted particular interest owing to their sustainable origins, economical costs and health benefits compared to animal-based counterparts. However, most of them have limited applications due to their inferior functionality, which is the consequence of poor-aqueous solubility, complexity and sensitivity to environmental stress conditions such as pH, salt and temperature. Additionally, plant proteins are often embedded in hemicellulose, lignin and other poorly digestible polysaccharides, which further reduce their bioavailability. Therefore, the modulation of plant proteins to improve their technological and industrial applications, and make them more accessible in general, is highly sought after. The modification of plant proteins by altering their physicochemical properties provides the possibility to improve and diversify their techno-functionality and biological activities as well as addressing their limitations. The selection of protein modification method should be done carefully from the final application view especially in food products since it can influence the protein functional, nutritional and organoleptic properties. Therefore, discussing different modification methods with their advantages and disadvantages is particularly timely. This review highlights and discusses the modification methods for plant proteins in order to make their applications in foods more feasible by improving their flavor, nutrition and techno-functional attributes, which will open up new opportunities within different plant-based food products.

1. Introduction

Plant-derived materials have recently attracted growing attention in food and pharmaceutical industries due to their advantages over their animal counterparts, such as a lower incidence of infection and contamination, less cultural and religious food habit limitations, targeting vegetarian consumers, along with their versatility and lower cost (Jafari, Sedaghat Doost, Nikbakht Nasrabadi, Boostani, & Van der Meeren, 2020; Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Roman, et al., 2019). Another considerable advantage of plant-based materials is their higher sustainability from the environmental and agricultural point of view (Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Roman, et al., 2019). The utilization of plant-based materials also respects the animal welfare and has a higher intrinsic ethical profile. Therefore, moving away from animal-towards plant-based materials is also driven by consumers and environmental concerns. Proteins are critical ingredients in human diets since they are essential for keeping muscles in good condition, control the immune responses, repair cells and improve their signaling (Wen et al., 2019). In addition to providing diverse amino acids in the human diet, proteins are also considered as very important components in food formulations due to their desirable functionalities, including thickening and gelling ability, emulsifying, foaming, water holding and fat absorption (Y. Cao, Bolisetty, Wolfisberg, Adamcik, & Mezzenga, 2019; Y. Cao & Mezzenga, 2019). Therefore, proteins are versatile ingredients in several food products. Animal-based proteins are used in food industry in different applications due to their desirable properties including their high yield and essential amino acids content with right balance (Wen et al., 2019). Despite of these advantages, finding renewable and sustainable alternatives for animal-based proteins has attracted considerable interest because of their adverse effects on the environment, high cost, limited availability and limited acceptance by some consumers such as vegetarians and vegans. Moreover, the consumption of animal proteins may have negative effects on the human health such as high blood pressure and

* Corresponding author.
E-mail address: raffaele.mezzenga@hest.ethz.ch (R. Mezzenga).

https://doi.org/10.1016/j.foodhyd.2021.106789
Received 2 January 2021; Received in revised form 24 March 2021; Accepted 26 March 2021
Available online 4 April 2021

obesity (F. B. Hu; Richter, Skulas-Ray, Champagne, & Kris-Etherton, 2015). There is also a concern about potential allergenicity of animal proteins, which may be greatly reduced with vegetable proteins (Sá, Moreno, & Carciofi, 2020; Wen et al., 2019). As mentioned above, plant proteins are considered as more environmentally sustainable and renewable alternatives for the animal-based ones. Moreover, their production is associated with less deforestation and climate changes since it requires much less land and emits much less greenhouse gases compared to the animal husbandry (Poore & Nemecek, 2018). Some plant-based proteins from different sources are displayed in Fig. 1. They can be isolated from sustainable and cheap sources such as plant-derived wastes from agriculture and by-products of crop and oil industries, which can be also impactful on the reduction of food waste (Pojić, Mišan, & Tiwari, 2018; Sá et al., 2020).

2. Plant-based proteins and their utilization challenges in food products

Plant-based proteins are considered as functional ingredients with various roles in food formulations, including thickening and gelling agents, stabilizers of emulsions and foams, binding agents for fat and water. Moreover, some proteins have biological activities such as antioxidant or antimicrobial characteristics (Jafari et al., 2020; Sedaghat Doost, Nikbakht Nasrabadi, Wu, A'Yun, & Van der Meeren, 2019; Warnakulasuriya & Nickerson, 2018). Vegetable proteins can also be utilized for the production of bioactive peptides. However, most of the plant-based proteins are intractable due to their poor aqueous-solubility, complexity and susceptibility to pH, ionic strength and temperature, limiting their applications (Y. Cao & Mezzenga, 2019; Warnakulasuriya et al., 2018). Most of the plant proteins are a mixture of different proteins with variable fractions, having a wide range of isoionic point (pI) instead of a single point, such as flaxseed (Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Dewettinck, & Van der Meeren, 2019; Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Roman, et al., 2019), soy (Lee et al., 2016) and pea proteins (Adal et al., 2017). Therefore, the modulation of their characteristics in order to improve their functionality is highly required. Another limitation of plant-based proteins is the presence of some specific plant residuals which are believed to be as anti-nutrients. These compounds are synthesized in plants with certain biological roles of protecting seeds and plants from insects, viruses, fungus and other organisms. Some of these modification methods can also be effective on reducing or eliminating the adverse effects of these anti-nutrients (Avilés-Gaxiola et al., 2018). Furthermore, some plant-derived proteins have limited applications in food products due to their undesirable tastes, such as bitterness, which may be masked by some modulation methods (Zeeb et al., 2018). The challenges of the application of plant-based proteins in food products which should be faced by their modification are schematically displayed in Fig. 2.

In fact, the modification methods should be selected carefully especially for the food and pharmaceutical applications since these methods can have some noticeable effects on the nutritional value, functional, and organoleptic properties of plant proteins. Therefore, this review discusses the different modification methods which have been used for the modulation of plant-based proteins and their advantages and disadvantages. Different modulation methods to widen their applications in food sectors, including physical, chemical, enzymatic, complexation and the most recent and efficient one, which is fibrillization, will be discussed with their pros and cons.

3. The modification approaches

The term "protein modification" refers to the process of altering the molecular structure or a few chemical groups of a protein by special methods for the purpose of their techno-functionality and bioactivity improvement. Modification of plant-based proteins provides the opportunity to make them multi-functional ingredients for food systems by changing their physicochemical properties and addressing their limitations. Generally, the protein modification methods can be classified into physical, chemical, biological and other novel methods as schematically illustrated in Fig. 3.

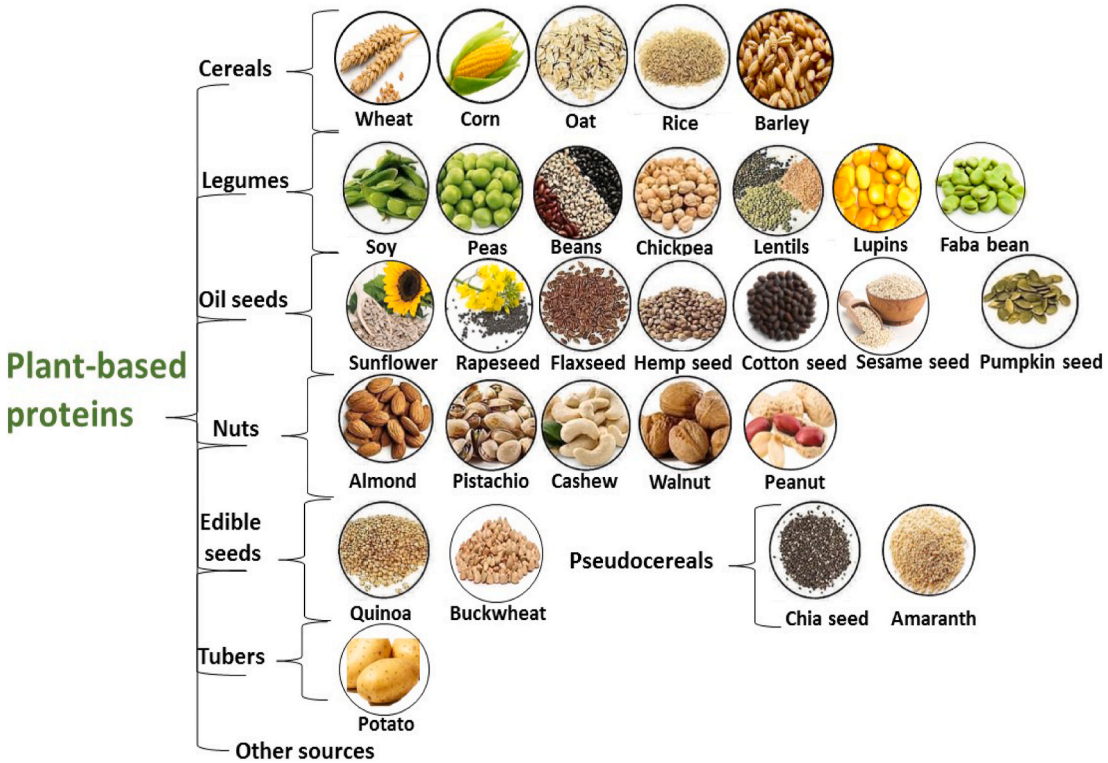


Fig. 1. Major sources of plant-based proteins.

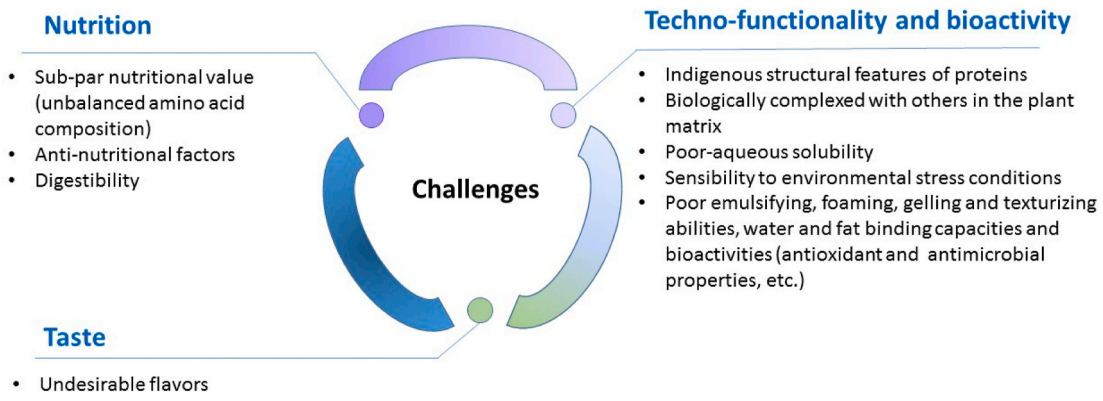


Fig. 2. Challenges of the utilization of plant-based proteins in food products.

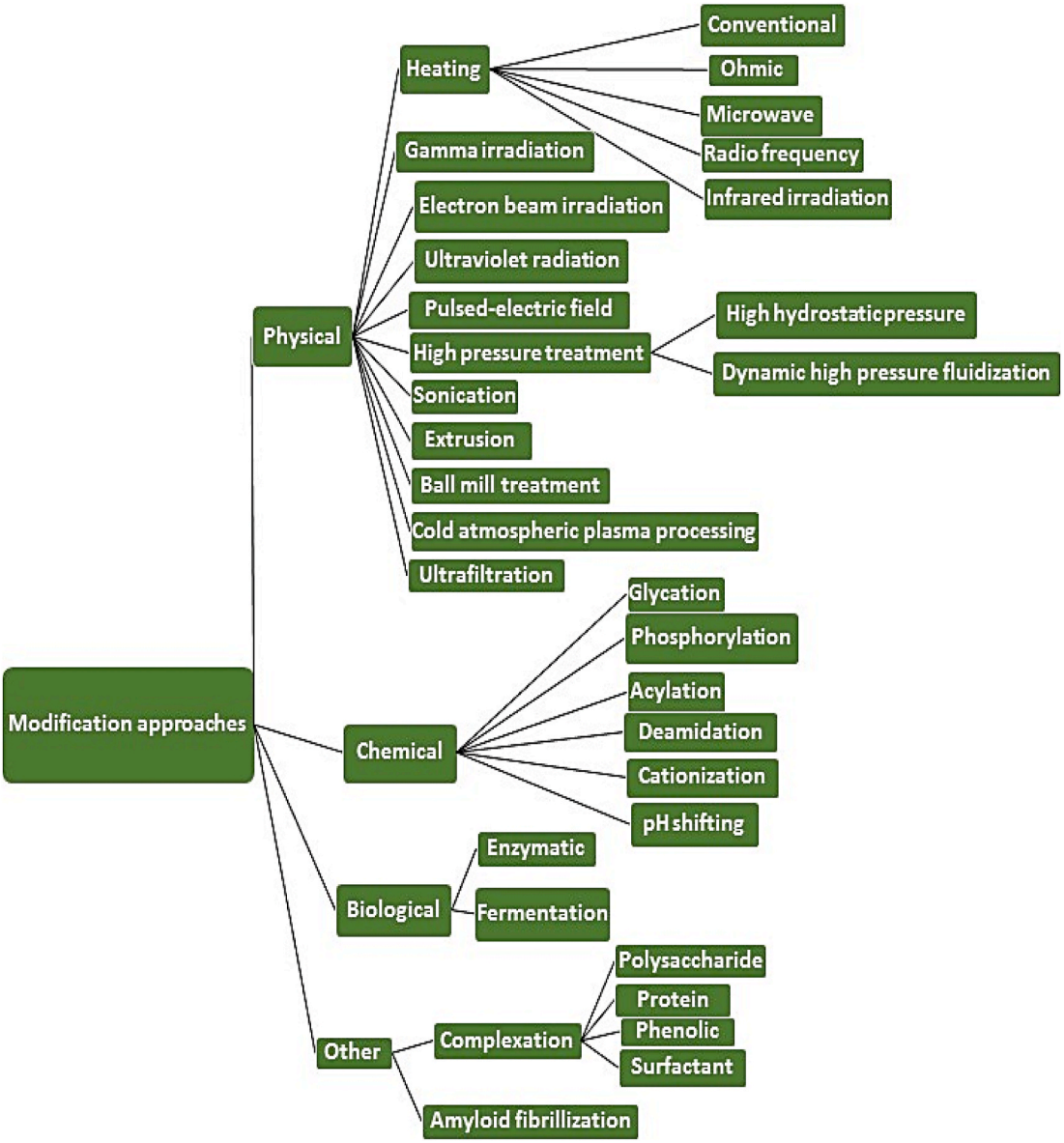


Fig. 3. Plant-based proteins modification approaches scheme.

3.1. Physical modification

Physical methods to expand the functionality of proteins are simple approaches that are not based on chemicals or enzymes. Since there are no chemicals used in the processing, these methods of protein modifications have gained significant interest, avoiding harmful consequences

of possible chemical residuals. The physical methods of modifications which have been used to date for the plant-based proteins are introduced as follows, and the advantages and limitations of these technologies will be individually highlighted. The recent studies about the modification of plant-based proteins using physical approaches are given in Table 1.

3.1.1. Heat treatment

3.1.1.1. Conventional thermal treatment. Conventional heating is one of the common methods for physically modifying plant-based protein structural and functional properties. Mild thermal condition promotes the protein unfolding, leading to an intermediate molten globule state with enhanced functionality. However, extreme thermal treatment causes irreversible changes in the protein structures, resulting in its denaturation and aggregation through different bonds including disulfide, hydrophobic and electrostatic, leading to a decrease on their functional properties. These heat-induced assembles have been exploited in pharmaceutical and food industries due to their improved functionality (Aryee, Agyei, & Udenigwe, 2018, pp. 27–45). During the thermal treatment of a protein solution, as a consequence of the unfolding of polypeptide chains, the internal sulfhydryl groups and the hydrophobic side chains, previously buried in the core of the native-state structure, become more exposed (Aryee et al., 2018, pp. 27–45; K.-Q.; Wang et al., 2017). These structural changes will extend the techno-functionality of plant derived proteins. For instance, the preheating of plant proteins can increase their thermal stability as Wuchao Ma et al. (2020) and C. Sun, Dai, He, et al. (2016) showed in the case of soy protein and zein, respectively. Although there are several researches on the positive effect of heat treatment on the foaming ability of plant-based proteins (Shao, Lin, & Kao, 2016; M.; Zhao, Xiong, Chen, Zhu, & Wang, 2020), there are also some researches showing its adverse effect on this functional performance (Chao et al., 2018; S.-W.; Lv, Sun, Zhao, & Bao, 2017). In the case of gelling properties, the thermal treatment was successfully improving the ability of cowpea protein to form gels (Felicitas Peyrano et al., 2017; F. Peyrano et al., 2016). The gelling properties of album protein isolates were also improved by controlled heat treatment at 100 °C for 30 min in a study conducted by Mir et al. (2020). The water holding capacity of faba bean protein was modified using dry heat method at temperatures from 75 to 175 °C by Bühler, Dekkers, Bruins, and van der Goot (2020).

Thermal treatment has been reported to be an efficient way for reduction or elimination of adverse effects of anti-nutritional compounds in plant proteins (Avilés-Gaxiola et al., 2018). For instance, thermal treatment was used for by Avilés-Gaxiola et al. (2018) for the inactivation of trypsin inhibitors in chickpea and soy bean and the combination of heating and use of metabisulfite as a reducing agent inactivated the trypsin inhibitors up to 99.4%. Trypsin inhibitors are considered as one of the anti-nutritional factors which limit the consumption of legumes since they prevent the action of pancreatic trypsin and chymotrypsin in the gut resulting in several disorders such as complicated digestion, poor protein digestion and absorption, pancreatic enlargement, growth retardation and muscle wasting (Avilés-Gaxiola et al., 2018). Thermal treatment can also improve the digestibility and nutritional properties of plant-based proteins. Mir et al. (2020) improved the digestibility of album protein isolate up to 87.55% and increased the availability of its essential amino acids by heating at 100 °C for 30 min.

In addition to thermal treatment as an individual modification method, heating can also be used to trigger other changes due to the higher rates of chemical or enzymatic reactions. For example, Drozłowska, Weronis, and Bartkowiak (2020) applied high temperatures and high pressure for the modification of potato protein isolate and the treated samples showed improved emulsifying ability without any change in their chemical composition. L. Liu et al. (2020) studied the complex action of heating at 60 °C and ultrasound for the modification

Table 1
A summary of recent studies related to the physical modification approaches of plant-based proteins.

Physical modification approach	Protein type	Modified characteristics	Reference
Conventional heat treatments	Album (*Chenopodium album*)	Improved thermal stability	Mir, Riar, and Singh (2020)
	Pea	Enhanced emulsifying properties	Chao and Aluko (2018) and W. Peng, Kong, et al. (2016)
	Soy		Q. Li, Zheng, Ge, Zhao, and Sun (2020)
	Cowpea	Gel forming characteristic	Felicitas Peyrano, de Lamballerie, Avanza, and Speroni (2017) and F. Peyrano, Speroni, and Avanza (2016)
	Album protein isolates		Mir et al. (2020)
High pressure	Zein	Structural changes	C. Sun, Dai, Liu, and Gao (2016)
	Faba bean		J. Yang, Liu, Zeng, and Chen (2018)
	Soy flour	Hydrophobicity and sulfhydryl content	H.-H. Liu and Kuo (2016)
Sonication	Pea protein isolate	Foaming properties	Xiong et al. (2018)
	Walnut	Higher solubility and emulsifying ability	Zhu et al. (2018)
	Acid-induced soy	Size reduction	Huang, Ding, Li, and Ma (2019)
	Wheat and soy	Secondary structural changes and better emulsifying ability	O'Sullivan, Park, and Beevers (2016)
	Flaxseed	Emulsifying, foaming, and gelling ability	Juodeikiene et al. (2020)
	Buckwheat protein isolates	Changes in the secondary and tertiary structure	J. Jin, Okagu, Yagoub, and Udenigwe (2021)
	Album seed protein	Solubility, foaming and thermal properties	Mir, Riar, and Singh (2019a)
	Soy	Reduction in protein trypsin inhibitor activity and improvement in digestibility	Vanga, Wang, and Raghavan (2020)
		Decrease in the allergenicity	H. Li, Zhu, Zhou, Peng, and Guo (2016)
Extrusion	Corn, soy, chickpea and Yellow Pea	Digestibility and decrease or even eliminating the anti-nutrients	Devi et al. (2020), Omosebi, Osundahunsi, and Fagbemi (2018), and Yin et al. (2019)
	Pea nut isolate	Increased accessibility for protease hydrolysis and improved emulsifying properties	L. Chen, Chen, Yu, Wu, and Zhao (2018)
	Pea	Improved antioxidant performance	Zhou, Liu, and Feng (2017)
	Legume-derived proteins	Reduction of unfavorable beany flavor	Simons et al. (2015)
Cold atmospheric plasma	Different types	Increase in the content of disulfide bonds via changes in	S. Dong et al. (2017), S. Dong et al. (2017), and

(*continued on next page*)

Table 1 (*continued*)

Physical modification approach	Protein type	Modified characteristics	Reference
		their secondary structure and rheological properties	J.-j. Yu, Huang, et al. (2020)
	Pea nut	Unfolding and the alteration of secondary structure	Ji et al. (2018)
	Flaxseed	Emulsifying, foaming and antioxidant properties	Xu et al. (2020)

of soy glycinin, and were successful in improving its emulsifying characteristics.

3.1.1.2. Ohmic heating. Ohmic heating, which was first used in 1920 for milk pasteurization, is a thermal processing method applying alternating electric currents straight away into a semi-conductive medium. By passing a moderate and alternating electric current through the product which acts like a resistance in an electrical circuit, a direct or volumetric heat is generated in the products based on Joule's law. By providing a fast and uniform heating as well as electrical effects, ohmic heating can result in unfolding, denaturation and the formation of uniform-sized protein aggregates with distinctive techno-functional properties. Therefore, this electro-heating method can be considered as an alternative for conventional heating methods without less adverse change on the protein quality and amino acid content (Mesías, Wagner, George, & Morales, 2016; Pereira et al., 2018). Although there are several studies on the effects of ohmic heating on animal-based proteins (Moreira, Pereira, Vicente, & da Cunha, 2019; Pereira et al., 2018), more emphasis should be placed on evaluating the application of this thermal process on the quality and properties of plant-based proteins. Recently, X. Li, Ye, Tian, Pan, and Wang (2018) compared the effect of ohmic heating and conventional heating on the structure and techno-functionality of proteins in soybean milk. Their results showed that ohmic heating was successful in decreasing the heating time and improving the emulsifying ability of the protein. On the contrary, the foaming property of the protein decreased due to the decrease in its surface hydrophobicity.

3.1.1.3. Microwave heating. The microwave heating technology is based on electromagnetic waves with wavelengths and frequency in the range of 1 mm to 1 m and 300 MHz to 300 GHz, respectively; it has gained considerable popularity in food processing due to its uniform heating, high heating rates, safety, simple, rapid, and clean operation, and low maintenance. Furthermore, this technique of heating has less effect on flavor and nutritional quality of food products in comparison to conventional heating. This method can induce protein unfolding by splitting disulfide and hydrogen bonds (non-covalent bonds) with consequent effect on the secondary and tertiary structures of protein (Han, Cai, Cheng, & Sun, 2018; Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019). For instance, Xiang, Zou, Liu, and Ruan (2020) reported significant changes in secondary structure of wheat gluten after microwave treatment at 1000 W for 5 min without any reduction in the amount of essential amino acids. The effect of microwave process as a techno-functional modification treatment has been studied for different plant-based proteins. It has been used for improving the gelling properties of soy protein (Mu et al., 2020) and emulsifying properties of lotus seed protein isolates (X. Zhang, Wang, Li, & Yan, 2020). Microwave process can modulate the protein without destroying its primary structure since the microwave energy is lower than the energy of chemical bonds, making it interesting as a pretreatment method before other modification techniques (Han et al., 2018). For example, Gohi, Du, Zeng, and Cao (2019) used microwave heating as a pretreatment for enzymolysis of lotus seed protein which was successful to increase the efficiency of enzymatic modification by breaking the protein sub bonds and unfolding its secondary structure resulted in its susceptibility to papain. Microwave process also was shown to assist the chia seed protein enzymatic hydrolysis with improved bioactivity (antioxidant activity)and functionality (emulsification and foaming properties) obtained in a shorter time in comparison to conventional hydrolysis methods (Urbizo-Reyes, San Martin-González, Garcia-Bravo, López Malo Vigil, & Liceaga, 2019). In another study conducted by Meng et al. (2019), microwave treatment was also used for assisting the modification of rice dreg protein through glycation with sodium alginate by wet heating.

Microwave process has been also used for immunomodulation of different plant-based proteins. H. Lee et al. (2016) showed a significant reduction (24.7%) in soy protein allergenicity by applying microwave heating at the power of 600 W for 10 min. There are also some studies introducing microwave treatment as a way for abolishing gluten toxicity for celiac patients (Lamacchia, Landriscina, & D'Agnello, 2016). However, the results of some other studies displayed the inefficiency of this treatment for the purpose of detoxifying of gluten (Mahroug et al., 2019).

A similar contradiction has been reported in terms of the digestibility of plant-based proteins affected by microwave heating. K. Liu, Zheng, and Chen (2019) reported 10.3% and 16.8% reduction of the gastric protein digestibility and gastrointestinal protein digestibility of microwave heated rice protein due to the formation of intramolecular disulfide linkages which results in protease resistance. Xiang et al. (2020) also reported the decreased *in vitro* gluten digestibility after microwave heating at 1000 W for 5 min as a result crosslinking between amino acids. In contrast, there are some studies showing the positive effect of microwave treatment on the digestibility of plant-based proteins such as the study conducted by Vanga et al. (2020) and X. Sun, Ohanenye, Ahmed, and Udenigwe (2020) in the case of soymilk proteins and pigeon pea protein, respectively.

3.1.1.4. Radio frequency treatment. Similar to microwave, Radio frequency (RF) is also based on volumetric heating, and is effective on the structure of proteins by the action of the free radicals produced by dipolar and ionic motion under the effect of RF field. Both RF and microwave heating methods can address the problems of low heating rate effects which are typical of conventional heating methods due to their volumetric heating (Han et al., 2018). However, RF has longer wavelength compared to microwaves, and occupies a region between 1 and 300 MHz, whereby only 13.6, 27.1, and 40.7 MHz are allowed for the purpose of industrial, scientific, and medical uses by the US Federal Communications Commission (C. Guo, Wang, & Wang, 2018; Ling, Cheng, & Wang, 2019). RF heating performance can be influenced by many factors, including dielectric properties or penetration depth, pH, protein concentration, temperature as reported by C. Guo et al. (2018) in the case of soy protein, and more in general power density of electromagnetic waves and types of heating systems (Ling, Cheng, & Wang, 2019).

RF heating was reported to be effective on the structure of soy protein through breaking disulfide bonds and increasing the surface hydrophobicity (C. Guo et al., 2017). Ling, Ouyang, and Wang (2019) displayed significant changes in secondary structure of rice bran protein according to increase in random coil and decrease in β-sheet, α-helix and β-turn after RF treatment and they also showed changes in the tertiary structure of protein due to decrease in tryptophan fluorescence and enhancement of surface hydrophobicity. RF may be efficient to improve the functionality as shown by Hassan, von Hoersten, and Mohamed Ahmed (2019) that the oil holding capacity and emulsifying properties of maize protein after exposure to RF energy at 300 W without any change in the protein profile was improved. Similarly, Ling, Ouyang, and Wang (2019) also displayed the positive effect of RF on emulsifying

properties of rice bran protein.

3.1.1.5. Infrared irradiation. Infrared radiation (IR) is a range of the electromagnetic spectrum which falls in between the visible region and microwaves, with wavelength extending from 0.5 to 1000 μm. The penetration of these waves results in vibration of water molecules at a frequency of 430 THz down to 300 GHz which subsequently triggers heating. IR technology is another way of heating which is more energy efficient, environmentally friendly and less water consuming in comparison to conventional ways of heating. Moreover, IR possesses higher heating rates, uniform heating, rapid heating and higher ability to maintain the food quality and safety. It is reported that the exposure of proteins to IR heating denatures them or makes them more susceptible to denaturation and aggregation due to the unfolding of their molecules (Ogundele & Kayitesi, 2019). The submission of African legumes to IR heating resulted in an increase in their digestibility and decrease in the amount of their anti-nutritional factors such as α-galactosides, protease 21 inhibitors, tannins and/or lectins (Nti, 2009).

To the best of our knowledge, there has not been much work performed on the modulation of plant-based proteins using this method. However, this method has been used in combination with other techniques for other kinds of proteins (G. Hu et al., 2017).

3.1.2. Gamma irradiation

Gamma irradiation is considered as a non-thermal process of extending the shelf-life of food products. In addition to the reduction of microorganisms, this novel technique has the ability to cause chemical changes in proteins, including crosslinking and fragmentation, beyond the physical aggregation. Hydroxyl and superoxide anion radicals formed during the gamma irradiation process are responsible for changes in primary, secondary, tertiary or even the quaternary structures of proteins (Han et al., 2018). These changes can be evidenced through different methods. For instance, Baccaro, Bal, Cemmi, and Di Sarcina (2018) confirmed the gamma irradiation-induced changes in the secondary and tertiary structure of rice protein through UV-VIS spectra and luminescence measurements. They also used pH measurements to confirm the changes in the chemical composition due to the reaction among tryptophan and tyrosine amino acids in irradiated samples. In a study conducted by Malik, Sharma, and Saini (2017), changes in the secondary and tertiary structures of sunflower protein isolate were shown after gamma irradiation according to changes in α-helix and β-sheet contents and changes in fluorescence spectra, respectively. These structural variations resulted in conformational and chemical changes, combined with protein aggregation and crosslinking, with consequent improvement in the protein functionality. Their results revealed an increased thermal stability of sunflower protein after irradiation. In another study, an increased surface hydrophobicity and improved antioxidant ability, oil binding capacity, emulsifying and foaming properties were found after introduction of gamma irradiation to sunflower protein isolate (Malik & Saini, 2017). In another study conducted by Hassan, Mahmoud, Elmamoun, Adiamo, and Mohamed Ahmed (2018) the improvement of emulsifying ability was observed in irradiated sesame proteins. However, there are some reports on the impairing effect of gamma irradiation on some functional properties of plant-based proteins. For example, Malik and Saini (2017) showed the decreased water binding capacity of sunflower protein after gamma irradiation. Presumably, these differences can be related to different dosage of exposure to gamma radiation.

Gamma irradiation was shown to be used for increasing the digestibility of plant-based proteins such as sesame protein (Hassan et al., 2018). It can also be used for the reduction of allergenicity of plant-based proteins especially in combination with other processing techniques. Kasera et al. (2012) evaluated the effect of different processing conditions such as boiling, gamma irradiation or the combination of both, on the allergenicity of different legume proteins including kidney bean, black gram and peanut. Their results showed that the gamma irradiation alone did not significantly change the allergenic potential of legume proteins while its combination with boiling was successful in attenuating allergenicity. P. Meinlschmidt, Ueberham, Lehmann, Schweiggert-Weisz, and Eisner (2016) also observed reduced immunoreactivity of soy protein after gamma irradiation. They also reported that the high dose of irradiation above 25 kGy could be successful in eliminating most of soy allergens. However, to date, the maximum allowed dose rate for specific commercial food items is 10 kGy according to food and drug administration (FDA) Food and Drug (2004).

3.1.3. Electron beam irradiation

Electron beam irradiation (EBI) technology is the exposure of food to high-energy electrons generated by machine sources and can be considered as a controllable non-thermal process. EBI is cost- and energy-efficient and has been used for the sterilization of food materials as well as assisting extraction processes. The application of EBI for the improvement of proteins quality and safety has gained considerable interest. Electrons due to their high energy are capable to affect the chemical and molecular bonds in the protein structure, leading to its unfolding and denaturation. Due to these structural changes in proteins, their functional properties and bioactivity can be improved (L. Wang, Yang, Fan, Zhang, & Chen, 2019; X. Zhang et al., 2020). L. Wang, Yang, et al. (2019) evaluated the relationship between these changes in the structure of wheat germ protein hydrolysates induced by EBI and its improved functionality and bioactivity performance. Their results revealed that EBI-triggered structural changes in this protein caused decrease in surface hydrophobicity and molecular weight, which positively affected its emulsifying and foaming properties. Moreover, the antioxidant ability increased as a function of EBI dose. Increased antioxidant ability after exposure to EBI was found by X. Zhang, Wang, Li, and Yan (2020) for rice protein at dose of 30 kGy, which was attributed to be the result of changes in its secondary structure due to the destroying effect of EBI on the α-helix structure. Interestingly, the puncture pores and fragmentation on microcosmic surface of walnut protein flour after exposure to EBI (at a dose of 5.0 kGy) have been shown by Y. Zhao, Sun, et al. (2017) which was along with aggregation and cross-linking, resulting in the increased thermal stability of this plant-based protein. Aggregation and cross-linking of rice protein peptides was also shown after EBI treatment at 75 kGy in another study, resulting in the improved emulsifying activity of the peptides from 145% to 204% (T. Li, Wang, et al., 2019). Nutritional value of the plant-based proteins is another factor that may be improved by EBI as a study conducted by V. Kumar et al. (2017) showed improved digestibility of soy protein and decreased content of trypsin inhibitor after exposure to EBI.

EBI can be potentially used as a pretreatment for other modification methods of plant-based proteins. For example, T. Li, Cui, et al. (2019) used EBI-assisted enzymatic hydrolysis to modify rice proteins, where an improved solubility and emulsifying ability was observed. X. Zhang, Wang, Li, and Yan (2020) also used EBI as a pretreatment for increasing the efficiency of rice protein enzymolysis.

3.1.4. Ultraviolet radiation

Ultraviolet (UV) light with the wavelength of 200–280 nm has been used for the inactivation of bacteria in water, air, surfaces, and packaging materials. It has been also used for some liquid food products such as milk and whey. UV treatment can be achieved by passing a liquid through a UV-penetrable tube or thin film with turbulent or laminar flows, respectively. Since the UV light can be absorbed by aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, the UV irradiation can be effective on proteins (Y.-F. Liu, Oey, Bremer, Carne, & Silcock, 2019). Therefore, UV irradiation can induce chemical modification in plant-based proteins which can improve their techno-functional properties. The cross-linking effect of UV light was

evaluated on the film forming properties of sesame protein isolate and it was reported to be successful in improving the mechanical properties of the formed films with more compact structure, that is without pinholes and cracks compared to the films obtained from untreated protein solutions (Fathi, Almasi, & Pirouzifard, 2018). A. Kumar, Nayak, Purohit, and Rao (2020) confirmed the UV light-induced changes in the primary chain of Osborne protein in wheat flour and its conformational changes through SDS-PAGE and FTIR, respectively, leading to an increase its solubility and sulfhydryl content.

Pulsed-light treatment, also known as pulsed UV, with a broader wavelength range (180–1100 nm) is also used in short pulses (flashes) for 3.0 ms, which is more effective than continuous UV treatment (Y.-F. Liu, Oey, et al., 2019). This process may also be capable to decrease the allergenicity of plant-based proteins in addition to its ability to modify their structure and functionality (Panozzo, Manzocco, Lippe, & Nicoli, 2016). Panozzo et al. (2016) showed the effect of this treatment on decreasing the immunoreactivity of wheat gluten as well as modifying its functionalities due to its partial depolymerization and unfolding of the monomeric fractions by disulfide exchange.

3.1.5. Pulsed-electric field

The pulsed-electric field (PEF) technology has been used as a non-thermal process for the purpose of microorganisms and enzymes inactivation. In this approach, the food sample is subjected to short high-power electrical pulses (μs or ms) between electrodes (Sedaghat Doost, Nikbakht Nasrabadi, Sadžak, & Van der Meeren, 2020). A PEF system consists of a chamber, electrodes, high-voltage pulse generator, and a computer for monitoring and controlling devices. A strong electric field is generated between two electrodes due to their electrical potential difference. The produced electrical energy during the PEF process can result in the protein unfolding and subsequent increased interactions with the solute. This can increase the protein solubility and subsequently affect its functional properties. L. Zhang et al. (2017) reported that PEF treatment of canola seeds increased the solubility, emulsifying and foaming properties of the extracted protein. However, depending on the intensity and time duration of the PEF process, it can cause denaturation and aggregation which leads into the reduction of solubility (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019). PEF process is capable of altering the secondary and tertiary structure of plant-based proteins. L. Zhang et al. (2017) confirmed the PEF-induced changes in the secondary structure of canola proteins by alteration in the proportions of α-helices, β-sheets, and β-turns in the amide I region, as revealed by Infrared spectrometry. The same authors also highlighted the changes in tertiary structure of the protein by showing the increased amount of free sulfhydryl groups and surface hydrophobicity. Changes in the secondary structure of peptides obtained from pine nut protein were also reported along with their antioxidant activity (Liang, Cheng, & Wang, 2018).

3.1.6. High pressure treatment

3.1.6.1. High hydrostatic pressure. High hydrostatic pressure (HHP), which applies hydrostatic pressures in range of 100–800 MPa for a few minutes, is a non-thermal process originally introduced for the milk preservation (Hite, 1899). HHP has been used for different purposes such as inactivation of microorganisms, texture changes and emulsification. Another application of this technology is its ability to modify food proteins by the breakage of hydrophobic and electrostatic interactions as well as the formation of new bonds which result in their aggregation and subsequently gelation (P. Lv et al., 2020; Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019).

The exposure of different plant-based proteins to HHP treatment was shown to affect their denaturation, aggregation, and interactions. HHP treatment typically increases the protein hydrophobicity and decreases its solubility due to its ability to exposing buried sulfhydryl groups after unfolding and denaturation, which is followed generally by aggregation, gelation or improvement of its techno-functional properties (Queirós, Saraiva, & da Silva, 2018). In terms of these structural changes, H. Lee et al. (2016) demonstrated an increased surface hydrophobicity and sulfhydryl group content of ginkgo seeds protein as well as secondary structural changes after HHP treatment, which resulted in improved heat stability and emulsifying properties. Although there are some studies showing decreased solubility of plant-based proteins after HHP, especially at higher applied pressures (i.e., >400 MPa) due to aggregation (Condés, Añón, & Mauri, 2015; J.; Zhao, Zhou, Zhang, Ni, & Li, 2015), some other works indicate a positive effect of this process on solubility (Liu et al., 2020d). For instance, B. Cao, Fang, Liu, Min, and Liu (2017) displayed improvement of solubility, water holding capacity and oil holding capacity of pine nuts protein after 200 and 400 MPa HHP, respectively. In the case of kidney bean protein, HP treatments higher than 600 MPa had also significant effect on the secondary structure of protein according to the FTIR spectroscopy which significantly improved water holding capacity, foaming, and emulsifying properties of this protein (Ahmed, Al-Ruwaih, Mulla, & Rahman, 2018). In order to evaluate the position of HHP as compared to the other methods, Piccini, Scilingo, and Speroni (2019) compared HHP and thermal treatment for modification of calcium-added soy protein where their results revealed that HHP improved the protein solubility and its colloidal stability, which was not the case in the conventional thermal treatment. HHP-treated samples also were able to form transparent cold-set gels with excellent water holding capacity. In a different study, Z.-K. Zhao et al. (2018) evaluated the combined effect of salt addition and HHP on sweet potato protein and reported the increased ability of the formed gels in improved textural properties and water holding capacity. Similarly, the authors also observed the positive effect of sulfur-containing amino acids additives and HHP as a modification method on the textural properties of sweet potato protein gels (Z.-K. Zhao, Mu, Zhang, & Richel, 2019).

HHP was also evaluated as an approach for increasing the nutritional value of plant-origin proteins. For example, H. Lee et al. (2016) showed HHP as the most successful approach in reducing the allergenicity of soy protein isolate for using in infant formula compared to other methods such as high-intensity ultrasound, microwaving and high-pressure homogenization, respectively. H. Lee et al. (2016) also observed a significant decreased allergenicity of proteins from ginkgo seeds after HHP at pressure ranging from 300 up to 700 MPa.

HHP could be also used as a pretreatment for enzymatic hydrolysis of plant-based proteins due to its ability to make them susceptible of increased efficiency of enzymes. HHP can increase the degree of enzymatic hydrolysis as Franck et al. (2019) revealed in the case of trypsin hydrolyzing flaxseed protein. Moreover, their results revealed the generation of smaller peptides with higher antioxidant performances after HHP pretreatment. Al-Ruwaih, Ahmed, Mulla, and Arfat (2019) also used HHP (300–600 MPa for 15 min) for assisting enzymatic proteolysis (alcalase as protease) of kidney beans protein isolates leading hydrolysates with improved functionality and antioxidant activity.

3.1.6.2. Dynamic high-pressure fluidization. Dynamic high pressure (DHP) is a technology with pressures up to 250–300 MPa based on forcing a fluid through a narrow orifice. Due to the high velocity and the sudden pressure reduction, intense shear, turbulence and cavitation will occur. DHP fluidization, which is also known as high pressure homogenization (HPH), is widely used for the formation of colloidal dispersions such as nanoemulsions (Sedaghat Doost, Dewettinck, Devlieghere, & Van der Meeren, 2018). It was also used for the non-thermal inactivation of microbial cells as an alternative for conventional thermal treatments in different food products such as milk, beverages and juices. Moreover, this technique can be used for the modification of biopolymers including proteins and carbohydrates (Porto, Tribst, & Cristianini, 2018; Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019). The application of DHP as an individual technology as well as

pretreatment for improving versatility and functionality of plant-based proteins has been studied by literature. Due to its mechanical forces which results in fragmentation of macromolecules, DHP, can reduce the size of the plant-derived protein particles and hence, more particle-solvent interactions rather than particle-particle interactions can occur. For instance, Primozic, Duchek, Nickerson, and Ghosh (2018) reported the decreased size of lentil protein isolate nanoparticles after DHP which made them sufficiently small for stabilizing oil-in-water nanoemulsions. Saricaoglu (2020) also used HPH for the modification of lentil protein isolate, decreasing the nanoparticle size and increasing solubility, emulsifying and foaming properties up to 100 MPa. However, at pressures higher than 100 MPa there was a significant decrease in these functional properties. In fact, their results revealed that HPH only had significant decreasing effect on the particle size up to 100 MPa and then there was only a slight decrease up to 150 MPa. In their previous study, the same authors observed similar results for the modification of hazelnut protein by HPH, illustrating a significant effect of HPH on decreasing the size only up to 50 MPa; however beyond this pressure and up to 150 MPa, there were not significant changes in the nanoparticle size (Saricaoglu, Gul, Besir, & Atalar, 2018). Depending on the type of the protein and the solvent used, there would be a maximum pressure of DHP at which the smallest nanoparticles can be obtained, beyond which there may be an increase in particle size due to the protein denaturation. This denaturation is the result of mechanical forces in the pressure drop and the elevated temperatures reached in the homogenizer valve (Porto et al., 2018). The structural changes induced by DHP can be more pronounced at higher pressures (>150 MPa) or when heat treatment is also applied. DHP treatment can also improve the digestibility of plant-based proteins as Primozic et al. (2018) showed in emulsions which were stabilized by HPH-treated lentil protein isolate. This method has been used also to reduce the allergenicity of different animal proteins (Porto et al., 2018). It can also be used to alter the immunoreactivity of plant-based proteins or as a pretreatment for other modification methods. For example, it was successfully used at 120 MPa as a pretreatment for soy protein isolate before enzymatic hydrolysis, which improved the pancreatin efficiency due to the increase in the accessibility of some soy protein subunits and improved the emulsifying ability of the ensued hydrolysates (L. Chen, Chen, Yu, & Wu, 2016).

3.1.7. Sonication

Sonication is a green, novel, innovative and sustainable technique based on high sound waves of frequencies (>16 kHz) which cannot be detected by human ear. This process has several advantages in comparison to conventional thermal processes including higher efficiency, higher rate, easier and cheaper application and operation, lower equipment contamination, and higher quality and functionality of the processed foods (Gharibzahedi & Smith, 2020; Sedaghat Doost, Nikbakht; Nasrabadi, Wu, et al., 2019; Wen et al., 2019). The cavitation phenomenon created by mechanical waves generate regions with high temperatures and pressures with very fast cooling in liquid, which can induce or accelerate chemical reactions. Cavitation can also increase the rate of mass and heat transfer (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019; F. Yang, Liu, Ren, et al., 2018). During the sonication process the produced radicals and superoxide may result in the creation of cross-linking in protein molecules (Wen et al., 2019). Sonication has the ability to induce structural changes due to the disruption of non-covalent bonds (but it is inert towards the covalent bonds). Therefore, it can destruct the secondary structure and can partially denature the tertiary and quaternary structure of proteins without any significant change on their primary structure (Gharibzahedi et al., 2020). Zhao et al. (2018) showed that sonication with power levels of 200, 400 or 600 W for 15 or 30 min, changed the secondary and tertiary structure of walnut protein isolate without any effect on its primary structure since the process could not break the covalent bonds. Mir, Riar, and Singh (2019b) also found that the sonication-induced structural changes in quinoa seed protein isolate provided favorable water binding capacity, oil binding capacity, emulsifying and gelling properties. This process can also affect the functionality of proteins by changing their free sulfhydryl groups content. Most of the studies showed the increased number of free sulfhydryl groups content after sonication process of plant-based proteins due to their partial unfolding and the surface exposure of their buried –SH groups. Moreover, more –SH groups can be formed due to the reduction of disulfide (S–S) bonds because of cavitation (F. Yang, Liu, Ren, et al., 2018; J.-j. Yu, Ji, et al., 2020). The enhanced exposed hydrophobicity reported for pea protein isolate after high intensity ultrasound (20 kHz, at varying amplitude 30%, 60%, 90% for 30 min) resulted in its improved foaming properties due to reduced surface tension at the interface of air and water (Xiong et al., 2018). Zhao et al. (2018) also found an increased amount of –SH groups after sonication of walnut protein with improved solubility and emulsifying activity. Similarly, F. Yang, Liu, Zeng, and Chen (2018) also found an increased amount of sulfhydryl group contents and surface hydrophobicity of soy protein isolate after sonication. In contrast, some researchers reported a reducing effect of sonication on –SH groups content, especially at higher sonication powers and times. F. Yang, Liu, Zeng, and Chen (2018) also showed that 5–10 min sonication of soy protein isolate decreased the amount of –SH groups due to their conversion into S–S bonds through oxidation of the hydrogen peroxide (H_2O_2) formed in gas bubbles.

Sonication process can be used to reduce the size of plant-derived protein aggregates, due to the disruption of non-covalent interactions, which results in increase solubility. F. Yang, Liu, Zeng, and Chen (2018) demonstrated the ability of sonication in reducing the size of soy protein, improving its solubility and emulsifying properties. However, there are some studies showing that too intense sonication conditions, especially longer ultrasonication times, can result in the formation of large aggregates (Mir et al., 2019b). Sonication was reported in the literature as an approach for decreasing the amount of anti-nutrients and improving the digestibility of plant-derived proteins. H. Lee et al. (2016) displayed that high-intensity ultrasound decreased the allergenicity of soy protein by 18.9%. Vanga et al. (2020) showed that ultrasonic treatment (25 kHz, 400 W, 1–16 min) decreased soymilk protein trypsin inhibitor activity by 52% and improved its digestibility. J. Jin et al. (2021) showed the improved digestibility of buckwheat protein isolates after sonication following the conditions: 20 kHz, on-time pulsed 10 s, off-time 5 s, amplitude of 60% and duration of 10 min.

This technology can also be used for the modulation of plant-based proteins functionality combined with other modification techniques since it can be effective on protein chemical structure. de Oliveira et al. (2020) used the complex action of pH adjustment and ultrasonication for the modification of pea protein without adverse effects on its digestibility with the best observed emulsifying ability at 562.5 W/426.66 s at pH 6.8. Huang et al. (2019) combined the effect of acid and ultrasound treatment for the modulation of soy protein which had a synergistic effect on the structure and emulsifying ability of this protein. Dabbour, He, Mintah, Xiang, and Ma (2019) evaluated the effect of enzymolysis and ultrasonication action on the structure and functional properties of sunflower protein. According to their results ultrasonication as a pretreatment had a considerable impact on the protein structure and improved its solubility, foaming capacity, emulsification properties and antioxidant ability while decreased the foaming stability. L. Chen, Ettelaie, and Akhtar (2019) also used thermo-sonication as a pretreatment for enzymatic hydrolysis of peanut protein isolate in order to increase its enzymatic accessibility with the highest degree of hydrolysis under 475.0 W at 72 °C. The ultrasonic modification approach of plant-based proteins is one of the most popular methods available. There are several studies on the application of ultrasonication in the field of plant proteins such as extraction assistance and modification. Although the sonication process has been widely evaluated for the purpose of plant-based protein modification by itself or in combination with other techniques in different researches, it is not yet commercialized. Whereas large scale sonifiers are currently used in food industry mainly for the extraction of high-value bioactive compounds, their

application for the modification of plant-derived proteins is still at the laboratory scale. Using the ultrasonic modification approach in large scales is limited due to some challenges which should be addressed. For instance, extreme conditions of sonication such as elevated temperatures can have adverse effects on the structure and quality of proteins. Moreover, extreme conditions can create an uncontrollable cell cavitation which results in the generation of free radicals with high reactivity formed via the decomposition of water molecules ($H2O \rightarrow H + \bullet OH$). Furthermore, destructive oxidation reactions can be increased in these circumstances (Gharibzahedi et al., 2020; F.; Yang, Liu, Ren, et al., 2018). Therefore, finding the optimum process conditions based on time, temperature, intensity or applied power, frequency, and amplitude is really sough after for the case of food proteins modification, especially for plant-based proteins. The results and yield of the batch and lab scale production do not usually match the large-scale studies. Moreover, more emphasis should be placed on the economic analysis of the large-scale application of ultrasonication in food industry.

3.1.8. Extrusion

Extrusion is the combination of mechanical shear, heat and pressure whereby the mixed ingredients are exposed to a continuous mixing and high mechanical stresses arising by a large rotating screw under high pressures (1.5–30.0 MPa) and high temperatures (90–200 °C). Since this process can be considered as a high temperature short time (HTST) cooking process, it has been used for different purposes including inactivation of microorganisms, enzymes, and naturally occurring toxic substances as well as gelatinization of starch or shaping food materials (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019). Extrusion process can also induce unfolding, denaturation and realignment of vegetable protein molecules, and thus it not only improves their techno-functionality but can also generate a texture mimicking that of meat. Thus, these texturized vegetable proteins can be used as meat analogues in food products (Zahari et al., 2020; J.; Zhang, Bolisetty, et al., 2019). According to the literature, extrusion can change the interactions responsible for the initial conformation of the protein while the peptide bonds as the major chemical bonds remain unchanged (Osen, Toelstede, Eisner, & Schweiggert-Weisz, 2015; J.; Zhang, Bolisetty, et al., 2019). High temperatures during extrusion can cause the unfolding of proteins due to the breakage of hydrogen bonds. By further enhancement of the temperature, the breakdown of the intramolecular disulfide bonds and the creation of new intermolecular ones, the formation of protein aggregates with subsequent increase in their collective molecular weight can be achieved (Beck, Knoerzer, & Arcot, 2017; Wenjun Ma, et al., 2018). Beck et al. (2017) observed the changed molecular weight of pea protein after low-moisture extrusion and they confirmed the changes in the secondary structure by the identification of formed α-helices, β-sheet, non-covalently bonded β-turn or anti-parallel β-sheet structures, as revealed by FTIR and SDS-PAGE.

High temperatures and high pressures applied during extrusion have the capability to destroy anti-nutrients and improve the digestibility of plant-based proteins by increasing the availability of their amino acids. This process can also be used as a pretreatment for other protein modification methods such as enzymatic hydrolysis (L. Chen et al., 2018; Wenjun Ma, et al., 2018; Zhou et al., 2017) and glycation (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019) by making available the amino acids folded in the inner part of the proteins.

3.1.9. Ball mill treatment

Ball mill treatment is another physical process which is usually used for size reduction in food processing. It has been used for decreasing the particle size of plant-originated proteins leading to an ensured higher solubility. This process is the combination of collision, friction, shear, and also the heat generated during the treatment which can be mechanically and chemically effective on the protein secondary and tertiary structure and results in the availability of more hydrophobic patches (Ramadhan & Foster, 2018; Vogel, Scherf, & Koehler, 2018). These changes can influence the functional properties of plant-derived proteins especially their emulsifying, foaming and gelling ability. It has been also revealed that the ball mill treatment of soy protein isolate altered the secondary and tertiary structure of the protein without any change in its primary structure (Ramadhan et al., 2018). The combined effect of this treatment on the structure of soy protein isolate and the decrease in its particle size, resulted in its improved gelling properties (B. Liu, Zhu, Guo, Peng, & Zhou, 2017).

3.1.10. Cold atmospheric plasma processing

Cold atmospheric plasma processing (CAPP) is based on the application of cold plasma which is the fourth state of matter and can be achieved under a wide range of temperatures and pressures through the combination of thermal, mechanical, nuclear and electrical energy sources. The advantages of this process is to provide uniform treatment without any thermal damage and lack of requirement for hazardous solvents. It has been used for the inactivation of microorganism, spores and viruses present on the surfaces of food since plasma cannot penetrate inside the products. CAPP can induce the cleavage of covalent bonds or initiation of chemical reactions, due to its high energy. It has been used for the modification of surfaces and biopolymers (Sarangapani, Patange, Bourke, Keener, & Cullen, 2018; Tolouie, Mohammadifar, Ghomi, & Hashemi, 2018). It is reported that CAPP can be used for the improvement of the techno-functional properties of plant-based proteins. It has been shown in literature that CAPP has the ability to decrease the size of the plant-based protein particles and aggregates. Mahdavian Mehr and Koocheki (2021) showed that applying higher voltage and longer CAPP processing time resulted in the fabrication of smaller grass pea (*Lathyrus sativus* L.) protein nanoparticles with the smallest size under 18.6 kVpp 600s condition. Plasma treatment was also reported to be able to increase the content of disulfide bonds in plant-based proteins resulting in changes in their secondary structure and rheological properties. revealed the decreased amount of free SH groups and increased surface hydrophobicity of grass pea protein after CAPP. Ji et al. (2018) showed that unfolding and the alteration of secondary structure of pea nut protein induced by cold plasma resulted in improved solubility, emulsifying ability and water holding capacity. In a recent study conducted by Xu et al. (2020), the emulsifying, foaming and antioxidant properties of flaxseed protein were improved after CAPP by jet devices. Mahdavian Mehr et al. (2021) observed the improved surface activity of the fabricated grass pea protein using CAPP. CAPP improved the gelling properties of pea protein by providing the ability to form gels when heated below 90 °C (S. Zhang, Huang, Feizollahi, Roopesh, & Chen, 2021). Although CAPP can be considered as a non-thermal, cheap and energy-efficient process, there are some limitations associated with its food industrialization including its complexity of action, a measurable dose for food products, scaling up and its safety (Sarangapani et al., 2018).

3.1.11. Ultrafiltration

Ultrafiltration (UF) is a non-thermal technology which is based on forcing a fluid through the pores of a membrane by applying pressure or electric field. UF has been used for the separation and isolation of proteins. This process is not only capable of increasing the protein content, but also can be effective on the structure and functionality of proteins depending on the membrane molecular size cut-off, pH, and the applied pressure or electric field (Aryee et al., 2018, pp. 27–45). Eckert et al. (2019) observed further improvement of emulsifying, foaming and oil holding capacity of faba bean protein after UF treatment of the hydrolysates obtained from enzymatic hydrolysis. However, there are some reports in contradiction to these results indicating better functional properties of isolates extracted by conventional methods. For instance, Arogundade, Mu, and Akinhanmi (2016) showed higher surface hydrophobicity, emulsifying and foaming ability of African yam bean (*Sphenostylis stenocarpa*) protein isolate extracted by isoelectric precipitation in comparison to UF.

As discussed before, one of the main limitations of some plant-based proteins for the human consumption is their bitter taste and the existence of anti-nutritional agents. Ultrafiltration can be used as an efficient process for decreasing or totally eliminate these compounds from proteins due to their lower molecular weight. Hadidi, Khaksar, Pagan, and Ibarz (2020) displayed that the simultaneous application of ultrafiltration and ultra-sonication was efficient in removing the anti-nutritional compounds (saponins and phenolic compounds) of alfalfa protein isolate.

3.2. Chemical modification

Chemical modification of food proteins has been widely used due to its efficiency, low cost and ease of operation. The chemical modification of proteins can be achieved by the addition of new functional moieties or elimination of components from the protein structure. Most of the chemical modification methods have got regulatory and clean label concerns since they use chemicals and produce chemical by-products in most cases. Therefore, some of these methods are not favorable for food applications (Zhang et al., 2019). Different chemical modification approaches will be discussed in this review with examples provided specifically in the context of plant-based proteins. Moreover, a part of the discussion will be devoted to the toxicity of chemically modified food proteins, since most of them may contain other groups either as an integral part of their structure or as impurities.

3.2.1. Glycation

Glycation, also referred to glycosylation, is a common food-grade reaction which is widely used to improve protein functionalities since it is safe and it usually does not require exogenous chemicals (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019). Glycation is inspired by nature as there are some naturally occurring covalent bonds between proteins and polysaccharides in some biopolymers, such as Arabic gum, which provide desirable functional properties for this gum (Maryam Nikbakht Nasrabadi, Goli, & Nasirpour, 2015; Maryam Nikbakht Nasrabadi, Goli, & nasirpour, 2016). Glycation can be achieved chemically through Maillard reactions or it can be obtained by cross-linking enzymes such as transglutaminase or laccase. The latter is discussed in the enzymatic modification section (3.3.1) in details. The chemical glycation occurs by covalent conjugation between a free amine group of a protein, peptide or amino acid and the carbonyl group of a reducing sugar through controlled heating in the presence of water in a chemical-reagent-free, safe and mild process which is achieved by different methods, including wet-heating, dry-heating and molecular crowding. Among these conventional methods, the dry-heating method is the most common approach (Sedaghat Doost et al., 2020; Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al. (2019); Quing Zhang et al., 2019). Novel approaches of preparing protein-carbohydrate conjugates have recently attracted attention as reviewed by Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al. (2019) for the enhancement of whey proteins functionality and will be discussed here for plant-based proteins along with conventional methods. Novel approaches which have been used for the glycation of plant-based proteins with saccharides are ultrasonication (L. Chen, Chen, Wu, & Yu, 2016; Qu, Zhang, Chen, et al., 2018; Wen et al., 2020; Xue, Wu, Tong, Zheng, & Li, 2017), electrospinning (Kutzli, Griener, Gibis, Grossmann, et al., 2020; Kutzli, Griener, Gibis, Schmid, et al., 2020), microwave (Meng et al., 2019; Xiang et al., 2020), spray drying (Mao et al., 2018), HHP (D. Liu, Li, et al., 2020), irradiation (W. Li, Dai, et al., 2020; Yuying Wang et al., 2020b,c), and cold plasma (J.-j. Yu, Ji, et al., 2020).

The nature and reactivity of the saccharides are important factors for improving the techno-functionality of plant-based proteins through Maillard reaction. (Li et al., 2016), L.-H. Wang, Sun, Huang, and Xiao (2018) found xylose more reactive compared to fructose in conjugation with soy protein isolate under controlled wet-heating conditions leading to the formation of more complex and larger conjugates with more relaxed structure. Liu, Dai, et al. (2020) compared the conjugation of black rice glutelin with different carbohydrates including arabinose, sodium alginate, maltodextrin, and lactose and their results revealed higher glycation degree for arabinose and maltodextrin. The chain length of the carbohydrate was also reported to be dependent on the structure and functionality of the conjugated protein (Liu et al., 2020a).

Glycation-induced structural changes in different plant-based proteins have been reported in the literature which resulted in improved functionality (Table 2). For instance, Saatchi, Kiani, and Labbafi (2019) confirmed secondary structural changes in sesame protein after dry-heating conjugation with maltodextrin through CD spectroscopy. This treatment provided higher solubility of the protein with subsequent improved emulsifying ability at a wide range of studied pH, especially at the Ip. Wet heating glycosylation of black soybean protein isolate with chitosan improved its emulsifying ability due to the induced secondary structural changes. Although most of the studies showed the improved emulsifying performance of plant-based proteins after conjugation with different sugars due to the formation of thicker adsorbed interfacial layer providing steric and/or electrostatic hindrance (Hiller & Lorenzen, 2010; Kutzli, Griener, Gibis, Grossmann, et al., 2020; W.; Li, Zhu, Zhou, Peng, & Guo, 2016), some researchers reported the negative effect of this treatment on this functionality. For example, L.-H. Wang, Zhang, Zhang, Ju, and He (2018) showed the deceasing effect of the wet-heating conjugation of soy protein isolate with xylose or fructose on its emulsifying activity despite its positive effect on solubility. Other functional properties of plant-based proteins were also reported to be improved after glycation with different saccharides. The foaming properties of soy protein isolate as well as its emulsifying properties improved after wet-heating glycation with glucose (R. Li, Cui, et al., 2019). The improved foaming ability was also reported for soy protein isolate after conjugation with lentinan via ultrasonic-assisted Maillard reaction, together with solubility, emulsifying ability and thermal stability (Wen et al., 2020).

Glycation was used as an approach for the reduction of beany flavor in some plant-based proteins such as pea protein conjugated by Arabic gum (J. Zhang, Bolisetty, et al., 2019). In another study, Q. Sun et al. (2019) revealed the improved flavor profile of pea Protein Hydrolysate after glycation with Arabic gum. This can promote the application of plant-based proteins as ingredients in food formulations without any unfavorable effect on their organoleptic properties.

Glycation can also be used for the immunomodulation of plant-based proteins due to the molecular structural changes, according to Meng et al. (2019), who showed the strong immunomodulatory properties of rice dreg protein-sodium alginate conjugates. Despite the efficiency and safe conditions of glycation method of protein modification, some

Table 2
A summary of chemical modification approaches of plant-based proteins.

Chemical modification approaches	Protein type	Modified characteristics	Reference
Glycation	Oat protein isolate	Increased in bioactivity	Zhong et al. (2019)
	Pea protein isolate		Q. Sun, Ma, Zhang, Ma, and Kong (2019)
	Black bean		H. Jin et al. (2019)
	Pea	Solubility and emulsification properties	Q. Sun et al. (2019), Zha et al. (2019), and Qing Zhang et al. (2019)
	Oat		Zhong et al. (2019)
	Rapeseed protein isolate	Solubility, emulsifying ability and thermal stability	Qu, Zhang, Han, et al. (2018)
	Black rice glutelin		Liu et al. (2020)
	Soy protein isolate		A. Kumar et al. (2020)

adverse effects on the nutritional value of proteins due to the loss of bioavailability of lysine, have been reported (Oliver, Melton, & Stanley, 2006).

Among the chemical modifications of plant-based proteins, glycation is the most desirable for food applications since there is no need for applying chemicals and there is no production of side chemicals. Therefore, in terms of consumer preferences, clean label and commercialization, this method can be a desirable chemical modification approach for plant-based proteins. However, the commercialization of glycated plant-based proteins is not feasible yet since most of the preparation approaches remains at lab-scale or require expensive devices or technologies such as freeze-drying, ultrasonic, high pressure techniques and alike (Sedaghat Doost, Nikbakht Nasrabadi, Wu, et al., 2019).

3.2.2. Phosphorylation

Phosphorylation can be considered as a chemical modification approach for plant-based proteins that allows keeping their nutritive bioavailability. Phosphorylation is the introduction of phosphate groups into protein primary sequence, which can regulate their functionality to a significant extent. An enzyme, called protein kinase, catalyzes the covalent bonding of phosphate moiety to specific amino acid residue of proteins. As a result, the shape of the protein is changed, modifying its activity and stability. Moreover, the phosphorylation can affect the interactions with other proteins by allowing other proteins to bind via the phosphorylated regions (Liu et al., 2020b). Dephosphorylation can also be induced by using protein phosphatases. The phosphorylation as a chemical method is inspired by nature for the modification of proteins since this method is used by the cells in order to activate proteins. Moreover, there are several phosphoproteins in nature which are used as food functional ingredients including casein in milk and ovalbumin in egg white (Aryee et al., 2018, pp. 27–45; Miedzianka & Pęksa, 2013). By the addition of phosphate groups, the negative charge and hydration of protein increase, leading to the improvement of its functional properties. Phosphorus oxychloride ($POCl_3$), sodium trimetaphosphate (STMP), and phosphoric acid have been used for the phosphorylation of food proteins (Aryee et al., 2018, pp. 27–45). STMP is an inorganic salt which is accepted as a food additive with FDA approval and is generally regarded as safe (GRAS) (Yu et al., 2015). STMP has not only less toxicity and physiological impacts, but is also more efficient compared to other straight-chain polyphosphates and can be used to solve many problems related to the use of $POCL_3$ (Miedzianka et al., 2013; Sánchez-Reséndiz et al., 2018). Due to these advantages, STMP is the most common phosphorylation reagent that has been used for the phosphate modification of plant-based proteins. Miedzianka et al. (2013) found that the modification of potato protein isolate through STMP phosphorylation is pH dependent. It was also reported previously that STMP phosphorylation of soy protein isolate was influenced by pH, temperature and concentration (Sung, Chen, Liu, & Su, 1983). Sánchez-Reséndiz et al. (2018) evaluated the effect of these variables on the STMP phosphorylation of peanut and soy protein isolate and their results revealed the higher degree of phosphorylation that can be achieved at higher pH, temperature and STMP concentrations. J. Zhang, Bolisetty, et al. (2019) also evaluated the effect of STMP concentration on the modification of rice glutelin showing the linear increasing trend of phosphorylation reaction with increasing salt concentration. Y. Li, Dai, et al. (2020) also optimized the STMP phosphorylation conditions of pea protein and the best condition set was reported to be at pH 12, 70 °C and 7.0% (w/v) concentration of STMP.

Functional properties of plant-based proteins were reported to be improved after their phosphorylation due the considerable increase in their electronegativity and electrostatic repulsion between protein molecules. Sánchez-Reséndiz et al. (2018) showed the improvement of emulsifying activity of peanut and soy protein isolate after phosphorylation using STMP. The phosphorylated peanut and soybean protein isolate showed increased *In-vitro* digestibility as well. J. Zhang et al. (2019) observed the improved solubility, emulsifying and foaming properties, thermal stability and higher apparent viscosity of rice glutelin due to the increased negative surface charge and decreased particle size after STMP phosphorylation. The viscoelasticity and thermal aggregation of rice glutelin were also improved after STMP phosphate modification according to another study conducted by Y.-R. Wang, Yang, et al. (2019).

3.2.3. Acylation

Acylation is the introduction of an acyl group to the protein using acyl anhydrides and halides. The process can be classified into acetylation and succinylation based on the used acylating agent which are acetic or succinic anhydride, respectively. All the nucleophilic groups within the protein structure will react with the reagents but the ε-amino group of lysine has the highest affinity. During succinylation, net positive charges are replaced with a net negative charge at hydroxyl and lysine amino group of proteins, whereas in acetylation basic groups are converted into neutral ones (Aryee et al., 2018, pp. 27–45). The surface charge of plant-based proteins changes after acylation, and this can be effective on the solubility and functional properties. For example, C.-B. Zhao, Sun, et al. (2017) observed the decreased zeta potential of oat protein at neutral pH after acylation, which was more pronounced for succinylation. The decreased zeta potential of mung protein isolate at neutral pH was also reported after succinylation, improving its emulsifying properties (Charoensuk, Brannan, Chanasattru, & Chaiyasit, 2018). Acylation can change the secondary structure and tertiary conformation of plant-based proteins turning them into more hydrophobic, with possible improvement of their functional properties without any adverse effect on their nutritional value (Goulet, Ponnampalam, Amiot, Roy, & Brisson, 1987; C.-B.; Zhao, Zhang, et al., 2017). According to C.-B. Zhao, Sun, et al. (2017), the results of FTIR and intrinsic fluorescence spectroscopy revealed the induced changes in the secondary structure and tertiary conformation of oat proteins after both acetylation and succinylation whereby the latter was more effective. Moreover, succinylation also increased the molecular weight of the oat protein. The increased hydrophobicity and molecular weight of rapeseed protein isolate were also reported, led to an improvement of its thermal stability and gelling properties (Z. Wang, Zhang, et al., 2018). The gelling property of rapeseed protein was another functionality which was evaluated by C. Chen et al. (2020) when acylation was applied. Their results showed the structural changes in the protein structure after the treatment, with improved gelling properties. The enhanced thermal stability, solubility and emulsifying properties were also reported due to the succinylation-induced changes in the secondary structure of male date palm pollen protein concentrate (Sebii et al., 2020). Likewise, in a study conducted by Shah, Umesh, and Singhal (2019), the improved solubility, foaming properties, emulsion stability and water holding capacity of pea protein were understood to be a result of the effect of succinylation on its secondary structure.

3.2.4. Deamidation

Deamidation is the transformation of amide groups of glutamine and asparagine residues into carboxyl groups within a protein, enhancing the negative charge of the protein. Unlike acylation and alkylation that require chemicals, deamidation can be achieved under mild conditions and without additional molecules. Therefore, it is considered as a safe modification method of proteins in food systems. Since cereal and legumes have very high proportions of glutamine and asparagine, deamidation can be a proper modification method especially for their application in food products. Deamidation can be achieved by different methods such as acid, alkali, enzyme, and cation-exchange-resin treatment among which the acid and alkali treatment are the most and least common ones, respectively (Kumagai & Urade, 2019, pp. 3–11). He, Yang, and Zhao (2019) used acid deamidation using acetic acid, tartaric acid, and citric acid for masking the bitterness of wheat gluten hydrolysates. In another study conducted by B. Y. Liu, Zhu, et al. (2017) acid

deamidation was also used for depressing the bitterness of wheat gluten hydrolysates. Alkaline deamidation was applied by Guan et al. (2017) for improving the solubility of rice bran protein. Although the acidic deamidation is the most common method, the enzymatic deamidation can be considered as the most appropriate one for the modification of plant-derived proteins due to its specificity of the substrates, moderate reaction condition, and minor side-chain reaction (Hadidi, Ibarz, & Pouramin, 2021). In the case of enzymatic deamidation, the only deamidase that is industrially produced for food purposes is extracted from *Chryseobacterium proteolyticum*. Transglutaminase and some proteases such as papain, chymotrypsin and pronase can also partially deamidate proteins under certain conditions (Z.-q. Jiang et al., 2015). The most studied enzyme in the literature for the deamidation of plant-based proteins is glutaminase. Glutaminase-deamidated oat protein showed improved solubility and emulsifying properties (Z.-q. Jiang et al., 2015). Kunarayakul, Thaiphanit, Anprung, and Suppavorasatit (2018) also observed improved solubility, emulsifying and foaming properties of coconut protein after glutaminase deamidation. Hadidi et al. (2021) improved the solubility, water holding capacity, emulsifying and foaming properties of evening primrose (*Oenothera biennis* L.) protein obtained from the oil processing by-product through glutaminase deamidation. Glutaminase deamidation was also used for solubility and techno-functionality improvement of pea protein isolate as well as its flavor with reducing its beany flavour, grittiness and lumpiness (Fang, Xiang, Sun-Waterhouse, Cui, & Lin, 2020). The bitter taste of wheat gluten hydrolysates was also masked by the application of glutaminase deamidation (B. Y. Liu, Zhu, et al., 2017). For some plant-based protein fractions which are soluble in aqueous alcohol such as gliadin and glutenin, the enzymatic deamidation cannot be used due to the inactivation of enzymes in an alcohol solution. In these cases, cation-exchange resins can be applied. The anionic groups of cation-exchange resins, sulfonate or carboxyl groups, have the ability to induce deamidation of proteins which has been used for wheat gliadin (Abe et al., 2018; Kumagai et al., 2007), and soy protein (Kumagai, Ishida, Koizumi, Sakurai, & Kumagai, 2002; Kumagai et al., 2004).

There are also some reports on the ability of deamidation in decreasing the allergenicity of plant-based proteins. Gliadin, which is considered as the major allergen of the wheat, showed lower oral allergenicity in both *in vitro* and *in vivo* systems after deamidation (Abe et al., 2018; Abe et al., 2014). The transdermal allergenicity of wheat gliadin was also reported to decrease after deamidation (Abe et al., 2020).

3.2.5. Cationization

Cationization is another chemical protein modification method which is achieved by the addition of quaternary ammonium groups. The addition of positively-charged groups on the surface of the protein can modify its techno-functionality. This method has been rarely considered for the modification of vegetable proteins; it was applied in soy protein and sunflower modification in order to improve their encapsulating properties (Nesterenko, Alric, Silvestre, & Durrieu, 2014; Nesterenko, Alric, Violleau, Silvestre, & Durrieu, 2014). Moreover, Nesterenko, Alric, Silvestre, and Durrieu (2014) reported improved solubility and emulsifying properties for soy protein after cationization. Based on the results of Nesterenko, Alric, Silvestre, and Durrieu (2014), the degree of cationization is understood to be positively affected by the amount of the glycidyl trimethylammonium chloride reagent and the temperature of the reaction. In another study, the same authors also showed that the degree of cationization can also depend on the protein type as soy protein and sunflower protein showed different degree of cationization due to the different protein conformation and NH_2 group accessibility. Besides these works, there are not many reports carried out on plant-based proteins cationization in the literature.

3.2.6. pH shifting treatment

Since the pH of the liquid matrix in which proteins are dissolved is one of the key effective factors on its structure, acidic or alkaline treatments can trigger changes in structural and functional properties of proteins. At extreme basic pH values, proteins are denatured and unfolded, exposing sulfhydryl and hydrophobic patches in their structure, which open-up for new protein interactions. Alkaline conditions can be achieved easily by the addition of chemical additives, including NaOH, NH_4OH or urea (Yildiz, Andrade, Engeseth, & Feng, 2017; Yildiz, Ding, Andrade, Engeseth, & Feng, 2018). Wheat gluten and potato protein were modified by adjusting the pH to basic values using NaOH, which resulted in changes in their molecular and secondary structure, and in improved extensibility and tensile properties of their formed films (Muneer, Johansson, Hedenqvist, Plivelic, & Kuktaite, 2019). Acidic treatment is also a chemical treatment in which protein is exposed to low pH values which may promote its unfolding; this can be followed by reversible refolding by pH readjustment. Hydrochloric acid (HCl) is commonly used for providing acidic conditions. pH shifting treatments can also be used as a pretreatment for other modification methods since they induce protein reactivity by promoting its unfolding (Lee et al., 2016). Most of the researchers have used pH shifting prior or in combination with ultrasound treatment for plant-based proteins. Lee et al. (2016) applied pH shifting at 12 followed by ultrasonication for the preparation of nano-sized soy protein aggregates (average size of 22 nm) with improved functionality, such as enhanced solubility, surface hydrophobicity and emulsifying ability. In another study conducted by Yildiz et al. (2017), the combination of pH shifting at 12 and mano-thermo-sonication was also used for the formation of soy protein isolate nano-aggregates with average size of 27 nm with improved solubility, surface hydrophobicity, antioxidant activity, rheological and emulsifying properties. Rice protein solubility, emulsifying and foaming properties were also improved by the combination of alkali and ultrasound treatment (L. Zhang, Yin, et al., 2018). S. Jiang et al. (2017) also displayed that the solubility, surface hydrophobicity and the emulsifying properties of pea protein improved after pH-shifting at pH 12 and ultrasound combined treatment. The combined treatment of pH-shifting and sonication was also used for extending the application of pea protein (S. Jiang et al., 2019) and peanut protein (J.-j. Yu, Ji, et al., 2020) for the encapsulation of bioactive compounds by addressing their poor solubility problem and improving their techno-functionality. Since proteins have the highest solubility at higher pH values, most of the studies in literature were done by shifting pH to basic values. S. Jiang et al. (2017) evaluated both acidic and alkali treatments in combination with ultrasonication on pea protein and according to their results the improvement of protein solubility and functional properties were more pronounced under alkali conditions.

3.3. Biological modification

Biological modification, relying on enzymatic and fermentation processes, is another popular modification technique since it is environmentally friendly and less energy-consuming without production of toxic by-products (Table 3). Yet, cost of the enzymes and cultures should be taken into consideration for large scale applications. In addition to the modulation of protein functionality, these approaches have the ability to improve their nutritional quality including digestibility, bioavailability, as well as antioxidant and antimicrobial properties.

3.3.1. Enzymatic modification

Enzymatic modification is one of the most popular methods of protein modification, particularly for their incorporation into food systems, since the reactants and by-products are non-toxic. Moreover, this kind of modification can be achieved under mild conditions with few by-products. In comparison to chemical modification, the chemical composition of the initial protein will be preserved after the enzymatic modification. Another advantage of enzymatic modification over chemical approaches is the fast reaction time and specificity of the enzymes. This method of protein modification can be classified into

Table 3
A summary of the biological and other modification methods of plant-based proteins.

Biological and other modification approaches	Protein type	Modified characteristics	Reference
Enzymatic	Peanut	Gelling properties and oil binding ability	S. B. Zhang, Wang, Li, and Yan (2020)
	Coconut protein	Mechanical strength, thermal and the barrier properties	Sorde and Ananthanarayan (2019)
	Black soybean protein isolate	Solubility, rheological and emulsifying properties	Y. Zhang et al. (2018)
	Walnut glutelin	Solubility and emulsifying properties	Q. Sun et al. (2019)
	Pea protein isolate	Foaming and emulsifying abilities	García Arteaga, Apéstegui Guardia, Muranyi, Eisner, and Schweiggert-Weisz (2020)
	Quinoa and amaranth	Antioxidative and antihemolytic activities	Mudgil, Omar, Kamal, Kilari, and Maqsood (2019)
	Pea	Oil and water holding capacity	Konieczny, Stone, Korber, Nickerson, and Tanaka (2020)
	Lupin protein isolate	Foaming properties	Schlegel et al. (2019)
Protein-polysaccharide complexation	Gliadin	Emulsifying properties	Y. Jiang, Zhu, Li, Li, and Huang (2020)
	Pea	Solubility	Pillai, Morales-Contreras, Wicker, and Nickerson (2020)
	Pea protein isolate	Solubility and thermal stability	Lan, Chen, and Rao (2018)
	Soy protein isolate	Water holding capacity and gelling properties	W. Wang et al. (2020a)
Amyloid fibrilization	Soy	Formation and size	Yajuan Wang et al. (2020b)
	Soy	Gelling properties	Yajuan Wang et al. (2020c)
	Rice bran	Emulsifying properties	Pang, Shao, Sun, Pu, and Tang (2020)
	Rice glutelin	Stability against in-vitro pepsin and pancreatin hydrolysis	S. Li, Zheng, Ge, Zhao, and Sun (2020)

enzymatic hydrolysis and enzymatic cross-linking methods. The latter is achieved by the enzymatic formation of covalent bonds using transglutaminase and laccase by catalyzing acyl transfer reaction between γ-carboxamide group of protein-bound glutamine and lysine, while enzymatic hydrolysis is obtained by breaking peptide bonds enzymatically. Cross-linking can be performed using different enzymes for the modification of plant-derived proteins. Different enzymes due to the different mechanism of action might have different end product properties and functionality. Nivala, Mäkinen, Kruus, Nordlund, and Ercili-Cura (2017) evaluated the action of two different crosslinking enzymes on faba bean and oat protein and their results showed that tyrosinase had limited crosslinking ability on both proteins, while transglutaminase acted more efficiently. Therefore, transglutaminase, which is commonly isolated from specific bacteria such as *Streptoverticillium mobaraense*, has been used as the most common enzyme for the cross-linking of plant-based proteins. The microbial transglutaminase treatment was used to improve the emulsifying ability of faba bean protein isolate by Z.-Z. Xu, Huang, Xu, Liu, and Xiao (2019) and according to these results, the time is an important factor since the incubation beyond 60 min resulted in accelerated protein oxidation and decrease in its emulsifying properties. In addition to incubation time, the composition, structure and conformation of proteins can be affected by the transglutaminase treatment, which should be taken into account in the case of plant-based proteins since most of them are mixture of different fractions (Zeeb, McClements, & Weiss, 2017). For example, Djoullah, Husson, and Saurel (2018) showed that the albumin and globulin fractions of pea protein displayed different behavior during and after microbial transglutaminase treatment and albumin fraction was not a good candidate for gelation by enzymatic treatment. The changes in the conformation and structure of plant-based proteins were reported to be responsible for the observed improved techno-functionality in the literature. Protein hydrolysis results in the increase in the number of ionizable groups and the exposure of the masked hydrophobic patches. Moreover, the resultant hydrolysate has lower molecular weight and superior functional properties and bioactivity compared to the original protein (Aryee et al., 2018, pp. 27–45; Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016). The type of the utilized enzyme (e.g. alcalase, trypsin, neutrase, chymotrypsin, chymosin, pepsin, flavoenzyme, and papain) plays an important role in the final properties of the modified plant proteins, since their molecular differences translate into different specific cleavage sites (Abe et al., 2020; Wouters, Rombouts, Fierens, et al., 2016). For example, trypsin can induce splitting only near the positively-charged amino acids lysine and arginine without disrupting hydrophobic groups of amino acids, which in turn provides favorable surface-active properties. This specific cleavage pattern of trypsin led to the improvement of pea protein solubility and interfacial properties (Klost & Drusch, 2019). Tryptic hydrolysis also resulted in the formation of oat protein peptides with improved foaming ability (Brückner-Gühmann, Heiden-Hecht, Sözer, & Drusch, 2018). In contrast to trypsin, chymotrypsin tends to break next to hydrophobic amino acids such as tryptophan or phenylalanine and according to García Arteaga et al. (2020), it had the least degree of pea protein hydrolysis among evaluated enzymes. Pepsin is another enzyme with cleavage sites similar to chymotrypsin, but it can generate hydrophobic peptides even more hydrophobic than trypsin, possibly due to the solubilization of hydrophobic amino acids after the action of pepsin. This can cause the increase in the hydrophobicity and surface-active properties of the generated hydrolysates (Wouters, Rombouts, Fierens, et al., 2016; Wouters, Rombouts, Legein, et al., 2016). Eckert et al. (2019) observed the improved foaming capacity and oil holding capacity of faba bean protein after pepsin hydrolysis. Papain, a proteolytic enzyme extracted from the raw fruit of the papaya plant, was reported to generate surface-active peptides with improved solubility according to L. Chen et al. (2018) and Schlegel, Sontheimer, Eisner, & Schweiggert-Weisz, 2019 in the case of peanut protein isolate and lupin protein isolate, respectively. Papain-induced hydrolysis was found to improve the bioactivity of plant-based proteins. The papain hydrolysis of chia seed expeller produced hydrolysates with improved antioxidant ability (Cotabarren et al., 2019). According to Esfandi, Willmore, and Tsopmo (2019), Papain showed the highest ability to increase the *in vitro* antioxidant and metal chelating properties of oat bran proteins among a series of enzymes including alcalase, protamex, and flavourzyme Alcalase and flavourzyme enzymes. Alcalase, as an endoenzyme, can release the internal hydrophobic amino acids, while flavourzyme, as an exopeptidase, has the ability to expose the ones located at the protein C and N terminals (Schlegel et al., 2019). Gomes and Kurozawa (2020) evaluated the individual action of alcalase and flavourzyme hydrolysis on rice protein and investigated the effect of generated hydrolysates on the oxidation of linseed oil. Their results showed that alcalase-induced rice protein hydrolysates were more successful in depressing the linseed oil oxidation in comparison to their flavourzyme counterparts. That was due to the release of smaller peptides by the action of alcalase, resulting in the exposure of more antioxidant amino acids. Justus, Pereira, Ida, and

Kurozawa (2019) showed that the combined action of alcalase and flavourzyme produced okara protein hydrolysates with better antioxidant ability in comparison to their counterparts generated by the individual action of these enzymes.

The main limiting factor for using plant-based hydrolysates as an ingredient in the food industry is their bitter taste, caused by low-molecular-weight peptides containing high amounts of hydrophobic amino acids. Since the bitterness of hydrolysates is correlated with the DH, it can be decreased by designing the hydrolyzing conditions in order to control the DH (García Arteaga et al., 2020; Schlegel et al., 2019). Alcalase was reported to generate hydrolysates with more bitterness due to its higher DH (Schlegel et al., 2019). However, regardless of its DH, the type of the proteolytic enzyme can also be considered as an important factor in the bitterness of the hydrolysates. For example, flavourzyme generates peptides with low bitterness, despite having the ability of hydrolyzing to high extents. This can be due to the fact that the hydrophobic amino acids and peptides which are located at the N or C terminus of the protein can induce lower bitterness compared to their counterparts located in the internal parts of the protein. Therefore, flavourzyme, an exopeptidase which cleaves at the N or C terminus of the proteins and peptides, yielded hydrolysates of lower bitterness in comparison to endopeptidases such as alcalase (Schlegel et al., 2019). The bitterness of hydrolysates can also be addressed through other modification approaches such as deamidation. For instance, He et al. (2019) and B. Y. Liu, Zhu, Guo, Peng, and Zhou (2017) applied deamidation for decreasing the bitterness of wheat gluten hydrolysates.

3.3.2. Fermentation

Fermentation has been used as a traditional energy- and cost-effective biological method for plant-based proteins modification. Different starter cultures have been used for the fermentation of plant proteins such as lactic acid bacteria, yeast, mold, and Bacillus strains among which lactic acid bacteria are the most common (Schlegel et al., 2019). Fermentation was reported to improve soy protein solubility, water and oil holding capacity as well as foaming properties (Meinlschmidt, Ueberham, Lehmann, Schweiggert-Weisz, & Eisner, 2016). The increased solubility and the improvement of functional properties was also reported after the solid state fermentation of lupin protein using *Pediococcus pentosaceus* (Klupsaite et al., 2017). Fermentation has been not only used for the improvement of plant-based proteins structure-functional properties, but also to promote their nutritional properties. X. Yang et al. (2016) showed a considerable increase in the nutritional parameters and digestibility of peanut meal with balanced content of amino acids after fermentation using *Bacillus licheniformis*. The increased nutritive functionality of chickpea protein after fermentation using the dominant autochthonous lactic acid bacteria strains in chickpea flour as well as *Pediococcus pentosaceus* and *Pediococcus acidilactici* strains was reported by Xing et al. (2020) due to the α-galactosides and phytic acid reduction. It was also reported that the fermented plant-derived proteins possessed enhanced biological activities as shown by X. Yang et al. (2016): a better antioxidant performance of peanut protein after fermentation. Fermentation can decrease the allergenicity of plant proteins by the degradation of their allergens and anti-nutritional compounds or due to the induced changes in their primary and higher structure. The decreased immunoreactivity of soy protein isolate after fermentation using *Bacillus subtilis, Rhizopus oryzae, Saccharomyces cerevisiae,* and *Lactobacillus helveticus* was reported by Pia Meinlschmidt, Ueberham, Lehmann, Schweiggert-Weisz, and Eisner (2016). Among the evaluated starter cultures, *Lactobacillus helveticus* was the most successful in their study with up to 100% of immunoreactivity reduction in soy protein isolate. Fermentation induced by *Lactobacillus plantarum* strains also decreased the immunoglobulin E reactivity of soy protein by 83.8–94.8% (Rui et al., 2019).

Fermentation has been found to be successful in decreasing the bitter and beany off-flavors of different plant-based proteins. Lactic fermentation of lupin protein extracts reduced or even masked its beany off-flavor by newly produced compounds (Schindler et al., 2011). Schlegel et al. (2019) also displayed the decreased bitterness of lupin protein isolate after fermentation with eight different microorganisms, among which *Lactobacillus brevis* was the most successful one. Liquid state fermentation of soy protein isolate also decreased its bitterness and beany flavor (Pia Meinlschmidt, Ueberham, Lehmann, Reineke, et al., 2016).

3.4. Others

3.4.1. Complexation

The chemical diversity of proteins enables them to undergo several molecular interactions including hydrophobic, electrostatic, hydrogen bonding, van der Waals, steric repulsion, and disulfide bridges with different substances, allowing the formation of micro- and nano-particles with new or improved technical and functional stability. Therefore, the complexation of plant-based proteins with other proteins or other substances is gaining interest as a method of protein modulation as well as assembling and designing novel bioparticles with tunable properties, with immediate significance in the pharmaceutical and food industries.

3.4.1.1. Protein-polysaccharide. The pH-induced electrostatic interaction between plant-based proteins and polysaccharides has been widely used to broaden the functionality of these proteins, as reviewed by Warnakulasuriya et al. (2018). Proteins can electrostatically interact with polysaccharides with opposite charges, forming the assembly of insoluble or soluble coacervates with new surface, structural and functional characteristics (Fan, Zhang, Tai, & Yuan, 2020; Sedaghat Doost, Nikbakht; Nasrabadi, Kassozi, et al., 2019; W.; Wang et al., 2020a). The self-assembly of plant protein-polysaccharide complexes, their characteristics and the strength of the interactions depend on several factors including the pH, ionic strength, mixing ratio, total concentration, temperature, shearing rate, charge density, polysaccharide type, its chemical and molecular composition (Sedaghat Doost, Nikbakht Nasrabadi, Kassozi, et al., 2019; Warnakulasuriya et al., 2018). Several research attempted to design novel bioparticles with desired techno-functional properties by tailoring these parameters. For instance, the effect of pH, mixing ratio and total biopolymer concentration on the formation and properties of flaxseed protein and flaxseed gum was investigated (Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Roman, et al., 2019; Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, & Van der Meeren, 2020). The authors reported that pH as one of the most important factors since it is effective on the ionization of functional groups of biopolymers and their surface charge in solution. Moreover, the proportions of the flaxseed protein and polysaccharide was effective on the charge balances within the mixture with subsequent effect on their particle size; more precisely the addition of gum resulted in smaller particles with more negative surface charge (Maryam Nikbakht Nasrabadi, Goli, et al., 2020). These results were in agreement with the results of Amine, Boire, Kermarrec, and Renard (2019) in the case of rapeseed napin protein and pectin.

The association of plant-based proteins with polysaccharides can modulate their techno-functional properties and address issues such as physical stability around their isoelectric point. For example, the solubility and reduced susceptibility of flaxseed protein to aggregation near its pI was improved after complexation with flaxseed gum as well as its emulsifying and foaming properties (Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Roman, et al., 2019; Maryam Nikbakht Nasrabadi, Goli, et al., 2020). In another study, Maryam Nikbakht Nasrabadi, Goli, Sedaghat Doost, Dewettinck, and Van der Meeren (2019) reported the improved stability of flaxseed protein against temperature, ionic strength and pH after its electrostatic interaction with flaxseed mucilage, especially around its pI. The same authors reported for the same complexes improved performance as Pickering emulsions stabilizers.

The complexation of plant-derived proteins with polysaccharides can also delay their digestion due to their hindered gastric proteolysis compared to the native protein, as reported by Maryam Nikbakht Nasrabadi, Sedaghat Doost, Goli, and Van der Meeren (2020) in the case of flaxseed protein and mucilage. Y. Xu et al. (2020) also showed the delayed *in-vitro* release of lutein encapsulated in rice protein hydrolysate-carboxymethylcellulose nanocomplexes, due to their improved stability against stomach digestion compared to the native rice protein hydrolysates. Unpleasant tastes and bitterness of plant-based proteins are other factors that need to be considered. For instance, the complexation of pea or potato proteins with apple pectin was shown to successfully mask their bitterness. It was also shown that the bitterness was decreased by increasing the pectin proportion due to the shifting of the proteins surface charges from positive to negative values (Zeeb et al., 2018). Yavuz-Düzgün, Zeeb, Dreher, Özçelik, and Weiss (2020) also demonstrated the considerable reduction in the bitterness of potato protein isolate after its electrostatic interaction with high-esterified pectin.

3.4.1.2. Protein-protein. Heteroprotein complexation is another electrostatically driven assembly, which can also be associated with hydrogen bonding and hydrophobic reactions, that occurs between at least two different proteins with opposite charges in a pH range in between their pI and at low ionic strength (Zheng, Gao, et al., 2020). This process can also be induced by external triggers such as heat treatments, enzymatic hydrolysis and/or high-pressure treatments (Alves & Tavares, 2019). Different plant-derived proteins have been modified by complexation with either other plant-based proteins or animal-based proteins. Alves et al. (2019) have recently reviewed mixing plant-based proteins with animal-based ones as a modification method for their techno-functionality and discussed this as a possible way for partial replacement of animal-derived proteins in food systems. There are also some more recent studies on the above mentioned heteroprotein complex coacervates (Zheng, Gao, et al., 2020; Zheng, Tang, Ge, Zhao, & Sun, 2020). However, in this review we will focus on the interaction of plant-based proteins with other plant proteins in order to design plant-based protein composites with improved functionality. There is not much research yet on the formation and characterization of heteroprotein complex coacervates that only consist of plant-originated proteins. T. Wang, Xu, et al. (2018) conducted a research on the complexation of rice and soy protein and according to their results, the water solubility of rice protein was considerably increased from 1.84% to more than 82% through its interaction with soy protein isolate upon a mass ratio of 1:0.1 at pH 12 for 1 h, followed by readjusting the pH to 7 at room temperature.

3.4.1.3. Protein-phenolic. A new method of complexation of proteins with phenolic compounds has been recently used for the modification of proteins. Phenolic compounds are secondary metabolites in plants classified into flavonoids and non-flavonoids and naturally exist in fruits, vegetables, and green tea. These compounds which possess biological activities, including antioxidant, antimicrobial, anticancer, antiallergenic, anti-inflammatory, and etc., can interact with proteins through covalent and non-covalent bonding (hydrogen bonding, hydrophobic and electrostatic interactions) (B. Hu et al., 2018; J. Liu, Oey, et al., 2019). These covalent and non-covalent interactions between plant-based proteins and phenolics provide an opportunity for their structural and functional improvements, which may also offer health-beneficial effects. The covalent bonding between proteins and phenolics can be achieved at alkaline conditions where the phenolics are oxidized to quinones. Quinones have the ability to covalently bind to the sulfhydryl and amino groups of proteins through their phenolic rings. Free radical mediated grafting, enzyme catalyzed grafting and chemical coupling methods are other types of reactions for preparing covalent bonding between proteins and (poly)phenolic compounds. Unlike the covalent bonding, other interactions of proteins with these compounds including hydrogen and hydrophobic bonding, and van der Waals forces are reversible and can be prepared conveniently (J. Liu, Oey, et al., 2019). The association of plant-based proteins with phenolic compounds may induce changes in their secondary and tertiary structures (J. Liu, Oey, et al., 2019). F. Liu, Ma, et al. (2017) compared the covalent and non-covalent addition of epigallocatechin gallate, quercetagetin and chlorogenic acid to zein. They found that both interactions could modify the structure of protein. Covalent complexes exhibited higher thermal stability and antioxidant activity compared to their non-covalent counterparts. In another study, Sui et al. (2018) also investigated both covalent and non-covalent interactions between soybean and black rice protein isolates with anthocyanins. It turned out that both interactions induced structural and conformational changes in soy and black rice proteins. It was also found that the covalent interactions with soy protein with anthocyanins were more likely to form.

Generally, previous studies have shown that the bonding of polyphenolic compounds is associated with a reduction in their solubility as well as an adverse effect on some of the functional properties of plant-based proteins. For example, the emulsifying properties of flaxseed protein was found to be worse after its complexation with flaxseed polyphenols and hydroxytyrosol (Pham, Wang, Zisu, & Adhikari, 2019). However, there are more observations on the improvement of emulsifying ability of plant-derived proteins after their interaction with phenolic compounds (Dai et al., 2020; Sui et al., 2018). Bioactive properties, particularly antioxidant ability, are other features that are reported to be affected through the complexation of the plant-based proteins. The improved antioxidant ability of flaxseed protein after complexation with flaxseed polyphenols and hydroxytyrosol was observed in O/W emulsions stabilized by these complexes (Pham et al., 2019). Also, pea protein-tannic acid non-covalent complexes were shown to delay the oxidation of flaxseed O/W emulsions (R. Li, Dai, et al., 2020). Resistance to digestion can also be obtained for plant-based proteins after the addition of polyphenols. Delay in the initial stages of lipid digestion of flaxseed O/W emulsions stabilized by pea protein-tannic acid complexes was reported in comparison with native protein stabilized emulsions by R. Li, Dai, et al. (2020).

3.4.1.4. Protein-surfactant. Proteins can interact through different mechanisms, including electrostatic and hydrophobic interactions with ionic and non-ionic surfactants. The exposure of surfactants to plant-based proteins can tune their amphipathic properties by modulating their hydrophobic or hydrophilic degrees. For example, the amphipathic properties of zein was tuned by SDS combined with heating, improving its water dispersibility, as shown by a study carried out by S.-R. Dong, Xu, Tan, Xie, and Yu (2017). In another study, L. Chen et al. (2019) showed the improved pH, salt, and thermal stabilities as well as foaming properties of gliadin after the addition of phospholipids. It seems that the addition of surfactants might be a short-term and practical solution to overcome some of the functional challenges in the utilization of plant-based proteins. This was also shown by S. B. Zhang, Wang, Li, and Yan (2020) who found the improved solubility and emulsifying properties of peanut protein isolate simply by the addition of Tween 20.

The properties of the formed protein-surfactant complexes are dependent on the nature of both the surfactant and the protein as well as their relative concentrations (Jung, Savin, Pouzot, Schmitt, & Mezzenga, 2008; Mehan, Aswal, & Kohlbrecher, 2015; Sedaghat Doost et al., 2020). Q. Guo et al. (2021) evaluated the complexation of pea protein isolate with different surfactants, including rhamnolipid, tea saponin and ethyl lauroyl arginate hydrochloride. Based on their results, rhamnolipid was found to be more effective on improving the physiochemical properties of complexes, including encapsulation efficiency, physical-, photo-, acid- and thermal stability as a delivery system for curcumin. It is noteworthy to mention that the concentration of surfactant is another important factor. L. Chen et al. (2019) observed the increased foaming

performance and stability of gliadin protein by increasing the phospholipid content. Moreover, the same authors showed that the complexes with the highest amount of phospholipid had the highest stability against the environmental stress conditions such as changes in pH, salt, and temperature. Although there are some studies on the positive effect of surfactant level on the functional properties of the plant-derived proteins, there are also some reports pointing at the adverse effect of increasing the surfactant content in the complex. For instance, S. B. Zhang, Wang, Li, and Yan (2020) showed decreasing trends of surface hydrophobicity and gel strength for pea protein isolate by increasing the Tween 20 concentration, which may be explained by the hydrophobic interactions between Tween 20 with protein. It should be also noted that some surfactants are in fact synthetic or petroleum based such as polysorbate emulsifiers, which might not be in line with clean labelling and natural based products. Moreover, if certain surfactants need to be added at relatively high concentrations, some health concerns might be raised (Sedaghat Doost, 2020; Sedaghat Doost et al., 2020).

3.4.2. Amyloid fibrillization

Protein amyloid fibrils are aggregates that can be achieved by various paths in compliance with food processing. The most common method implies heating at acidic conditions, which results in protein unfolding and hydrolysis, followed by 1D assembly of peptides in a typical cross-beta pattern, the hallmark of amyloid aggregation (Adamcik & Mezzenga, 2012). The 1D polymerization of these peptides as main building blocks of the amyloid fibrils proceed through several steps, involving nuclei and their further elongation (Smith, Fernandez-Rodriguez, Isa, & Mezzenga, 2019; Wei et al., 2017). Akkermans et al. (2008) reported that the β-lactoglobulin (BLG) fibrils obtained after heating (20 h at 85 °C) at pH 2 were composed of a part of the peptides which were yielded as a result of hydrolysis during the process, and not by intact BLG. These results were expanded by Lara, Adamcik, Jordens, and Mezzenga (2011) to β-lactoglobulin and lysozyme and consistent with later results by Josefsson et al. (2019) and Josefsson et al. (2020) in the case of soy protein and potato protein amyloid fibrils. The universal feature of amyloid fibrils, which applies also to fibrils obtained by food proteins, is the presence of β-sheets which are composed of unbranched and ribbon-like β-strands with same length that are interacting with each other through hydrogen bonds (N–H···O═C) between two consecutive peptide backbones (Y. Cao & Mezzenga, 2019). These self-assembled β-sheets are interdigitated pairwise forming and stabilizing a dual β-sheet structure, i.e., a steric zipper spine through additional forces such as a) the attractive van der Waals energy from the tight-mating steric zipper interface between the β-sheet layers, b) hydrogen bonds gain from amide groups that run up and down the sheets and are mutual polarized, c) hydrophobic interactions, and d) the formation of ladders of bonding side chains on the surfaces of the paired sheets. These dual/cross β-structures provide the nanofibrils a rigid structure with a very high stability and mechanical strength which has no analogue among the other protein aggregates (Y. Cao & Mezzenga, 2019). Protein fibrillization can greatly improve protein functionalities due to the nanofibrils excellent properties, including high aspect ratio, high stiffness, accessibility of the functional groups, and high stability against harsh conditions, which expand their potential in functional colloidal dispersion (Y. Cao & Mezzenga, 2019; Knowles & Mezzenga, 2016; Wei et al., 2017). Therefore, the fibrillization method offers a great potential for modulation of proteins for different applications such as drug and nutraceutical delivery platforms (Shen et al., 2017), foam and emulsion Pickering stabilizers (D. Peng, Yang, Li, Tang, & Li, 2017; J. Peng, Simon, Venema, & van der Linden, 2016; Schlegel et al., 2019), degradable films (Dunge, 2019), ultralight aerogels (Gohi et al., 2019), gels (Y. Cao, Bolisetty, Adamcik, & Mezzenga, 2018; Y. Cao & Mezzenga, 2020) and water purification filters (Bolisetty & Mezzenga, 2016; QingruiZhang et al., 2019; Zhang et al., 2019). Up to now, much research has been carried out on the fibrillization of animal-based protein, especially whey proteins (mostly β-lactoglobulin). However, the propensity to form amyloid fibrils is a generic property for all proteins (Mezzenga & Fischer, 2013), including plant proteins, which will be addressed shortly in what follows.

The Fibrillization process and the properties of the assembled fibrils depend on different factors including the protein source, protein concentration, temperature, pH, ionic strength, stirring, heating time, incubation time and the presence of other substances. The nature of the protein and its amino acid composition have an influence on the formation of amyloid fibrils and their characteristics. J. Liu and Tang (2013) compared the fibrillization of vicilin from three different sources, including mung, red and kidney beans during heating at 85 °C at pH 2.0 for 1–24 h. Their results showed that the vicilin obtained from mung had the highest potential for the amyloid fibril formation while kidney bean had higher ability to form highly ordered fibrils. The differences in their amino acid composition and propensity of their polypeptides to acid hydrolysis was responsible for these differences. S.-R. Dong, Xu, Li, Cheng, and Zhang (2016) evaluated the fibrillization of β-conglycinin (7S globulin) and glycinin (11S globulin), two different fractions of soy protein, and discussed the different required conditions for each fraction. The acidic subunits from glycinin formed thin, long and flexible fibrils at shorter time, higher temperature and wider pH value in comparison to β-conglycinin. Their results also revealed that the synergistic effect of β-conglycinin and the adverse effect of glycinin and its basic subunits on the fibrillization of the acidic subunits. The fibrillization of proteins from three different legume sources including cowpea, chickpea and lentil was investigated in a study conducted by T. Li et al. (2020): based on their results vicilin of cowpea protein had the highest ability to form amyloid fibrils with superior rheological properties, due to their longer and flexible morphology compared to semiflexible and rigid fibrils of lentil and chickpea proteins, respectively. Higher protein concentrations in general result in more fibrils (Lambrecht et al., 2019; J. Liu et al., 2013). The protein concentration below which no amyloid fibrils are assembled is called critical protein concentration, and differs from one protein to another. The protein concentration also affects the morphology of the assembled fibrils. Higher amount of fibrils can be formed at higher temperatures while too high temperatures might have negative impact on the process due to the competition with amorphous aggregation (Lambrecht et al., 2019). As mentioned above, pH is another effective factor on this process. Wan and Guo (2019) investigated the effect of pH (2-10) on the formation of soy protein isolate amyloid fibrils and their results showed different shapes of the assembled constituent peptides at different pH values, in agreement with earlier works on β-lactoglobulin (Jung et al., 2008). Their results also showed the lack of β-sheet structures in the assembled aggregates above pH 6 which made them susceptible to dissociation, and which effectively discard the aggregates as amyloids. More precisely, among the pH values below 6 only at pH 2 the formed β-sheets assembled into amyloid fibrils. Salt concentration can also influence the fibrillization process and the morphology of the formed fibrils. Tang, Wang, and Huang (2012) found the enhancement of formation and length of soy β-conglycinin fibrils by increasing the salt concentration from 0 to 300 mM NaCl during heating at 80 °C and pH 2.0 for 2–16 h. This could be attributed to the effect of salt on tertiary and quaternary structure of proteins and increasing the β-sheet formation, or more likely, to the screening of repulsive electrostatic interactions. By increasing the salt concentration, the critical protein concentration was also decreased and short, curvy and highly branched fibrils could be assembled. As far as the size of the amyloid fibrils is concerned, J. Liu et al. (2013) showed that the size of the amyloid fibrils obtained from kidney bean vicilin increased when increasing the salt concentration to 200 mM. On the other hand, the size of the fibrils from red and mung bean vicilin stayed unaffected. The heating time is another important factor with regards to the changes in the characteristics of amyloid fibrils. Xia et al. (2017) for example, showed that by heating soy protein isolate and soy protein β-conglycinin hydrolysates at 95 °C at pH 2 for 60 min, short worm-like fibrils and long semi-flexible fibrils were formed, respectively. The extension of heating

time to 360 min triggered clusters coexisting with fibrils, whereas heating for 720 min stopped the formation of fibrils in both protein samples, indicating that the appropriate heating time can also influence the structural and functional properties of the formed amyloid fibrils. Likewise, Pang et al. (2020) showed that the heating time had effect on the structural properties of rice bran protein fibrils in a way that by increasing the time to 420 min, the content of β-sheets and surface hydrophobicity increased. By contrast, the further increase in heating time up to 600 min had a downward trend. The other features such as the molecular flexibility and the emulsifying properties of rice bran protein fibrils were also influenced by the heating and formation time. It has been found that the heating time of 420–480 min is the most appropriate time for the optimum physicochemical and emulsifying properties.

Although fibrillization of plant-based proteins seems to be a very efficient, novel and attractive method of modification, the issue of safety of the amyloid fibrils and their potential health risks and hence their commercial exploit in food industry have been already widely discussed in literature. For example, Y. Cao and Mezzenga (2019) and Jansens et al. (2019) have reviewed the digestion and potential toxicity of food-derived amyloid fibrils. Lassé et al. (2016) investigated the *in-vitro* toxicity of food amyloid fibrils including whey, soy, kidney bean, and egg white fibrils and according to their results there was no reduction in either *Caco-2* or *Hec-1a* cells viability after exposure to amyloid fibrils except for fibrils obtained from kidney bean proteins, that triggered negligeable reduction (5–10%) in the viability of *Hec-1a*-epithelial cells. Likewise, S. Li, et al. (2020) observed no *in-vitro* cytotoxicity of rice glutelin fibrils. However, most of these available studies were *in-vitro*. To the best of our knowledge, the only *in-vivo* study available today is perfomed by Shen et al. (2017) on animal models, which concluded not only safe use and no toxicity for β-lactoglobulin amyloid fibrils, but also highlighted their possible use in nutrition for iron fortification, when combined with nutraceutical iron nanoparticles. There is currently still a lack of comprehensive information about the potential health risks of plant-based amyloid fibrils for human and their biosafety aspects as well as their digestion fate, which need to be carefully addressed in future studies, although all data at hands point to a safe use in food applications.

4. Conclusions and future perspectives

Plant-based proteins are becoming fast-growing and innovative ingredients in food industry due to their advantages over their animal-derived counterparts, especially with respect to sustainability aspects and ethical implications. There are also some other drivers of plant-based proteins such as population growth, variety, traceability and growing demand for hybrid, clean labeled and healthier products. However, as the nutritional value and functional properties of proteins determine their quality and technological use for food applications, plant proteins tend to have inferior functionality compared to animal-based proteins since they are more difficult to process and more susceptible to extrinsic factors including temperature, pH, and ionic strength. Moreover, plant proteins often contain anti-nutritional compounds with a strong off-taste. These problems have limited the application of plant proteins as a constituent in food systems. Therefore, in order to partially or fully replace animal-based proteins, efficient modification processes of plant-derived proteins are required. In this review we have discussed the current state of the art on processes available to this end and we have highlighted opportunities and challenges to move the field forward. Different physical, chemical biological methods as well as other approaches, such as complexation with other compounds and amyloid fibrillization, were discussed as modification methods due to their ability to induce chemical, structural and biophysical changes in plant-based proteins, leading to improved techno-functional properties. Each method has been discussed with its advantages and disadvantages. For example, chemical modification approaches are not ideal, -a priori-from the industry and consumers point of view, due to their need for chemicals any because they may be creating toxic side streams. Among the chemical modification approaches, glycation has no need for exogenous chemicals and does not produce any chemical or toxic by-products. Therefore, in terms of food-safety regulations, this method can be a desirable chemical modification approach for plant-based proteins, fully in line with the increasing trend of "clean-label" ingredients. Most of the physical and biological methods can be used at the industrial level for the improvement of plant-based proteins with benefits for scaling up and overall costs. For instance, some physical methods such as heat treatments and high-pressure techniques have been already used in food processing for a long time. However, the energy and cost efficiency of these techniques should be re-evaluated in order to fully align with sustainable developing goals. Improving the quality of plant-based proteins using biological methods such as enzymes and fermentation is also attracting interest since this implies environmentally friendly and low energy-consuming processes which furthermore do not lead to the production of toxic by-products. However, the cost of enzymes and cultures should also be taken into consideration for large scale applications. Moreover, as mentioned before, protein hydrolysates, obtained from enzymatic modification, are often associated with a strong bitter and/or astringent after-taste. One of the most recent and attractive methods for plant protein modification seems to be amyloid fibrillization which is both effective and efficient; yet, as this method is new in food technology, research is still ongoing to fully assess their biosafety as well as their digestion fate: all indications available to date, nonetheless point to a safe use in food applications. It is anticipated that the rational design of modified plant-based proteins with improved nutritional, sensorial and techno-functional properties can open up new opportunities within food and nutrition and allow the designing of novel complex foods based on plant proteins and their hybrids with dairy and animal counterparts.

CRediT authorship contribution statement

Maryam Nikbakht Nasrabadi: Writing – original draft, All authors wrote the manuscript and approved its final version. **Ali Sedaghat Doost:** Writing – original draft, All authors wrote the manuscript and approved its final version. **Raffaele Mezzenga:** Writing – original draft, All authors wrote the manuscript and approved its final version.

Declaration of competing interest

The authors declare no conflict of interest.

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