Pea protein ingredients: a mainstream ingredient to (re)formulate innovative foods and beverages.

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Highlights:

- Pea proteins as promising ingredient for food and beverage design
- Novel technologies for improving pea protein functionality and sensory perception
- Mitigation strategies for reducing/ masking off-flavors of pea proteins
- Pea proteins impact on nutritional and technological properties of foodstuffs

Abstract:

Background: Pea (*Pisum sativum*) proteins are emerging as a popular alternative to those conventional (deriving from animal and soy) due to their high protein content with interesting functionality, sustainability, availability, affordability and hypo-allergenicity. This popularity has been parallel to an intensive research from protein isolation to their applications. Pea protein ingredients can be obtained through wet extraction, dry fractionation or more recently mild fractionation. As such, commercial pea proteins ingredients include flour (20-25% protein), concentrate (50-75% protein), and isolate (>80% protein). Beside protein content, these ingredients differ in their chemical composition, thereby affecting their functionality.

Scope and Approach: In this perspective, this review offers the lastest update on essential knowledge for developing innovative food and beverages using pea proteins through emphasizing the production and the characteristics of pea proteins, addressing the efficiency of pea proteins as functional ingredients in foodstuffs making, and discussing the challenges encountered for pea protein popularization.

Key Findings and Conclusions: Current research indicates the importance of developing extraction and drying technologies to reach target techno-functional and organoleptic attributes of pea proteins. A better modulation of processing steps can enable designing high-quality pea protein rich food and beverage.

Keywords: pea proteins, isolate, concentrate, functionality, processing, food industry

1. Introduction

The global protein demand is expected to grow rapidly in the coming years due to an increasing world population. Currently around one billion people in the world do not have access to a diet providing enough protein and energy. To keep up with this demand, new initiatives are underway to increase the production of high quality, functional, affordable and sustainable protein sources, which can partially substitute those mainly deriving from animal products (*e.g.*, whey proteins, caseins and gelatin) (Bogahawaththa, Bao Chau, Trivedi, Dissanayake, & Vasiljevic, 2019). In terms of the global pressure on the demand for water and energy, consumption of plant-based proteins is more environmentally friendly and a more sustainable source due to their lower carbon footprint than animal proteins (Apostolidis & McLeay, 2016).Over the last years, there is a remarkable movement toward plant derived proteins as preferred alternatives to animal protein due to growing concerns surrounding health, ethical and/or environmental impacts (Kornet et al., 2020). Plant-based diets have been shown to deliver health benefits by lowering both cholesterol level and blood pressure, balancing blood sugar, and even reducing the risk of developing certain cancers (Gravely & Fraser, 2018). Additionally, decreased use of animal proteins can be driven by consumer dietary restrictions (lactose free) or ethical choices (vegan, vegetarian and flexitarian). Another important stake is providing a balanced amino acid composition similar to the reference pattern described in FAO/WHO recommendations. Several sources of plant proteins were characterized by a balanced nutritional quality and high protein content suggesting their use for human nutrition (Sá, Moreno, & Carciofi, 2020).

In this context, pulses, dry edible seeds of *Leguminosae* crops (beans, peas, chickpeas and lentils), present environmental benefits such as nitrogen fixation to the soil, minimal requirement for fertilizers, low carbon and food wastage footprints, water efficiency, and low cost of production (Acquah, Zhang, Dubé, & Udenigwe, 2020; Boukid, Zannini, Carini, & Vittadini, 2019). As well, pulses are a rich source of bioactive compounds such as polyphenols and dietary fibers (Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019). Pulses are remarkably rich in protein (20-25%) with interesting nutritional and functional properties (*e.g.* solubility, emulsification capability and foaming) (Boukid et al., 2019). Pulses also contain anti-nutrients (*e.g.* proteinase/amylase inhibitors, phytic acid, lectins, tannins, oxalates, and saponins) that may play both desirable and undesirable effects on health and protein digestion depending on the ingested quantity (Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). Anyway, the content of these compounds in the final products is usually reduced during the common pre-treatment and processing operations (*e.g.* dehulling, soaking, cooking, etc.) (Boukid et al., 2019; Kumitch et al., 2020). So, for their agronomic and compositional characteristics, pulses have been gaining interest as functional ingredients for foods and beverages applications including gluten-free products (Chan, Masatcioglu, & Koksel, 2019).

Dry peas (*Pisum sativum* L.) are the second most important pulse crop covering more than one third (34.2%) of the total area under dry pulse (Eurostat, 2020). In 2019, a total of 7, 166, 876 hectares of pea were harvested globally providing 14, 184, 249 tons, where Canada, Russia, United States, India are the top producers (Eurostat, 2020). Pea is a cool season crop, while soybean thrives in warm crop. Depending on the cultivar, pea seeds contain about 23–31% of proteins, 60–65% carbohydrates, and 1–2% of fat(Bogahawaththa et al., 2019; Rempel, Geng, & Zhang, 2019). Pea protein attracted a great deal of attentions as a promising substitute for traditional protein ingredients (animal proteins and soy protein) due to its low allergenicity, non-transgenic status, high nutritional value and availability and deriving from a sustainable crop (Chaudhary, Marinangeli, Tremorin, & Mathys, 2018; Ding, Liang, Yang, Sun, & Lin, 2020; Gao et al., 2020; Warnakulasuriya, Pillai, Stone, & Nickerson, 2018). analysis . Pea protein can be considered a high-quality protein owing to its balanced amino acid ratio, and all essential amino acids, except for methionine, that can fulfil FAO/WHO recommendations (Gorissen et al., 2018). As such, the global pea protein market size was valued at USD 215.5 million in 2019, and is projected to expand at a compound annual growth rate (CAGR) of 7.6% during the forecast period from 2020 to 2027 (Grandviewresearch, 2019).

Commercially, pea protein ingredients are available as flours, concentrates or isolates. In spite of the great interest of this products, the inclusion of pea proteins in foods and beverages is still a challenging task for the food industry, mainly as a consequence of the pea protein's inherent distinct beany flavor and impact on functional and technological properties (Trikusuma, Paravisini, & Peterson, 2020). Beany flavor volatiles (e.g., alcohols, aldehydes, ketones) in raw peas are formed during germination by lipolytic enzymes (mainly lipoxygenase) contributing to the oxidation of unsaturated fatty acid beside non-enzymatic oxidation.. In addition, undesirable volatiles (e.g. alcohols, aldehydes, hydrocarbons, ketones, sulfur compounds, terpenes, esters, and pyrazines) can be produced during harvest, storage and/or processing (Kornet et al., 2020). Beside off flavors development, secondary metabolites of lipid oxidation can react with pea proteins resulting in the loss of essential amino acids and changes in protein structure leading to loss of functionality (Estévez & Luna, 2017). For these reasons, conventional and innovative processing are being investigated to mitigate off- flavors and enhance the technological and physiological functionalities of pea protein ingredients to meet the requirements of the industry and the consumers expectations (Gao et al., 2020; Klost & Drusch, 2019; Kornet et al., 2020; Lan, Chen, & Rao, 2018).

Recently, more focus was attributed to the functional and structural properties of pea protein isolates (Lam, Can Karaca, Tyler, & Nickerson, 2018) or on the applications without emphasizing the relevant impact of processing (Lu, He, Zhang, & Bing, 2019). Therefore, a critical review based on the scientific literature published in the past decade was conducted to identify the status of the knowledge and how to move further with pea proteins industry. In this light, this review addressed the production chain of pea proteins (preprocessing, processing and postprocessing), functionalities and their implication on developing innovative foods and beverages using pea proteins. Therefore, this critical review presents the extraction methods used for pea protein extraction focusing on their advantages and limitations; then it offers insights on pea proteins structural, nutritional, biological and functional properties aiming to underline their potential use as food ingredient. Moreover, it aims identifying the different food applications and the main stakes associated with food formulation by linking the functional properties of pea protein ingredients to the quality of end products.

2. Production of pea protein ingredients

Selecting the appropriate processing for pea proteins extraction is essential to maximize the yield and to determine their structural, nutritional and functional properties which will greatly influence their applicability in the food industry. As illustrated in Figure 1, separation of pea proteins can be achieved by wet extraction (A), dry fractionation (B) or mild fractionation (C) (Adenekan, Fadimu, Odunmbaku, & Oke, 2018; Kornet et al., 2020; Pelgrom, Boom, & Schutyser, 2015a; Reinkensmeier, Bußler, Schlüter, Rohn, & Rawel, 2015; Rempel et al., 2019).

2.1. Pre-processing: for a better functionality

Prior to protein extraction, pea seeds can go through pre-processing steps such as cleaning, drying, sorting, dehulling or/ and splitting. Splitting and dehulling enables the detachment of the hulls and the cotyledons from whole pulses thereby facilitating protein extraction without affecting their techno-functional properties (Saldanha do Carmo et al., 2020). Even though pea seeds have a low lipid content, the oxidation of fatty acids significantly contributes into the generation of beany odor of protein ingredients (Murat, Bard, Dhalleine, & Cayot, 2013). Solvent alone or in combination with supercritical fluid extraction was used for the removal of lipids from pea flour resulting in removing undesirable flavors (Schutyser & van der Goot, 2011; Vatansever & Hall, 2020). Germination is a promising process to improve the functionality, nutritional value (mitigating anti-nutritional factors and boosting antioxidant capacity) and the flavor of seed storage proteins due to hydrolytic enzymes activated during pulses germination (Kaczmarska et al., 2018; Setia et al., 2019; Singh & Sharma, 2017; Xu et al., 2019). In the case of pea seeds, germination (up to 5 days) enhanced nutritional value and functional properties (emulsion activity and stability, foaming capacity and foam stability) (Setia et al., 2019). Xu et al (2020) indicated that germination longer than one day increased the beany-related odours (including hexanal, *(E,E)*-2,4-nonadienal, *(E,E)*-2,4-decadienal, 3-methyl-1-butanol, 1-hexanol, and 2-pentyl-furan) in protein-enriched flours, probably due to the increased activity of lipoxygenase on unsaturated lipid or as a consequence of the release of beany-related volatiles originally bound with protein (Xu, Jin, Gu, Rao, & Chen, 2020; Xu et al., 2019). Although not a new technology, fermentation processes have been used on pulses and particularly on peas to improve protein digestibility to reduce the levels of anti-nutrients compounds (*e.g.* tannins, trypsin, α-galactosides and chymotrypsin inhibitors) and to increase mineral bioavailability (Goodarzi Boroojeni et al., 2018). Although it has not been implemented yet, solid-state fermentation might be also a promising method to be applied in peas as it showed interesting results in other pulses like soybean and lupin (Villacrés, Quelal, Jácome, Cueva, & Rosell, 2020).

2.2. Wet extraction: the alkaline extraction-isoelectric precipitation method

Wet extraction is the conventional method for the production of commercial pea protein isolates (Stone et al., 2015). Extraction parameters such as pH, temperature, salt and ionic strength can strongly affect yield and proteins' thermal, structural and functional properties (Feyzi, Milani, & Golimovahhed, 2018; Klost & Drusch, 2019). In alkaline extraction-isoelectric precipitation method (Figure 1A), yellow pea seeds (20-25 g protein/100 g dry matter) are milled to fine flour, then dispersed (with continuous mixing) in water to enable the dissolution of proteins and the suspension of starch granules. The slurry passes through a hydrocyclone to separate proteins from starch granules; the protein rich-fraction is solubilized under alkaline condition to remove the insoluble residues and then precipitated at its iso-electric point (pH 4.8) to remove dissolved impurities. The precipitates are collected, re-suspended in water with the pH adjusted to 7.0 and finally pea protein isolates (>80 g protein/100 g dry matter) are obtained after a final drying step (Berghout, Pelgrom, Schutyser, Boom, & Van Der Goot, 2015; Gao et al., 2020). Extraction yield varied from 3.1% to 15.9% depending on the extraction parameters including pH (2.5–10), extraction time (20–80 min) and water: flour ratio (5-20 v/w) (Feyzi et al., 2018). The highest extraction yield was obtained at pH=9.96), water: flour ratio=15 v/w and extraction time=58 min. Also, drying methods (vacuum oven and freeze drying) had considerable effect on the protein structure, thermal stability and function. Particularly in vacuum oven drying, temperature could be adjusted below the denaturation temperature of protein isolate. Overall, wet extraction enables the complete extraction of protein isolates, but native functionality of the proteins is compromised, thus to maintain the functional integrity of the proteins some additional research for optimization should be undertaken (Pelgrom, Boom, & Schutyser, 2015b). In particular protein structure and integrity might be hindered leading to the formation of large aggregates of insoluble proteins (Chao & Aluko, 2018). Conversely, the whole process may induce the mitigation of volatile compounds initially present in pea flours (77 compounds were removed out of 124 volatile compounds) (Xu et al., 2020, 2019). In fact, 19 new volatile compounds were formed during extraction but none of them contributed in intensifying the beany flavor (Xu et al., 2020).

2.3. Dry fractionation: size reduction and air classification

As illustrated in Figure 1B, dry fractionation of peas involves two key steps, milling (size reduction) and air classification (size separation) (Geerts et al., 2018; Saldanha do Carmo et al., 2020; Schutyser et al., 2015). Milling pea seeds can be conducted using different methods (roller, stone, hammer, and pin milling), where the roller miller is the most standard method used. This results in breaking down seeds into small fragments thereby liberating starch granules from protein matrix (Pelgrom et al., 2015b). Depending on the intensity of the milling process, the resulting flour can be very fine (low roller gap) indicating that starch granules have been damaged and their size is severely reduced which results in difficulties in separation between starch and proteins, whereas larger roller gap results in coarse particles where proteins and starch are still mostly attached, and subsequent separation is not possible (Angelidis, Protonotariou, Mandala, & Rosell, 2016; Li et al., 2016). The appropriate roller gap must be selected to enable homogeneous size distribution and to avoid the disruption of starch granule structure and breakdown of amylopectin molecules that negatively impact starch pasting properties. Air classifying is the splitting of the flour of a mixed particle size into two size fractions at a predetermined cut point using air power to modify the particle size distribution. The cut point is the size at which a particle has a 50% chance to move either to the fine fraction or to the coarse fraction. In the case of pea, protein-rich particles (fine fraction; 1–3 μm) are separated from starch granules (coarse fraction; 2–40 μm) based on size, shape and density. The optimum cut point is around 15–22 μm, below the size of most pulse starch granules. A lower cut point may result in an increased purity of the protein fraction, however, at the expense of yield, but even 44% yield was considered manufacturing acceptable (Rempel et al., 2019). A pea protein concentrate (fine fraction) is obtained with 50–55 g protein/100 g dry matter and a pea starch concentrate (coarse fraction) is obtained with ~67 g starch/100 g dry matter (Pelgrom et al., 2015a). Compared to the wet extraction, dry fractionation is a chemical-free (no chemical residues in the flour fractions and no loss of the native functionality of the proteins), no use of water, effluent-free, cost-effective (less energy requirements) and therefore a more sustainable process (Rempel et al., 2019; Schutyser et al., 2015). Its major drawback lies in the lower purity of protein concentrate (50–55 g protein/100 g dry matter) compared to proteins isolates (>80 g protein/100 g dry matter) (Pelgrom et al., 2015a; Rempel et al., 2019; Schutyser et al., 2015).

2.4. Mild fractionation

A mild fractionation process (Figure 1C) was proposed for producing pea protein isolates using an hybrid approach (Geerts et al., 2017; Kornet et al., 2020; Pelgrom et al., 2015). The fine fraction of pea flour (recovered after dry fractionation) was suspended in water and then fractionated through a layer-by-layer separation using centrifugation forces or/ and additional purification (*e.g.* dialysis or ultra-filtration) to increase purity (up to 75-90 g protein/100 g dry matter) (Geerts et al., 2017).

As summarized in Table 1, both dry and mild fractionations involve the physical separation based on size and density distribution. Dry method is more sustainable (no water needed), where their yields (dry, 77 g/ 100g; mild, 55-65 g/ 100g) depended on the number of passages (milling-air classification)] still preserving its native form (Kornet et al., 2020; Pelgrom et al., 2015b). On the contrary, wet processing reduces the amount of non-protein materials and provides a more purified protein isolate (80-90% protein) and yield 80 g/100 g, but reduces native functionality and requires high quantities of water, chemicals and energy (Geerts et al., 2018; Wang et al., 2020).

2.5. Post-processing: for a better functionality and sensory perception

The presence of off-flavor compounds (beany and green notes) is closely associated with the natural presence of aldehydes, ketones, furans, pyrazines and alcohols in peas. As such, pea proteins are perceived as ‘green’, ‘grassy’, ‘hay-like’, ‘pea pod’ (Lan, Xu, Ohm, Chen, & Rao, 2019; Yousseef, Lafarge, Valentin, Lubbers, & Husson, 2016). These off-flavor compounds have the tendency to bond with pea protein during dry or wet pea protein processing (Lan et al., 2019). Modifying proteins structure through fermentation (bacteria, yeast, fungi), enzymes, chemical and thermal processing can reduce the number of accessible binding sites thereby reducing protein-flavor binding affinities and changing sensory perception (K. Wang & Arntfield, 2016).

Lactic acid fermentation has been applied to minimize the beany odors of pea concentrates (Yousseef et al., 2016). However, depending on the quantity of pea protein concentrate (0 to 40% addition) and the starters used (10 types), the green/beany flavors can either be reduced or the negative characteristics (astringency and bitterness) might increase during lactic fermentation (Yousseef et al., 2016). The change in the aroma profile of pea protein results from the generation of 23 highly odor-active compounds (such as *n*-hexanal, 1-pyrroline, dimethyl trisulfide, 1-octen-3-one, 2,5-dimethyl pyrazine, 3-octen-2-one, β-damascenone, and guaiacol) in fermented pea proteins (Schindler et al., 2012). *Lactobacillus plantarum* fermentation of pea protein concentrate results in proteins hydrolysis, thereby the formation of novel flavors, with a concomitant reduction of antinutrients and increase in bioactive peptides (Çabuk et al., 2018). This method also can enable tailoring the functionality of the fermented proteins depending on pH and duration of fermentation. For instance, fermented pea proteins improved emulsion stability (at pH=7 after 5 h of fermentation) and foam capacity (at pH=4 after 5 h of fermentation). Therefore, further investigation is needed to modulate the lactic fermentation and to extend the functionalities of the protein concentrates. By combining lactic acid bacteria and yeasts (*Kluyveromyces lactis*, *Kluyveromyces marxianus*, or *Torulaspora delbrueckii*), "green notes" were reduced and masked by the generation of a "yogurt-like" aroma owing to esters formation (El Youssef et al., 2020). Thus, this mixed culture can be further applied to improve the sensory perception of a pea protein enriched food and beverages (El Youssef et al., 2020). The fermentation of pea proteins (obtained from dry fractionation) by *Aspergillus oryzae* and *Aspergillus niger* increased phenolic content and decreased trypsin and chymotrypsin inhibitors activities. Also, *in vitro* protein digestibility was increased after fermentation but reduced decrease methionine and cysteine (Kumitch, 2019) (Kumitch, 2019). As well, fermentation improved water hydration and oil-holding capacities of pea proteins concentrates (Kumitch, 2019).

Chemical modification was also applied for improving the properties of pea proteins. Deamidation with glutaminase of pea protein isolates does not change the basic protein composition but enables its unfolding and conformational reorganization (Fang, Xiang, Sun-Waterhouse, Cui, & Lin, 2020). The deamidation leads to pea proteins with higher flexibility, solubility, homogeneity and dispersibility with reduced beany flavor, grittiness, and lumpiness compared to those of the untreated. Thus, the glutaminase treatment offers a promising approach for enhancing the applicability of pea proteins (Fang et al., 2020).

Solvent treatment of pea protein can modify the ketone flavors (2-hexanone, 2-heptanone and 2-octanone) and thus the protein-flavor binding can be modulated by varying the type and concentration of salt added (K. Wang & Arntfield, 2015). Addition of higher concentrations of non-chaotropic salts increased protein-flavor hydrophobic association, while lower concentration decreased flavor retention. At acidic condition (pH=3), the low binding capacity can be beneficial in formulating acidic protein-fortified beverages with lower flavors (K. Wang & Arntfield, 2015)

Wang & Arntfield (2016) investigated the effects of chemical (acetylation and succinylation) treatments on the binding properties of salt-extracted pea protein isolates to 2-octanone, octanal, hexyl acetate and dibutyl disulfide. They found that acetic and succinic anhydrides (up to 1 g) reduced the bond protein-octanal and hexyl acetate due to partial protein denaturation. At low concentration of dicarboxylic acid anhydrides (<0.1 g), the binding capacity (protein-2-octanone and dibutyl disulfide) increased, while at higher concentration, flavor retention decreased probably due to extensive protein denaturation (K. Wang & Arntfield, 2016).

Pea proteins can be subjected to hydrolytic and crosslinking enzymes. Hydrolytic treatments (alcalase, chymotrypsin, pepsin or trypsin) of pea protein concentrates results in the generation of peptides with α-amylase and α-glucosidase inhibitor activities, principally against α-amylase than α-glucosidase (Awosika & Aluko, 2019). Pea protein isolates hydrolyzed by alcalase releases bound ketone and ester flavors whilst bond aldehyde and disulfide flavors (K. Wang & Arntfield, 2016). As for crosslinking enzymes, transglutaminase enhances the shear strain or gel elasticity of pea isolates and does not alter its thermal properties (Shand, Ya, Pietrasik, & Wanasundara, 2008). Furthermore, treating pea protein with transglutaminase slows down the rate of heating and cooling thereby enhanced the rearrangement of pea protein and gel strength (Sun & Arntfield, 2011). This enzyme may provide opportunities for extending the properties of pea proteins when developing new food products.

Combined chemical-thermal treatment (gum arabic and maltodextrin during spray-drying) has been used to enhance the protein solubility and mitigate off-flavor of pea protein isolates. Particularly, this treatment improves the surface area/volume ratio hydrogen bonding and/or electrostatic interaction between protein and polysaccharides, mitigates the beany flavors and increases the solubility of the formed pea protein-polysaccharide complexes (Lan et al., 2019). Therefore, the solid dispersion-based spray-drying technique may be a useful tool to enhance both functionality and sensory attributes of pea proteins (Lan et al., 2019).

3. Pea protein ingredients characteristics

3.1. Structure

Yellow pea proteins are made up of albumin (10–20%) and globulin (70–80% of the total seed protein) (Acquah et al., 2020). Albumins (∼5–80 kDa, 2S) are water-soluble metabolic proteins and can be mainly classified into enzymes, enzyme inhibitors and lectins (Barac, Pesic, Stanojevic, Kostic, & Bivolarevic, 2015; Djoullah, Husson, & Saurel, 2018; Lan et al., 2018) Although albumins contain high amounts of tryptophan, lysine, threonine, and methionine compared to globulins, which is more interesting from the nutritional point of view, globulins offer more opportunities for obtaining functional ingredients. Globulin, salt-soluble storage proteins, can be further divided based on their sedimentation coefficients into legumin (∼300–400 kDa, 11S), vicilin (∼150–170 kDa, 7S) and convicilin (∼70 kDa, 7S) (Bogahawaththa et al., 2019; Gao et al., 2020). The vicilin/legumin ratio is generally within 0.5 and 1.7, the higher this ratio the lower the protein content is (Gueguen & Barbot, 1988). This ratio is closely related to genotype and environmental conditions. The legumins are a hexameric fraction that consists of six subunits (∼60 kDa), each a combination of an acidic α-chain (∼40 kDa) and a basic β-chain (20 kDa), linked via a disulfide bond. The hydrophilic α-chains are located at the molecule surface, whereas hydrophobic β-chains are buried at the interior. Vicilins are a trimeric fraction consisting of three subunits (α, β, and γ) connected by hydrophobic interactions (no disulfide bonds) (Acquah et al., 2020; Warnakulasuriya et al., 2018). Convicilin (7S) is a tetrameric fraction comprising four subunits (∼71 KDa) (Klost & Drusch, 2019). Legumins result with more rigid conformation due to the compact quaternary structure and disulfide bridges as well as hydrophobic interactions; while vicilins are characterized by a more flexible structure (Barac et al., 2015). Nutritionally, vicilins have higher amounts in arginine, isoleucine, leucine, phenylalanine and lysine compared to legumins; while this later is richer in sulfur-containing amino acids. Compared to vicilins, convicilins present cysteine in their amino acid sequences (Barac et al., 2015; Djoullah et al., 2018; Lan et al., 2018). From a functional point of view, no data was found reporting the functionality of convicilins. These structural and compositional differences result in different functionalities, where vicilin present better gelling and emulsifying properties than legumins due to structural flexibility. The authors also highlighted that stronger elastic gels are formed through more crosslinking of vicilin polypeptides (Djoullah et al., 2018).

3.2. Nutritional value and health benefits

On a dry basis, pea flour contained ∼51% starch, ∼20% protein, ∼2% lipid, ∼17% fiber and ∼3% ash (Geerts et al., 2017). Commercially available pea proteins show a great variability in their composition, because the percentage of protein and other nutrients may vary depending on pea variety, process conditions and the type of ingredient (concentrate or isolate) (Corgneau et al., 2019). As expected, increasing purity increases proteins content and reduces starch, fiber and fat contents. Typically, pea protein concentrates contain 8% starch, ∼55% protein, ∼3% lipid, and ∼34% other carbohydrates like cellulosic and hemicellulosic compounds (AM Nutrition, Stavanger, Norway). Pea protein isolates contain ∼79-89% protein, ∼0% starch, ∼1% lipid, and ∼6% ash (NUTRALYS® F85, Roquette, France).

Pea proteins are considered high-quality proteins as they are a rich source of essential amino acids including arginine, phenylalanine, leucine and isoleucine, and more importantly lysine, which is normally deficient in cereals (Çabuk et al., 2018; Gorissen et al., 2018; Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019). Pea proteins, however, are deficient in the sulfur-containing amino acids, mainly methionine and cysteine (Stone et al., 2015)**.** The amino acid scores (AAS) of pea protein isolates (1.56) is slightly lower than soy isolates (1.69) but higher than egg white (1.19) (Corgneau et al., 2019). Protein digestibility-corrected amino acid score (PDCAAS) of pea protein isolates and pea-protein concentrate was reported as good quality proteins (0.82 and 0.9, respectively) compared to whey proteins (1) and soy protein isolate (0.97-1) (Mathai, Liu, & Stein, 2017; Rutherfurd, Fanning, Miller, & Moughan, 2015). In 2013, Food and Agriculture organization (FAO) proposed to replace PDCAAS with digestible indispensable amino acid score (DIAAS), which is based on the digestibility of individual amino acids rather than the total digestibility of proteins (FAO, 2013). DIAAS of pea protein isolates (0.82) is lower than whey protein isolate (1.09) and soy protein isolate (0.8-0.9) (Rutherfurd et al., 2015)**.** Regardless of the score used, digestibility of pea protein ingredients is lower than animal proteins due to limiting sulfur amino acids (*e.g.* cysteine and methionine) (Akin & Ozcan, 2017; Gorissen et al., 2018) and this value could be further reduced (0.66) if those protein concentrates that are subjected to fermentation (Çabuk et al., 2018), because of that bacteria with limiting sulfur amino acid metabolism would be advisable for pea fermentation. The digestibility of unprocessed pea seeds was found lower with 64 PDCAAS and 73 DIAAS than protein isolate due to the presence of anti-nutrients reducing protein digestibility (Gorissen et al., 2018; Mathai et al., 2017). Overall, pea concentrates had higher AAS, lower digestibility and greater PDCAAS values than their isolate counterparts. As such, processes used in the isolation of pea protein increased digestibility, but may have led to shifts in protein composition, leading to a lower PDCAAS value (0.82) compared to pea protein concentrate (0.9) (Mathai et al., 2017)**.**

Proteins play a key role in many biological processes including satiety and building of muscles. As a satiety-inducing food ingredient, pea protein was compared to two dairy proteins, slow-digestible casein and fast-digestible whey under *in vitro* simulated gastric conditions and *in vivo (*male Wistar rats, n=9) (Overduin, Guérin-Deremaux, Wils, & Lambers, 2015). Pea protein induced weaker initial, but equal 3-h integrated ghrelin and insulin responses than whey protein, possibly due to the slower gastric breakdown of pea protein observed *in vitro*. *In vivo*, pea-protein-induced physiological signals relevant to satiety were similar to that of whey protein particularly cholecystokinin, glucagon-like peptide 1, and peptide YY). The supplementation with pea protein promoted a greater increase of muscle thickness as compared to placebo and especially for people starting or returning to a muscular strengthening program (Babault et al., 2015). Also, Babault et *al* (2015) found no differences in strength were observed between whey and pea protein groups. Likewise, ingestion of whey and pea proteins produced similar outcomes in terms of body composition, muscle thickness, force production, workout of the day performance and strength following 8-weeks of high-intensity functional training (Banaszek et al., 2019). Bioactive small peptides (< 4 kDa) with inhibitory activity towards angiotensin I-converting enzyme (ACE) have been also reported, although it must be stressed that their inhibition ability (IC50) is dependent on the protease used for the enzymatic treatment (Barbana & Boye, 2010), and the level of protease could be reduced by pretreating the protein concentrate with heat or high pressure (Chao, He, Jung, & Aluko, 2013). Small peptides of 2-6 amino acids, containing low concentrations of sulfur , were very effective in lowering the blood pressure of hypertensive rats (Girgih, Nwachukwu, Onuh, Malomo, & Aluko, 2016). Likewise, antioxidant activity has been reported in pea peptides (< 1 KDa), which sequences correspond to YSSPIHIW, ADLYNPR and HYDSEAILF (Ding et al., 2020). Even though vicilin and convicilin can trigger an immune response to some consumers, allergenic epitopes are potentially deactivated by thermal treatment (*e.g.* cooking) prior ingestion (Warnakulasuriya et al., 2018).

3.3. Functionality

Beside their nutritional benefits, pea proteins show peculiar functional benefits including solubility, emulsifying and foaming capacity and emulsion and foam stability as well as gel and film forming capacity. Anyway, due to the increasing interest in pea protein applications for (re)formulation of food and beverages products, a better understanding of their functional properties is still required.

3.3.1.Solubility

Pea protein solubility is one of the most important techno-functional properties as it can affect other proteins properties, such as foaming, emulsification and gelation (Bogahawaththa et al., 2019). Solubility can be affected by several parameters including pH value, temperature, ionic strength, solvent type and protein concentration (McCarthy et al., 2016). The solubility of pea protein is strongly pH-dependent, the highest is reached above pH 6.0 and below pH 4.0 (about 80%), while the lowest was reported to be between 4 and 6 (less than 30%) (Chao & Aluko, 2018; Yin, Zhang, & Yao, 2015) The extraction and dehydration steps may also play a crucial role on protein solubility, by affecting the protein surface hydrophobicity, exposing hydrophobic residues, and leading to increased hydrophobic interactions between proteins (McCarthy et al., 2016). In the case of wet extraction, commercial pea protein can have a lower solubility due to heat-induced denaturation (and potential aggregation) during spray-drying (Chao & Aluko, 2018). Beside wet extraction, several studies focused on mild fractionation (Kornet et al., 2020; Stone et al., 2015) and more innovative dehydration techniques (*e.g.* high hydrostatic pressure) (Chao, Jung, & Aluko, 2018) to preserve the native form of proteins and to enhance pea protein solubility. Controlled enzymatic hydrolysis (Klost & Drusch, 2019), use of additives (*e.g.* arginine) (Reinkensmeier et al., 2015) or ultrasound treatments (Jiang et al., 2017) have been also suggested as alternative strategies to improve pea protein solubility, although information is still limited.

3.3.2.Foam formation and stability

Several studies were carried out to evaluate and improve the foaming properties of pea proteins, but there is still a substantial lack of knowledge about the effects of the multiple factors involved (*e.g.* protein concentration and type, ionic strength, viscosity, temperature and pH of the medium, etc.) in determining the foam formation and stability of these ingredients (Mohanan, Nickerson, & Ghosh, 2020; Xiong et al., 2018).

Pea protein concentrates were found to be more suitable to generate stable foams than the corresponding isolates, probably due to their higher concentration of polysaccharide (Mohanan et al., 2020). (Chao et al., 2018) observed the highest foaming capacity of a pea protein isolate at pH 3.0, with a maximum value of 81%, and lower values at pH 5.0 and pH 7.0 (38% and 62% respectively). Stone et al. (2015) found that pea proteins isolates extracted by salt precipitation had better foaming properties than those obtained by alkaline extraction or micellar precipitation. High-pressure supercritical CO_2 extraction seems useful to improve the foaming properties of pea protein extracts (Saldanha Do Carmo et al., 2016), while additives (*e.g.* non-surface-active maltodextrin, guar gum and alginate) may considerably improve the foaming stability of pea protein isolates (Mohanan et al., 2020; Moll, Grossmann, Kutzli, & Weiss, 2019). Protein unfolding by high intensity ultrasound (20–100 kHz) increased the exposure of hydrophobic groups in the protein thereby promoting the adsorption dynamics at air-water interface and consequently improving the foaming capacity of pea proteins resulting in the formation of small and more homogeneous bubbles (O’Sullivan, Murray, Flynn, & Norton, 2016).

3.3.3.Emulsion ability and stability

Proteins can play an essential role in forming and stabilizing emulsions, due to their amphiphilic nature and film-forming abilities (Jarzębski et al., 2019). In an emulsion matrix, the adsorption of proteins to the oil/water interface occurs slowly compared to small molecular emulsifier and create compact layers around oil droplets (Jarzębski et al., 2019; McCarthy et al., 2016). Several factors can influence the emulsification ability of pea proteins including protein concentration, protein structure, homogenization temperature/ pressure, viscosity, pH and contact duration of protein-oil-water (McCarthy et al., 2016) (Jarzębski et al., 2019). As a function of pH values (3.0–9.0), pea protein had the lowest emulsification capacity at pH values close to its isoelectric point (around pH=5) (Chao et al., 2018; McCarthy et al., 2016); at pH values above 7, emulsification capacity was much improved (McCarthy et al., 2016); and it specially increased below pH=3, suggesting that pea proteins have better potential as emulsifiers in acidic conditions than at neutral or alkali pH (Jarzębski et al., 2019; Jiang et al., 2019). Acidic conditions increase protein absorption at the interface and induce the formation of strong viscoelastic interfacial films (Shao & Tang, 2016). In general, the application of pea protein as emulsifier is still limited compared with soy protein isolates (Shao & Tang, 2016). Several studies considerably improved pea proteins emulsion properties through heat treatment, high hydrostatic pressure and pH treatment by modifying protein structure (Chao & Aluko, 2018; Chao et al., 2018). Ultrahigh temperature has been also applied, being effective in increasing the emulsion properties when pea protein concentrates were subjected to microfluidization instead of sonication, to avoid the formation of protein aggregates (McCarthy et al., 2016; Qamar, Bhandari, & Prakash, 2019). Likewise, emulsion properties have been improved by creating a complex with different polysaccharides (*e.g.* carrageenan, xanthan gum, gum Arabic) (Vélez-Erazo, Bosqui, Rabelo, Kurozawa, & Hubinger, 2020). In this case, pea protein in combination with carrageenan or xanthan gum-based emulsions resulted in stable emulsion systems (Vélez-Erazo et al., 2020).

3.3.4. Gel forming capacity

Gelation properties of pea proteins are closely related to protein extraction conditions, *e.g.* : temperature, pH and salt composition (Mession, Roustel, & Saurel, 2017). During heating, the dissociation of legumin and their rearrangements via hydrophobic interactions and sulfhydryl/disulfide bonds reactions might result in the formation of high-molecular weight aggregates of random structure. Pea proteins cold gelation is a two steps process, where i) aggregates are formed by heating a low-concentrated protein solution (<10%) at a pH far from its isoelectric point and without salts; and after cooling, ii) these aggregates will assemble into structured network by lowering electrostatic repulsions. Instead of step 2, heat induced aggregates could form cold-set gels in the presence of acidifying agents such as glucono-δ-lacton due to heat-denatured legumin subunits re-association via non-covalent and new disulfide linkages (Mession, Chihi, Sok, & Saurel, 2015). Recent studies have reported the effect of transglutaminase on pea protein fractions gel formation (Djoullah et al., 2018). Other studies showed that globulin (native or denatured) is a good candidate for gelation by enzymatic treatment unlike albumin. Oher studies focused on heat-induced gelation of micellar casein suspensions in combination with pea protein isolates (Mession et al., 2017; Silva, Balakrishnan, Schmitt, Chassenieux, & Nicolai, 2018) or with pea protein fractions (vicilin 7S or legumin 11S enriched-fractions) (Mession et al., 2017). For acid induced gel via fermentation, the acidification led to a two-phase gelation process resulting in thick gels with weak rheological behavior (Klost & Drusch, 2019).

3.3.5.Film forming capacity

Biofilm materials from proteins (e.g. soy proteins, whey proteins, casein or zein) are commercially exploited in coating and bioactive components encapsulation (Garrido, Peñalba, de la Caba, & Guerrero, 2019; Muhoza, Xia, & Zhang, 2019). Given the poor moisture barrier properties of proteins, other polymers (e.g. chitosan, xanthan gum, gelatin or glycerol) are usually added to improve mechanical, barrier and thermal properties of proteins (Hedayatnia, Tan, Joanne Kam, Tan, & Mirhosseini, 2019). Previous studies revealed that pea protein isolates can be used in edible film formation (Carvajal-Piñero, Ramos, Jiménez-Rosado, Perez-Puyana, & Romero, 2019; Huntrakul, Yoksan, Sane, & Harnkarnsujarit, 2020). Blending pea protein (concentrates and isolates) with glycerol resulted in films with more surface structure homogeneity and limited light transmission compared to those based on whey proteins, while their physical and mechanical properties were comparable (Acquah et al., 2020). Other studies showed that blending pea protein with sorbitol can form films with good tensile strength and transparency (Kowalczyk, Gustaw, Świeca, & Baraniak, 2014; Kowalczyk et al., 2016). Alternatively, combined acetylated cassava starch-pea protein isolates formulation enhanced film formability and mechanical properties (Huntrakul et al., 2020). Particularly pea protein isolates increased film stability, tensile strength, protein aggregation and improved crystallinity, surface hydrophobicity and barrier properties against water vapor and oxygen. As a result, this film was an effective barrier for soybean and olive oil during storage (Huntrakul et al., 2020). Combining other ingredients (milk fat, candelilla wax, lecithin and oleic oil) with a blend of sorbitol-pea protein also resulted in edible emulsion films with reduced water vapor and increased oxygen permeability (Kowalczyk et al., 2016). Incorporating candelilla wax (2%) improved water vapor barrier properties and transparency and reduced the impact on oxygen permeability and mechanical strength of the films suggesting its potential use for coating (Acquah et al., 2020; Kowalczyk et al., 2016).

4. Pea protein ingredients in food and beverages applications

Through incorporation into staple food, pea protein ingredients could offer opportunities to enhance the protein content in the diet while providing some functionality (binder, emulsifier, stabilizer or extender) to the formulation (Zhao, Shen, Wu, Zhang, & Xu, 2020). This section aims to provide a better understanding of the impacts of pea protein on array of products (bread, pasta, baked goods, snacks, meat products and beverage) as summarized in Table 2.

4.1. Bread

The application of pea protein ingredients in gluten-containing bread increases protein quantity and quality, improving the amino acids profile as wheat flour lacks lysine (Erben & Osella, 2017; Millar, Barry-Ryan, et al., 2019). However, their functionality cannot replace gluten and when substituting 15% of wheat flour with pea protein isolates (85% protein), dough gluten-network weakens and decreases bread volume leading to compact crumb structure (small crumb cells) with hard texture (Hoehnel, Axel, Bez, Arendt, & Zannini, 2019).

Gluten-free bread is one of the more studied food matrices when it comes to the reformulation with proteins ingredients, looking for alternative proteins that could mimic the viscoelastic properties of gluten. In addition, gluten-free breads are usually made with high content of starchy ingredients, and consequently increasing proteins to such formulations will ensure a better nutritional composition . Generally, this kind of bread is obtained from versatile basic ingredients including starches and flours derived from gluten-free cereals or pseudocereals to mimic the role of gluten. Legume proteins have been seen as an attractive option to nutritionally enrich this type of foods, but also to contribute to the protein network, particularly pea proteins. In fact, 5% pea protein results in enriched breads with specific volume and thickness (4.00 mm and 6.89 mL/g, respectively) comparable to the control bread (based on rice flour and maize starch 50%-50%; 4.05 mm and 6.92 mL/g, respectively) (Pico, Reguilón, Bernal, & Gómez, 2019). This result can be attributed to the high water absorption capacity of pea proteins resulting in less loss of moitureness during baking as well their foaming capacity than enables gases retention resulting in a significant improvement of bread volume. Pea proteins modify the volatile profiles of breads, giving a rich volatile profile due to higher lipids oxidation (Pico et al., 2019). Pea proteins (5%) make appropriate functional blends with rice flour, increasing the viscoelastic properties of the rice doughs due to their foam forming ability enabling a better gases entrapment within the starch-protein network as well their emulsification property contributing into the formation of a stable and strong dough, that can be further intensified with transglutaminase (1%, w/w), creating inter-protein linkages that contribute to the dough network (Marco & Rosell, 2008). Even 10% of pea proteins (79.22% protein) has been used for partially substituted millet flour, combined with transglutaminase (0.5, 1.0 and 1.5% w/w based on the flour-protein blends) (Tomić, Torbica, & Belović, 2020). This strategy, besides the inherent nutritional benefit, improves the technological quality (structure strengthening, specific volume increase and sensory quality improvement) of millet bread, even increasing bread softness due to the high water absorption of pea proteins resulting in moisture preservation while mitigating the bitter taste originating from millet (Tomić et al., 2020). Pea protein functionality (emulsification and foaming capacities) has been also effective in starch-based recipes containing maize and potato, strengthening the dough structure (by increasing elastic and viscous modulus) with 10% pea protein isolate (85% protein) (Ziobro, Juszczak, Witczak, & Korus, 2016), although some bread volume reduction has been observed (Pico et al., 2019). Pea protein addition increases cell density leading to smaller gas cells, probably the emulsifying properties of these proteins might stabilize the air gas cells of the doughs, like it has been described for β-conglycinin in rice-based breads (Espinosa-Ramírez, Garzon, Serna-Saldivar, & Rosell, 2018). More nutritious gluten free breads have been formulated by using 30% pea protein (78.13% protein) (Sahagún & Gómez, 2018a). When using that high amount of proteins, water hydration must be adjusted due to the high water holding capacity of plant proteins, which allows reducing impact in crumb hardness (Sahagún & Gómez, 2018a). Bread made with blending maize starch and pea proteins (70:30) had higher slowly digestible starch and lower rapidly digestible starch values compared to the control (100% starch) (Sahagún, Benavent-Gil, Rosell, & Gómez, 2020).

4.2. Pasta

In pasta making, pea proteins have been used for nutritionally enriching the pasta varying the levels of addition up to 12.5% in combination with a range of ingredients. For instance, egg-free pasta (type *tagliatelle*) with acceptable firmness was formulated with pea protein (84–88% protein) in combination with extruded and non-extruded quinoa (red and white) flour, potato starch and tara gum (Linares-García, Repo-Carrasco-Valencia, Paulet, & Schoenlechner, 2019). Lower water absorption in pea protein enriched pasta may be a factor determining higher firmness and hardness of the cooked pasta.

Nevertheless, pea protein might have additional health contribution beyond nutrition, modulating the glucose release during digestion. This effect has been reported in wheat noodles reformulated by adding 7.5% thermally denatured pea proteins that were obtained by dissolving 5% native pea protein in water at 85°C for 30 min then freeze-dried for 48 h (Wee, Loud, Tan, & Forde, 2019). The denatured pea proteins did not affect the noodles texture and sensory perceived properties but attenuated glucose release in *in vitro* studies, which has been associated with stronger interaction between protein and starch that lowers the gelatinization degree. Although pea proteins interact with starches limiting the gelatinization process, those interactions depend on the pea proteins structure, whether denatured, hydrolyzed or crosslinked. In fact, interactions between hydrolyzed pea protein and maize or cassava starches decrease pastes apparent viscosity during heating and cooling and also lead to weaker starchy gels (Ribotta, Colombo, & Rosell, 2012). Conversely, starchy gels obtained with transglutaminase crosslinked pea proteins results in a network that better entraps water, showing lower syneresis during storage. Those interactions between pea proteins and starch might be also controlled with polyphenols, as it reported Song & Yoo (2017). Specifically, fried noodles containing 10% pea protein isolate (85% protein) and green tea extract (38.6%) had reduced peak viscosity, breakdown, and final viscosity but enhanced viscoelastic properties and reduced starch retrogradation; as a result, cooking loss of those enriched noodles was similar to that of the wheat noodle control (Song & Yoo, 2017).

Pasta like sheets based on blending pea protein isolate (86% protein) with pea fiber at different ratios (100/0, 90/10, 80/20, 70/30 and 50/50, respectively) was processed using a heat press machine (Muneer et al., 2018). Polymerization and extensibility were most pronounced for the blend made with 100% pea proteins, and both decreased with addition of the fiber. The negative impact of fiber on polymerization can be attributed to 1) high starch content of in fiber fraction (37 g/100g starch) competing with protein (7 g/100 g starch) for water absorption; 2) limited hydration of the blends due to pectic substances in the fiber resulting in less cross linking; and 3) bi-modal size distribution of fiber [small particle (30 µm) and large particles (>150 µm)] vs a more homogenous size distribution of pea protein (around 150 µm). Consequently, increased levels of fiber decreased the β-sheets and increased the nanostructure. As for cooking quality, the water uptake increased, and cooking loss decreased with increased fiber. On the other hand, the lack of strong covalently linked protein network in 100% pea protein pasta resulted in a weak overall pasta structure that facilitates penetration of water and hence starch swelling and significant leaching out of particles during cooking.

4.3. Baked goods

In baked goods different proteins have been used to increase protein content or produce changes in sensory attributes. In gluten-containing sponge cake formulation, increasing the level of pea proteins (85% protein) addition (from 10% to 40%) increased the elastic behavior, water binding capacity and batter stability due to higher gas retention and water retention attributed to foaming and water holding capacities of pea proteins. At microscopic level, pea proteins played the role of a filler resulting in the increase of rheological properties of the dough owing to is emulsifying and foam properties (Assad-

Bustillos et al., 2020; Assad Bustillos, Jonchère, Garnier, Réguerre, & Della Valle, 2020). Lin et al. (2017) formulated an egg-free cake by combining pea protein (80% protein), xanthan gum and mixtures of emulsifier. The eggless cake containing 12.5% pea protein isolates, 0.1% xanthan gum and 1% soy lecithin was found to be the closest formulation to the traditional cakes (control) in terms of specific gravity, crumb color and porosity (Lin, Tay, Yang, Yang, & Li, 2017).

Even though the incorporation of many different types of proteins has been well established in the bakery industry, these ingredients still play an important role in the case of gluten-free baked goods (Mancebo, Rodriguez, & Gómez, 2016; Matos, Sanz, & Rosell, 2014) and pea proteins are not an exception. Adding 17% pea protein (77.85% protein) to gluten-free muffins dough increased both elastic and viscous moduli compared to the control showing a similar effect to that of soy protein isolates and casein. As a result, pea proteins enriched muffins had desirable texture (increased softness and springiness) and aspect (increased yellow index) and similar specific volume compared to the control (Matos et al., 2014). Furthermore, adding 50% of pea proteins to gluten-free rice layer cakes resulted in batter with low density and high quantity of entrapped air resulting in good volume and harder crumb (Gularte, Gómez, & Rosell, 2012). An additional benefit of reducing the estimated glycemic index due the decrease of rapidly digestible starch.

In the case of gluten-free cookies, the addition of 20% pea proteins (80% protein content) modifies the rheology of dough, increasing hydration properties and consistency, and limiting its spreading during baking and those changes result in cookies with low hardness (Mancebo et al., 2016). Similar results were observed in terms of rheological changes for 30% pea protein (89.87% protein) supplemented cookies, but without the detrimental effect on hardness (Sahagún & Gómez, 2018b). Those enriched cookies showed similar sensory scores to the control, except for taste that scored lower. Compared to proteins from different sources (potato, egg white and whey), pea protein enabled the production of cookies appreciated by a consumers panel (Mancebo et al., 2016; Sahagún & Gómez, 2018b).

4.4. Snacks

Pea protein is among the major ingredients used to produce healthier snacks rich in proteins (Arribas et al., 2017; Maskus & Arntfield, 2015). Therefore, understanding the interaction of pea protein with different ingredients (fat, starches, minor cereals and cereals) can provide crucial knowledge to upgrade formulations and processing to produce protein-fortified snacks with a uniform structure and improved quality (Philipp, Emin, Buckow, Silcock, & Oey, 2018). Many different recipes have been reported about the inclusion of pea proteins in this type of food, but only the latest researches are mentioned to show the impact of pea proteins. Extruded snacks made from a blend of pea starch (50%), oat fiber (40%) and pea protein (10%) had high porosity (~76% of the pores among all samples have area within area class <0,2 mm) and brownish color (browning index ranged from 2.9 and 4.4) as well as appreciated texture during sensory tests (Saldanha do Carmo et al., 2019). Extruded snacks made with 13% pea protein level instead of rice flour showed high expansion ratio (6.33 vs 4.12 for control made with rice starch), crispiness, adhesiveness and uniformity and they were perceived with dominant rice flavor. Adding higher amounts, like 30% pea protein, resulted in snacks with non-uniform structure and shrinkage, which can be probably due to an increase in melt viscosity and a subsequent delay in its solidification (Philipp et al., 2018). However, beyond 45%, snacks were described as hard, dense and non-crisp, with an intense pea flavor (Philipp, Buckow, Silcock, & Oey, 2017; Philipp, Oey, Silcock, Beck, & Buckow, 2017). Extrudates containing 20% pea protein isolates exhibited the highest final expansion and no shrinkage was observed (Philipp et al., 2018). However, Beck et al (2018) found that the addition of 25% for pea protein isolate (85% protein) and 16% for pea fiber enhanced the expansion compared to the control (pure rice starch-based snacks). Although changing the blend ratio to 42% pea protein and 24% pea fiber led to low expansion due to the alignment of starch and protein into thin layer as well non fully hydrated fiber during extrusion increasing initial nucleation but following with the rupture of air cells during expansion (Beck et al., 2018). Therefore, up to 42% pea protein have been added to extruded products obtaining diversity of structures, offering an alternative for innovative foods varying the proteins levels and extrusion conditions.

The addition of 20% pea protein isolate (85% protein) to crackers based on dehulled oat flour increased protein content of crackers (24.66 g/100 g cracker) and reduced their hardness (Morales-Polanco, Campos-Vega, Gaytán-Martínez, Enriquez, & Loarca-Piña, 2017). Pea proteins improve air retention and expansion without collapsing during baking owing to their foaming and emulsifying properties resulting in crispy structure.

4.5. Meat products

Processed meat products have been traditionally enriched with a wide spectrum of ingredients (*e.g.* proteins, spices and starch) for their functional, flavoring and texturing properties. Pea proteins have showed good properties for producing processed meat products, although food features can be affected. For instance, the addition of pea protein (3%) increases the hardness of beef patties compared to control due to higher water holding capacity, gelling capacity and emulsion stability, but they have a strong rancid aroma during storage, which it is not present when rice proteins are used, likely because the former inhibits oxidative rancidity and those rice fortified beef patties have softer texture and are more stable during storage (12 days) (Baugreet, Kerry, Botineştean, Allen, & Hamill, 2016). In cooked restructured steaks the inclusion of pea protein isolate (8%) besides enhancing the protein content, increased hardness, chewiness, cohesiveness and gumminess due pea proteins ability to water and fat binding as well as gelling properties; and better when combined with transglutaminase uniform structure (Baugreet, Kerry, Allen, Gallagher, & Hamill, 2018), and high protein *in vitro* digestibility (high free amino acids isoleucine, lysine, phenylalanine and valine) were obtained (Baugreet et al., 2019). Cooked restructured steaks made with pea protein (10%) reduced cooking loss indicating that this ingredient could be useful to retain moisture in the product during cooking owing to its high water holding capacity (Baugreet et al., 2018). Probably pea proteins may form a well-structured protein matrix, or a gel enabled to trap water during cooking thanks to it gelling and water holding properties. Through combining transglutaminase (2%), pea protein isolate (8%), rice protein (9.35%) and lentil flour (4%), the texture of cooked restructured steaks was enhanced while sensory evaluation revealed that this product was less appreciated than the control due to the negative impact of non-meat ingredients on color parameters (darker compared red color control) (Coombs, Holman, Friend, & Hopkins, 2017). Hence, enhancing the visual appearance of raw restructured beef products is also a critical aspect to be considered beside taste and texture (Baugreet et al., 2018).

Chicken nuggets were enriched with pea protein isolates (83% protein) at 12% level raising the protein content (up to 39%) if compared to the control (35%), while pH and ash contents were not affected. In these products, pea protein again decreased cooking loss during cooking. Likely, it can be attributed to the high binding capacity of pea protein resulting in stronger network thereby less cooking loss. However, pea proteins-enriched nuggets showed sensorial issues related to green notes when high amounts (> 9%) of pea protein was used (Shoaib, Sahar, Sameen, Saleem, & Tahir, 2018). Therefore, some additional improvement would be required by exploring the methods for reducing beany or green odors.

Up to now, scientific literature has been reporting the use of pea proteins for increasing the level of proteins in meat products but current trends for replacing animal proteins for plant- based proteins open a range of possibilities, specifically for pea proteins. This application is even more demanding than the enrichment previously mentioned, since emulsifier and viscoelastic properties are required for developing textures resembling those accomplished with animal meat. Actually, there are a number of food products in the market made with a mixture of plant proteins from legumes and cereals, like those going under the brand “Beyond meat” (https://www.beyondmeat.com/products/) that use blends of pea, mung bean, faba bean and brown rice. In this context, the pre and post-processing methodologies previously reported could offer interesting alternatives to tailored made pea proteins for producing plant-based meat products.

4.6. Beverages

When developing beverages fortified with pea protein ingredients, the most critical functional properties are solubility, thermal stability and rheological behaviors of proteins (Lan et al., 2018). Considering those, several beverages have been developed based on fermentation and non-fermented processes.

Non-fermented beverages were developed by dissolving 3% of pea protein (80% protein) and 0.03% carrageenan in nano-filtered water and then subjected to ultra-high temperature processing (UHT). Pea protein based beverages have stronger aroma, which can be associated with the release of compounds deriving from lipid oxidation and the Maillard reaction pathways during the thermal treatment (Trikusuma et al., 2020). Roux et al (2020) found that an infant formula with pea protein and whey protein (50% - 50%) had similar protein hydrolysis degree and amino acid bio-accessibility to that made with 100% whey protein (Roux et al., 2020).

Fermentation as a new "old" process can enhance the quality of pea beverages particularly for the mitigation or masking the presence of off-flavor compounds associated with beany and green notes (El Youssef et al., 2020). Incorporation of 0.5% pea protein isolate in a dairy milk formulation improves protein and amino acid contents (Akin & Ozcan, 2017). It must be considering that during storage, viscosity and amino acid levels could increase, which has been attributed to pea proteins behavior during acidification (Lan et al., 2018; Yin et al., 2015). These beverages have been appreciated for their aroma intensity, appearance and sweetness (Akin & Ozcan, 2017). The emulsification and gelling properties of pea protein contribute into the formation of stable product with adequate rheological properties. The application of yeasts, *Candida catenulate* and *Geotrichum candidum,* triggered the formation of banana and apricot aroma in a cheese-like pea-based product (Ben-Harb et al., 2019). Furthermore, Ben-Harb et al (2020) combined lactic bacteria and yeasts for fermenting three formulations consisting of 100% pea protein, 100% milk protein and a mixture of both (50% - 50%). Nevertheless, fermented 100% pea protein has been described by undesirable aromatic notes (smoked/onion/garlic), while fermented 100% milk protein and 50% pea - 50% milk proteins were characterized by a dairy/cheese aroma.

Similarly, to the trends in meat products, non-dairy beverages are trendy and plant-based beverages, fresh and fermented are a growing market. Pea based milk has been already marketed (https://www.ripplefoods.com/products/), having the same protein content as the dairy milk. Nevertheless, this market is still dominated by nuts, cereals and soy, and the use of pea still incipient could have a long run ahead. Likely, biochemical process leaded by lactic acid bacteria, yeast and enzymes could confer better emulsifying, viscous and creaming properties as well as higher stability lowering syneresis, which could extend pea proteins applications to this range of products. Additionally, it must be stressed that the nutritional quality of plant-based beverages is lower than that of dairy milk (Musa-Veloso & Juana, 2020), and some diseases have been identified in infants with nearly exclusive consumption of plant-based beverages (Vitoria Miñana, 2017).

5. Conclusion

Plant proteins seem like they are taking the market by a storm, yet it is the result of a progressive evolution from marginal to mainstream. Plant protein diet is not anymore, a trend but a lifestyle, for vegetarians, vegan and flexitarians. Protein deficiency, increasing population, sustainability as well as increasing awareness over health and wellness are the main boosters of plant-based market. Anyway, it is still not clear which is the best economical, highly nutritional and environmentally friendly source of proteins. In recent years, public eye was more and more focused on pea proteins as a suitable ingredient to reformulate food and beverages and to maintain target protein intake instead of animal proteins and soy proteins.

Anyway, industry is still facing challenges related with taste, texture, functionality and nutritional properties of pea protein ingredients. Several approaches have been suggested to reduce vegetal notes, including ingredients, process, recipe (increasing sweeteners to reduce the bitterness), adjustment and use of masking agents. The combination of these techniques provides flexibility to fulfil food product requirements and to respond to consumers expectations. Creating portfolio of different proteins (balanced in terms of quality and quantity of proteins) can be the ground stone in tailored plant protein-based products and a way to mask off-notes, enhance the amino-acid composition and obtain the desired texture.

Current research indicates that the interesting functional properties of pea protein ingredients are strongly influenced by extraction (*e.g.* temperature and solvent) and production conditions (*e.g.* temperature and pH). These outcomes underlie the importance of developing functionality-driven extraction and drying technologies to reach target techno-functional and organoleptic attributes. Depending on the type and the level of inclusion, reformulation with pea protein ingredients can enhance the nutritional and technological properties of snacks, cereals-based and meat products, and beverages. However, there is still a lack of knowledge about the complex interactions between pea proteins and the other components of the food matrix (mainly starch, fiber and fat). A better modulation of these interaction as well as designing suitable processes can produce pea protein rich food without hindering the quality of the final product.

Likewise, an incipient market is exploring the healthy benefits of pea proteins, mainly exhibited by the peptides released from pea protein hydrolysis. Nowadays, different bioactivities have been reported but considering the large variety of peptides regarding size and amino acids sequences many of them could still be unexplored.

Declaration of competing interest

The authors declare no competing interests.

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Figure caption

Figure 1: From pea seeds to pea protein ingredients. A. Wet extraction; B. Dry fractionation; C. Mild fractionation. This figure illustrates the steps of processing enabling the obtention of pea proteins with different purity, isolates or pea protein concentrate

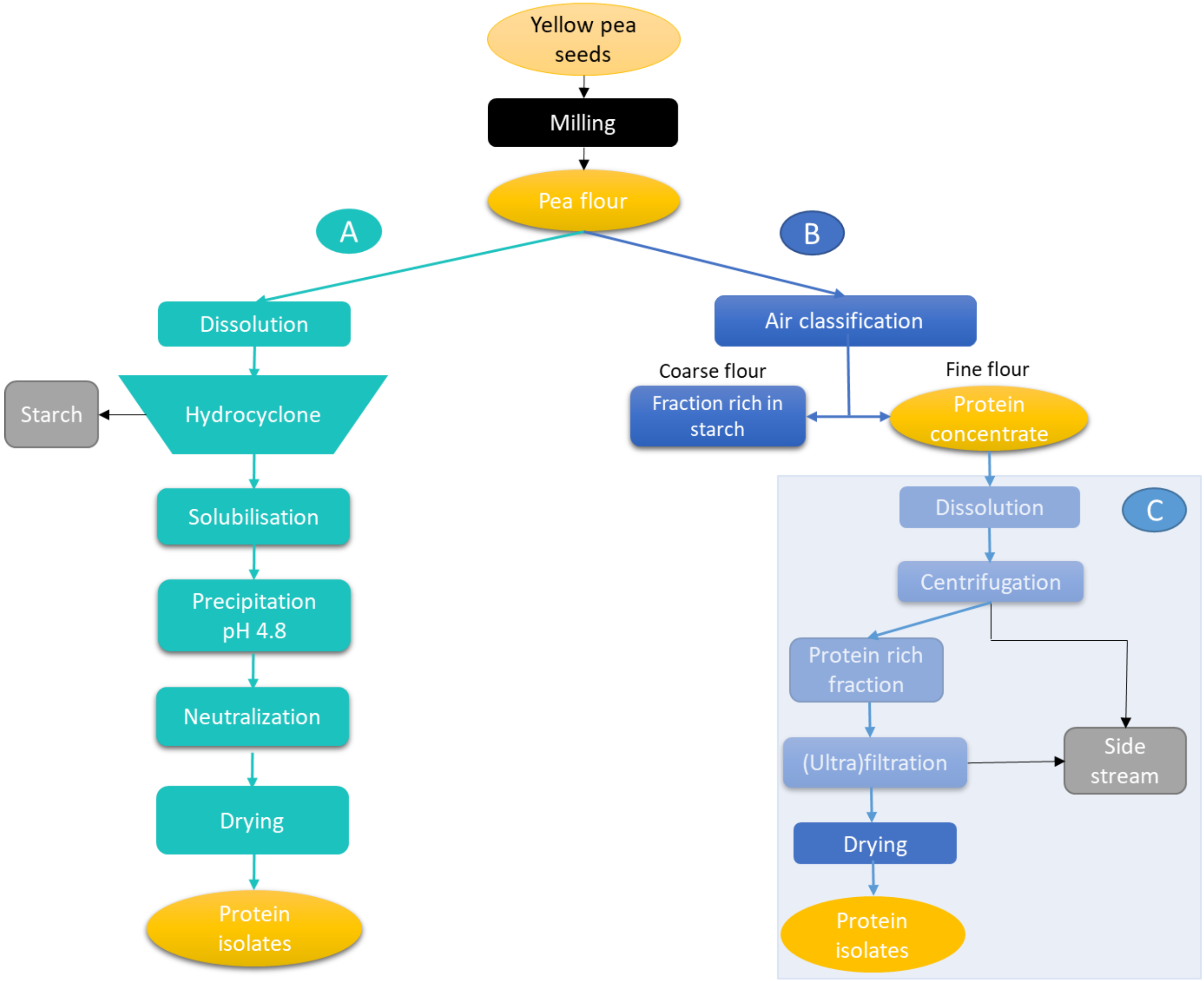
Yellow pea seeds
Milling
Pea flour
A
B
Dissolution
Hydrocyclone
Starch
Solubilisation
Precipitation pH 4.8
Neutralization
Drying
Protein isolates
Air classification
Coarse flour
Fraction rich in starch
Fine flour
Protein concentrate
Dissolution
C
Centrifugation
Protein rich fraction
Side stream
(Ultra)filtration
Drying
Protein isolates

Figure 1: From pea seeds to pea protein ingredients. A. Wet extraction; B. Dry fractionation; C. Mild fractionation (Pelgrom et al., 2015a; Reinkensmeier et al., 2015)**.**

1228 **Table 1: Characteristics of the principal industrial processes to obtain pea protein ingredients**

Characteristics	**Wet extraction**	**Dry fractionation**	**Mild fractionation**
Approach	Solubility	Density and size	Density and size
	Processing		
Number of processing steps	7 (milling+dissolution+precipitation+solubilisation+isoeletric precipitaion+neutralisation+drying)	2 (milling+air classification)	6 (milling+air classification+dissolution+centrifugation+filtration+drying)
Raw material	Dehulled split seeds	Dehulled split seeds	Fine flour obtained from dry fractionation
Chemical use	alkaline and acid solutions	no chemicals	no chemicals
Water use	High	no water	Medium
Energy use	High use of energy	Low use of energy	Medium use of energy
Sustainability	Low	High	Medium
	Product quality		
Product	Protein isolate	Protein concentrate	Protein isolate
Purity (w/dw% protein)	>80	50-75	>75
Protein yield (g/ 100g)	80	77	55–65

Protein form	• loss of the insoluble proteins • partial loss of native form (denaturation due to pH shifts and drying)	• no loss of the insoluble proteins • no loss of the native form of proteins	• no loss of the insoluble proteins • no loss of the native form of proteins
References	(Berghout et al., 2015; Gao et al., 2020)	(Avila Ruiz, Arts, Minor, & Schutyser, 2016; Pelgrom et al., 2015a)	(Avila Ruiz et al., 2016; Geerts et al., 2018)

Table 2: Application of pea protein in food and beverages

Application	Sub-category	Main contributions	Limitations	Potential solution
Bread	Gluten-containing	-increase protein quantity and amino acids (Erben & Osella, 2017; Millar, Barry-Ryan, et al., 2019).	Beyond 15% addition level: gluten dilution→dough weakening→ bread volume decrease+ hard and compact crumb (Hoehnel et al., 2019).	-Low addition (up to 10%) -adding masking agents -adding cross linking enzymes
	Gluten free	-increase protein content and amino acids+increase the viscoelastic properties (Ziobro et al., 2016) -enhance the volatile profile (Pico et al., 2019) -increases crumb porosity and decrease cell density (Espinosa-Ramírez et al., 2018). -enhance digestibility (Sahagún et al., 2020).	-Beyond 10% pea protein isolate→volume reduction (Pico et al., 2019) - 30% addition→ high water holding capacity but accurate water hydration can reduce crumb hardness (Sahagún & Gómez, 2018a).	-pea protein+ transglutaminase →enhance dough network (Marco & Rosell, 2008)+ improve structure , specific volume increase and sensory quality improvement+ mitigate the bitter taste originating from millet (Tomić et al., 2020).
Pasta	Gluten containing	-no effect on texture and sensory perception +enhance digestibility (Wee et al., 2019).		- pea protein isolate +green tea extract →enhance the viscoelastic properties +reduce starch retrogradation and cooking loss (Song & Yoo, 2017).
	Gluten free	-enhance pasta firmness (Linares-García et al., 2019).	-reduce viscoelastic properties (Ribotta et al., 2012). - pea proteins isolate + pea fiber→ increase cooking loss (Muneer et al., 2018).	- pea proteins +transglutaminase →enhance viscoelastic properties +reduce syneresis during storage (Ribotta et al., 2012). - pea proteins isolate

				+ pea fiber→enhance rheological properties (Muneer et al., 2018).
Baked goods	Gluten containing	-increase protein content+ increase the elastic behavior, water binding capacity and batter stability (Assad-Bustillos et al., 2020; Assad Bustillos et al., 2020).		pea protein+ xanthan gum + soy lecithin → substitute the role of egg in eggless cake + enhance specific gravity, crumb color and porosity (Lin et al., 2017).
	Gluten free	Muffins: increase dough viscoelastic properties + increase softness, springiness and aspect yellow index of bread (Matos et al., 2014) Cake: good volume + reduce glycemic index (Gularte et al., 2012).	- cookies: beyond 20% → increase hydration properties and consistency+ limit spreading during baking +reduce hardness + affect taste (Mancebo et al., 2016; Sahagún & Gómez, 2018b).	-Low addition (up to 10%) -adding masking agents -adding cross linking enzymes
Snacks	Extruded snacks	-enhance protein content (Arribas et al., 2017; Maskus & Arntfield, 2015) -increase porosity and brownish color (Saldanha do Carmo et al., 2019). -increase expansion, crispiness, adhesiveness and uniformity and acceptable flavor (Philipp et al., 2018).	Beyond 30% pea protein→ non-uniform structure and shrinkage (Philipp et al., 2018)+ intense pea flavor (Philipp, Buckow, et al., 2017; Philipp, Oey, et al., 2017).	pea protein isolate+ pea fiber)→ enhance expansion (Beck et al., 2018).
	Crackers	- increase protein content + reduce hardness (Morales-Polanco et al.,		

		2017).		
Meat products	Beef patties	- flavoring and texturing properties (Baugreet, Kerry, Botineştean, Allen, & Hamill, 2016).	-increase of hardness + a strong rancid aroma during storage (Baugreet, Kerry, Botineştean, Allen, & Hamill, 2016).	-enhance formulation
	Steaks	enhance protein content -increase hardness, chewiness, cohesiveness and gumminess+ reduce cooking loss (Baugreet et al., 2018)	Dark color (Baugreet et al., 2018).	-Pea protein +transglutaminase →uniform structure +high protein *in vitro* digestibility (S Baugreet et al., 2018; Sephora Baugreet et al., 2019) -pea protein +transglutaminase +rice protein + lentil flour →enhance texture + sensory perception (Coombs et al., 2017).
	Chicken nuggets	-increase protein content +decrease cooking loss during cooking (Shoaib et al., 2018)	Beyond 9% pea protein→ high green notes (Shoaib et al., 2018).	Additional improvement would be required by exploring the methods for reducing beany or green odors.
Beverages	Non-fermented	-enhance protein hydrolysis degree and amino acid bio-accessibility (Roux et al., 2020).	-strong aroma (Trikusuma et al., 2020)	-modulation of thermal treatment
	Fermented	- mitigation or masking the presence of off-flavor compounds associated with beany and green notes (El Youssef et al., 2020) -improve protein and	Beyond 50% pea protein→high off-flavor compounds	-Fermentation -adding masking agents

	amino acid contents (Akin & Ozcan, 2017). -increase viscosity (Lan et al., 2018; Yin et al., 2015). -improve aroma intensity, appearance and sweetness (Akin & Ozcan, 2017; Ben-Harb et al., 2020, 2019)	