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REVIEW ARTICLE

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How can processing technologies boost the application of faba bean (*Vicia faba* L.) proteins in food production?

Fatma Boukid | **Massimo Castellari**

Institute of Agriculture and Food Research and Technology (IRTA), Food Safety and Functionality Programme, Food Industry Area, Catalonia, Spain

Correspondence

Fatma Boukid, Institute of Agriculture and Food Research and Technology (IRTA), Food Safety and Functionality Programme, Food Industry Area, Finca Camps i Armet s/n, 17121 Monells, Catalonia, Spain.
Email: fatma.boukid@irta.cat and fboukid@clonbioeng.com

Present address

Fatma Boukid, ClonBio Group LTD, 6 Fitzwilliam Pl, DublinD02 XE61, Ireland.

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Abstract

Faba bean (*Vicia faba* L.) has attracted increasing interest as a high source of proteins for human nutrition. However, the application of faba bean proteins is still limited due to their low functional properties. Several processing technologies were used to enhance these features starting with the selection of the appropriate pretreatments to favorize protein extractability and to ensure their safety by removing/degrading the antinutritional factors; extraction methods (dry fractionation or wet extraction) and posttreatments to produce multifunctional protein ingredients. In this frame, this review aims to provide a better understanding of the production chain of faba proteins and discuss the impact of processing on protein characteristics based on a recent critical compilation of scientific literature. The different processing applied to faba proteins impacted different degrees of nutritional, functional, sensory, and biological properties. Depending on the final food product, a combination of technologies can be designed to meet specific properties. It must be kept in mind that besides quality, price, sustainability, and scaling up feasibility are relevant factors to consider in the selection of processing.

KEYWORDS

dry fractionation, faba bean, functionality, nutrition, wet extraction

1 | INTRODUCTION

The continuous growth of the world population creates an increasing pressure to meet the protein requirements (Fernandes et al., 2019). The Food and Agriculture Organization (FAO) predicts that meat demand will reach 455 million tonnes to feed 10 billion mouths by 2050. To ensure global food security, intensifying animal production will not be enough to keep up with population growth. This is directing interest toward a variety of alternative protein sources such as fungi, algae, seaweed, duckweed, and plants (Anzani et al., 2020; Boukid, 2021; Calabrese & Ferranti, 2018; Samaei et al., 2020). Plant proteins are in growing demand as alternative sources to meat, egg, and dairy-derived proteins (Dekkers et al., 2018; Di Paola et al., 2017). The intake of plant proteins is also increasingly promoted for several motivations: (i) to reduce the ecological footprint of animal protein production (Alavi et al., 2021); (ii) to increase animal welfare (Alonso et al., 2020); and (iii) to meet protein body needs with fewer calories and cholesterol (Mohamed et al., 2017).

Legumes are important food crops worldwide and represent good protein sources for humans and animals (Hoehnel et al., 2019). The use of legume proteins keeps increasing in the food industry for a multitude of reasons (Boukid et al., 2019; Felix et al., 2018). Legumes proteins are well accepted by the consumers and can be used for the development of a wide spectrum of food products compared with novel sources (Boukid, 2021; El-Sohaimy et al., 2020; Hoehnel et al., 2019). Compared with cereals, legumes have higher protein content (~20%–40% protein) and can deliver distinct nutritional and functional properties depending on the

type and extent of processing as well the interaction that might take place in each food matrix (Drulyte & Orlien, 2019; Felix et al., 2018).

Pulses, the dry family of legumes, have gained particular attention as a source of proteins due to their versatility, sustainability, high nutritional value, affordability, and availability (Boukid et al., 2019; Heusala et al., 2020; Saldanha do Carmo et al., 2020; Yang et al., 2018). Pulses are a wide variety of crops, which can grow in varied climatic zones, and with their nitrogen-fixing ability and soil preservation from degradation (Boukid et al., 2019; Multari et al., 2015). While soy proteins remain the most consumed plant proteins, proteins from pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), faba beans also called broad bean or fava bean (*Vicia faba* L.), or lupine (e.g., *Lupinus albus*, *Lupinus mutabilis* Sweet, *Lupinus luteus* L.) are gaining a relevant position in the market of alternative proteins owing to their high nutritive quality and good techno-functionalities (Boukid, Rosell, et al., 2021; Le Roux et al., 2020; van der Goot et al., 2016).

Among pulses, faba bean is extensively grown in the Mediterranean area, and is used for animal feeding and human food (Felix et al., 2018; Multari et al., 2015; Saldanha do Carmo et al., 2020). Faba beans are constituted by high amounts of carbohydrate (~55%), protein (~25%–30%), minerals (calcium, magnesium, and iron) and vitamins (thiamine, riboflavin, and pyridoxine), and low fat (<1%) (Martinez et al., 2016). As an emerging plant-based source of protein, faba bean has several advantages including (i) its high protein content particularly rich in lysine and threonine (Coda et al., 2017; Le Roux et al., 2020); (ii) easiness of cultivation and ability to grow in different environments and climatic zones (Samaei et al., 2020); (iii) affordability (Samaei et al., 2020); and (iv) beneficial effects on sustainable agroecosystems (Alavi et al., 2021; Boukid et al., 2019; Heusala et al., 2020). As such, the carbon footprint of faba bean proteins was found lower by 80%–90% per kg protein than dairy proteins and four times lower than oat protein thereby less energy consumption (Heusala et al., 2020; Vogelsang-O'Dwyer et al., 2020).

Anyway, the use of faba bean protein in food formulation still presents several challenges mainly attributed to the sensory attributes and the techno-functional properties when compared with animal proteins such as egg and dairy proteins (Nivala et al., 2020). Therefore, several strategies were developed to improve their nutritional, sensory, and techno-functional properties including physical (Saldanha do Carmo et al., 2020; Setia et al., 2019), chemical (Mendowski et al., 2019), and biological treatments (Coda et al., 2015; Nivala et al., 2017; Rizzello et al., 2019; Setia et al., 2020) as well as breeding (Khazaei et al., 2019; Rubiales et al., 2016). These strategies influence different aspects of protein quality and thus their use in the food industry. In this context, this review will address the current knowledge about faba bean proteins to deliver valuable insights on conventional and innovative production technologies, the impact of extraction on protein characteristics, the outcome of poststrategies, and their application in food formulation.

2 | PREPROCESSING OPERATIONS

2.1 | Breeding

In the past, faba beans had limited demand mainly due to the presence of anti-nutritional factors such as pyrimidine glycosides (vicine and convicine), condensed tannins, and protease inhibitors (Khazaei et al., 2017; Purves et al., 2018). Vicine and convicine are known for causing favism (acute hemolytic anemia) in susceptible individuals (suffering from glucose 6 phosphate dehydrogenase deficiency), and the reduction of animal production systems such as the reduction of egg size of chickens (Lessire et al., 2017). Breeding approaches enabled the selection of low/free vicine- and convicine genotypes. Nevertheless, these genotypes result in low yield as vicine and convicine have a beneficial effect on faba seeds due to their defense against fungi and insects (Verni et al., 2017). The faba bean line with low vicine and convicine was first identified in 1980 and used later to breed several genotypes worldwide (Khazaei et al., 2019). Plant breeding also focused on tannins and resulted in low-tannin genotypes having 0.01% tannins compared with genotypes-containing tannin (1%) (Zanotto et al., 2020).

2.2 | Processing technologies

A wide variety of pretreatments processes have been suggested to reduce the antinutritional factors, which can be classified as thermolabile (e.g., protease inhibitors and lectins) and thermostable (e.g., phytic acid, raffinose, tannins, vicine, and convicine) (Martinez et al., 2016; Rizzello et al., 2016; Sozer et al., 2019), and to enhance at the same time the extraction efficiency and the nutritional value of the faba bean protein (Table 1). Pretreatment processes must be tailored to selectively remove the antinutrients and to limit the negative effects on the physicochemical, structural, and nutritional characteristics of the proteins.

Removal of the hulls from faba bean seeds (dehulling) by friction reduces fiber and tannin and is usually the first processing step. Dehulling is generally a dry process but can be also carried out after a soaking phase (wet dehulling) to facilitate the separation of the hulls from the rest of the seed. Dehulling was reported to slightly increase the protein content of extracts (dehulled

TABLE 1 Suggested pretreatments of faba bean

Pretreatment	Pros	Cons	References
Soaking	– Reduce or inhibit antinutritional factors – Improve milling	– Loss of nutrients in the soaking water	Langton et al. (2020); Setia et al. (2019)
Dehulling	– Increase protein recovery – Enhance the color	– Do not significantly improve the techno-functional properties	Saldanha do Carmo et al. (2020).
Defatting	– Increase protein recovery	– Use of solvents	Martínez-Velasco et al. (2018)
Splitting	– Increase protein recovery	– Loss of nutrients	Nosworthy et al. (2017)
Ultrasound	– Improve rheological behavior, solubility, gelling and forming capability	– Decreased in vitro digestibility of protein.	Ouraji et al. (2020).
Microwave	– Improve milling properties of faba beans. – Decrease seeds hardness – Rapid treatment (950W for 1.5 min) inactivates peroxidase and lipoxygenase	– Long treatment (>2 min) reduces faba bean protein solubility and decrease of flour pasting viscosity.	Jiang et al. (2016)
Heat treatment	– Inactivate peroxidase and lipoxygenase – Reduce or inhibit antinutritional factors – Improve the in vitro protein digestibility	– Affect the milling quality, moisture content, protein extraction efficiency and flour pasting quality – Reduce soluble protein	Espinosa et al. (2020); Jiang et al. (2016); Nosworthy et al. (2018); Revilla (2015)
Extrusion	– Increase amino acid digestibility – Decrease the content of anti-nutritive factors.	– Energy consumption	Hejdysz et al. (2016); Nosworthy et al. (2018)
Fermentation using lactic acid bacteria	– Increase free amino acids content – Increase in vitro protein digestibility – Decrease the antinutritional factors concentrations	– Heterogeneity of the outcome	Jakubczyk et al. (2019); Rizzello et al. (2016); Rosa-Sibakov et al. (2018); Verni et al. (2019)
Solid-state fermentation	– Increase γ-aminobutyric acid and total amino acids contents	– Increase tannins	Polanowska et al. (2020)
Germination	– Enhance the functional properties – Enhance the in vitro digestibility of protein	– Did not improve in vitro protein digestibility corrected amino acid scores (IV-PDCAAS) due the drastic reduction of some amino acids such as threonine	Setia et al. (2019)

seeds: 60.9% protein vs. whole seeds: 60% protein) (Saldanha do Carmo et al., 2020). Soaking also removes the thermostable antinutrients soluble in water but it can result in the loss of soluble proteins (Langton et al., 2020; Setia et al., 2019). Following dehulling, seeds can go through splitting to detach the cotyledons from the whole seed to facilitate the milling process. Dehulled seeds can be also defatted but it is less common than soy or other pulses rich in fat as faba bean has low-fat content (<1%) (Vogelsang-O'Dwyer et al., 2020).

Thermal pretreatments can be applied to degrade thermolabile antinutrients and to inactivate the endogenous enzymes originally contained in the faba beans (e.g., lipoxygenase, peroxidase, and peroxygenase), which can trigger the generation of undesirable "beany flavor" from the oxidation of fatty acids such as linoleic and linolenic acids (Espinosa et al., 2020; Jiang et al., 2016; Revilla, 2015). Jiang et al. (2016) proposed microwave heating for pretreatment of faba bean seeds as a suitable step to reduce unpleasant "beany flavor" if compared with untreated beans. A quick microwave treatment (1.5 min at 950 W) was found more efficient in inactivating peroxidase and lipoxygenase compared with oven heating (170°C for 30 min) (Jiang et al., 2016). In addition, microwaving for 1.5 min decreased seed hardness and improved milling quality; whereas over 2 min or longer treatment decreased the protein solubility (Jiang et al., 2016).

Faba flour can be dissolved in water at a ratio of 1:10 (flour: water) and then subjected to ultrasonic waves. This pretreatment improves protein extraction and characteristics, particularly solubility. This can be due to the partial expansion of protein molecules favoring the protein/water interactions (Ouraji et al., 2020). Milling of uncooked beans, extrusion of the resulting flour, and milling of the extrudate decreased antinutrient factors and enhanced protein digestibility of milled extruded flours (Hejdysz et al., 2016; Nosworthy et al., 2018). Baked faba bean flours had a higher protein digestibility-corrected amino acid score (PDCAAS, 66%) than extruded and cooked faba beans (58% and 54%, respectively) (Nosworthy et al., 2018).

Lactic acid fermentation has been used to boost the nutritional profile of faba bean flour (Sozer et al., 2019), by drastically reducing (almost 89%) antinutritional factors (i.e., trypsin inhibitory compounds, phytic acid, tannins, vicine, and convicine) and increasing free amino acids content as well as in vitro digestibility of proteins of fermented faba flour (Jakubczyk et al., 2019; Rizzello et al., 2016; Rosa-Sibakov et al., 2018; Verni et al., 2019). Solid-state fermentation by *Rhizopus oligosporus* of ground faba seeds increased γ-aminobutyric acid and total amino acids contents of the resulting faba bean flours, while the content of tannins increased suggesting that more investigation is needed to define the bioprocessing condition and strains which can improve the nutritional value of fermented faba bean (Polanowska et al., 2020). Setia et al. (2019) demonstrated that germination was a suitable step to enhance the functional properties (emulsion activity and stability, foaming capacity, and foam stability) and the in vitro digestibility of protein of the flours (Setia et al., 2019).

3 | EXTRACTION OF FABA BEAN PROTEINS

3.1 | Wet extraction

The choice of extraction parameters (e.g., solvent, pH, and temperature) is crucial to determining the yield and the characteristics of protein extracts (Langton et al., 2020). Aqueous NaOH solution has been used for a range of plant proteins including pea, oat, chickpea, and lupine (Muranyi et al., 2016; Prosekov et al., 2018; M. Xu et al., 2020). Similarly, faba bean proteins are commonly extracted by alkaline solution followed by isoelectric precipitation (Alavi et al., 2021; Eckert et al., 2019; Langton et al., 2020). Dehulled (and defatted) flour is dissolved in an alkaline solution (pH = ~10.5) and then centrifuged (Ouraji et al., 2020; Singhal et al., 2016). The collected supernatant is acidified to the isoelectric point of faba bean proteins (pH 5.0–5.5) (Langton et al., 2020). The precipitated proteins are recovered after removing fibers and insoluble proteins by centrifugation. Proteins isolates are neutralized, washed with water (to remove any undesirable residue), and froze or spray dried. The protein content of faba bean protein isolates is ~88%–94% and yield varies between 18 and 25 g of protein/100 g of faba bean flour, with a protein extraction efficiency of 55%–77% (Eckert et al., 2019; Singhal et al., 2016). The major advantage of this method is the high extraction efficiency and the degradation of vicine and convicine (Table 2). However, it requires the use of large amounts of water and other chemical compounds such as hexane, HCl, or NaOH (Felix et al., 2018). Additionally, this process generates a large amount of side-stream products and causes the loss of the native structure in the extracted proteins (Eckert et al., 2019).

TABLE 2 Comparison between extraction methods of faba bean proteins

	Alkaline extraction	Acid extraction	Dry fractionation
Protein content %, dry matter (DM)	~88%–94%	90.1%	51%–66%
Protein yield	~18%–25%	~43%	~26%–49%
Protein extraction efficiency	~55%–77%	~21%	~16%–18%
Proteins structure	High impact	Medium impact	Low impact
Protein nutritional aspects	– Remove/reduce antinutritional factors	– Remove/reduce antinutritional factors	– Retain some antinutritional factors
Environmental impact	– High use of energy – Use of solvents – Generation of aqueous wastes	– High use of energy – Use of solvents – Generation of aqueous wastes	– Low use of energy – No use of chemical – No use of water
References	Eckert et al. (2019); Singhal et al. (2016)	Langton et al. (2020); Vogelsang-O'Dwyer et al. (2020)	Coda et al. (2015); Martinez et al. (2016); Saldanha do Carmo et al. (2020); Vogelsang-O'Dwyer et al. (2020).

As an alternative, faba bean protein isolates can be produced by acid extraction/isoelectric precipitation based on the patented method WO2012116703 (Andersen et al., 2012). Faba bean seeds are wet milled under heated acidic conditions and then go through centrifugal sieves to remove fibers and insoluble proteins. The resulting slurry is decanted to separate starch and proteins and finally, proteins are precipitated at pH 4.8. After increasing its pH up to 6.8, the precipitated proteins are dried. The protein content of these isolates is around 90% of dry matter (Vogelsang-O'Dwyer et al., 2020). During this process, the antinutritional components, such as trypsin inhibitor compounds, are drastically reduced, while vicine/convicine are completely removed (Vogelsang-O'Dwyer et al., 2020). Also in this case, although this process facilitates a high protein purity, a relevant amount of water, chemicals, and energy are used (Table 2). Furthermore, the use of heating during the extraction can negatively affect the tecno-functionality of the isolates.

3.2 | Dry fractionation

Dry fractionation comprises two steps, that is, milling and size separation. During milling, the starch granules are detached from the protein bodies (Felix et al., 2019b). Dehulled (and split) faba beans are milled into a very fine flour and then separated based on their density, size, and shape by means of airflow or centrifugation (also called densification) into two outlets: light fine fraction (protein-enriched) and a heavy coarse fraction (starch enriched) (Felix et al., 2018; Vogelsang-O'Dwyer et al., 2020). Protein-rich fractions have a protein content of ~51%–66% d.m., with a maximum protein recovery of ~26%–49% (Coda et al., 2015; Martinez et al., 2016; Saldanha do Carmo et al., 2020; Vogelsang-O'Dwyer et al., 2020). This dry process does not require the use of water or chemicals, thereby producing a low amount of waste (Felix et al., 2019b). As a result, based on life cycle assessment, dry processing was found more eco-friendly than wet extraction (Vogelsang-O'Dwyer et al., 2020). As well, the native structure and functionality are retained almost unaltered due to mildly processing steps (Bühler et al., 2020). On the negative side, the purity of the isolates is lower than that achieved by the wet extraction, and the antinutritional compounds (vicine and convicine) were not completely removed (Felix et al., 2018; Vogelsang-O'Dwyer et al., 2020).

4 | IMPACT OF EXTRACTION METHOD ON FABA BEAN PROTEINS PROPERTIES

4.1 | Structural properties

The storage proteins of faba bean are mainly globulins and albumins followed by prolamins and glutelins (Gürbüz et al., 2018). Globulins are the major storage protein of faba bean seeds (60%–80%) and are categorized into two classes according to their sedimentation coefficient: 11 S legumin (40%–45%, 11 S, M_w 200–500 kDa) and 7 S vicilin (20%–25%, 7 S, M_w 150 kDa) (Alavi et al., 2021). Legumins have a hexametric structure comprising subunits of 50–60 kDa (each containing acidic and basic polypeptides, ~40 and ~20 kDa, respectively) and contain α and β-chains (Nivala et al., 2017; Zhao et al., 2021). They have large proportions of arginine, glutamic acid, and aspartic acid, but are low in methionine and cysteine (Martinez et al., 2016). Vicins have a trimeric structure comprising 40–70 kDa subunits and are also glycosylated (Martinez et al., 2016). Legumin and vicilin types have a high degree of structural homology (Warsame et al., 2020). Convicilin is classified as 7 S globulin having different amino acid profiles but similar immunological properties to 7 S vicilin (Multari et al., 2015; Nivala et al., 2020; Yang et al., 2018). Albumin (M_w 12.4–13.7 kDa) is mainly regulatory protein being mainly involved in metabolic activities and enzymatic regulations (Warsame et al., 2020).

Regarding the impact of the extraction method on the structure of faba bean proteins, the results of electrophoresis suggest that the alkaline extracted proteins are mainly composed of globulin proteins 11 S and 7 S, while in those dry fractionated and acid extracted, three bands are observed ~68, ~59, and ~51 kDa corresponding to convicilin, legumin, and vicilin (Eckert et al., 2019; Vogelsang-O'Dwyer et al., 2020). This can be attributed to a potential dissociation of legumin into its acidic and basic subunits under reducing conditions. Surface charge and hydrophobicity of concentrates from dry fractionation were lower than those wet extracted due to the preservation of the native globular structure of proteins, while heating and pH lead to protein denaturation and more exposure of hydrophobic regions in wet extractions (Martinez et al., 2016; Singhal et al., 2016; Vogelsang-O'Dwyer et al., 2020). Dry fractionated may have maintained a more native protein structure due to the milder conditions of dry fractionation compared with alkaline extraction. Besides processing, the ratio of legumin/vicine depended on genotype and environmental conditions (Martinez et al., 2016; Singhal et al., 2016).

4.2 | Nutritional properties and health aspects of faba bean proteins

The main nutritional parameters of faba bean proteins as a function of the extraction method are shown in Table 3. The protein content of isolates (obtained from alkaline and acid extraction) ranged from 88% to 94% (Eckert et al., 2019; Singhal et al., 2016). Faba bean protein concentrates (obtained from dry fractionation) had lower proteins (~51%–69%) and higher total carbohydrates (~23%–38%) and ash content (~4%–5%) confirming that wet extraction was more effective protein

TABLE 3 Nutritional composition of faba bean proteins as a function of extraction method

Nutritional composition (g/100 g)	Proteins concentrates (dry fractionation)[a]	Protein isolates (acid extraction)[b]	Protein isolates (alkaline extraction)[c]
Moisture	8-12	6	-
Protein	51–69	90	88–94
Fat	2–3	4	0.1
Ash	4–5	5	2.9–5
Total carbohydrate	23–38	0.34	-
Starch	7–23	2	-
Fiber	10	-	2

[a]Dry fractionation (Coda et al., 2015; Vogelsang-O'Dwyer et al., 2020).
[b]Acid extraction (Vogelsang-O'Dwyer et al., 2020).
[c]Alkaline extraction (Eckert et al., 2019; Singhal et al., 2016).

purification (Coda et al., 2015; Vogelsang-O'Dwyer et al., 2020). On the other hand, nonprotein components such as starch and fiber can play a relevant techno-functional roles (thickeners, emulsifiers, and gelling agents) in food formulation. Fat content in protein concentrates was lower than isolates obtained from acid extraction (processing resulting in fat concentration) contrary to isolates obtained from alkaline extraction (less than 0.1%) (Eckert et al., 2019; Vogelsang-O'Dwyer et al., 2020). Compositional properties can vary as a function of the initial content of protein in faba bean genotype and its intrinsic properties that might impact the protein extractability (Martinez et al., 2016; Vogelsang-O'Dwyer et al., 2020).

Extraction processing also influences the amino acid profile of the faba bean protein isolates and concentrates (Table 4). There were slight differences between the three processing, except for cysteine (almost the double in alkaline extraction compared with that in the other two methods) and tryptophan (alkaline extraction almost half the amount of the other two methods). With respect to the requirement of FAO/WHO for adults (WHO/FAO/UNU, 2007), the amount of essential amino acids (i.e., the essential amino acid content to the total amino acid content ratio is higher than 36) is higher except a deficiency in methionine (lower than 1.7 g/100 g protein) as reported in several studies (Coda et al., 2015; Samaei et al., 2020; Vogelsang-O'Dwyer et al., 2020). Isolates from alkaline extraction had slightly higher total amino acids content than those obtained by acid extraction and dry extraction. All proteins have the essential amino acid content to the total amino acid content ratio of over 36% as recommended by FAO. Regardless of the extraction process, faba bean protein (isolates of concentrates) maintained an acceptable quality. PDCAAS (protein digestibility-corrected amino acid score) of faba beans (0.63–0.68) is lower than pea proteins (PDCAAS = 0.73) and soy protein isolates (PDCAAS = 1.0) (Espinosa-Ramírez & Serna-Saldívar, 2019; Nosworthy et al., 2017; Tavano et al., 2016). This suggests potential blends of faba bean protein (deficient in sulfur amino acids but rich in lysine and threonine) with other cereals (deficient in lysine and threonine) to compensate for the deficiency in sulfur amino acids and result in a blend with improved amino acids profile. For instance, cereals such as wheat have a low PDCAAS (48) but when mixed with beans (PDCASS = 79) resulted in a PDCAAS of 89.

4.3 | Technofunctional properties

Technofunctional properties as a function of extraction processing are summarized in Table 5.

4.3.1 | Solubility

Protein solubility depends on extrinsic (e.g., pH, temperature, and ionic strength) and intrinsic factors (amino acid composition and distribution, molecular flexibility, and surface charge) (Eckert et al., 2019; Lam et al., 2018; Martinez et al., 2016). Regardless of the extraction proteins, the solubility of faba bean proteins is minimum at pH 4–5 due to the lack of electric charge promoting hydrophobic aggregation and precipitation, while it is maximum at pH 10–11 due to the increased protein surface aggregation (Eckert et al., 2019; Martinez et al., 2016; Vogelsang-O'Dwyer et al., 2020).

At neutral pH, faba bean protein concentrates (dry fractionation) show a good protein solubility (~82% and 88%), in the same range of pea (92%) and soy (85%) protein isolates (Martinez et al., 2016) and higher than that observed in acid (32%–52%) or alkaline wet extracts (24.7%) (Bühler et al., 2020; Vogelsang-O'Dwyer et al., 2020). Several authors demonstrated that alkaline extraction generates protein aggregates with compact structure and thereby low solubility at neutral pH (Eckert et al., 2019; Langton et al., 2020), while dry fractionated proteins retain their native structure and do not form insoluble aggregates like those observed in alkaline extracted isolates (Eckert et al., 2019; Martinez et al., 2016; Vogelsang-O'Dwyer et al., 2020). This suggests that mild fractionation could enable the sustainable production of ingredient that can be used as functional substitutes for soy or pea protein isolates.

4.3.2 | Foaming properties

The foam capacity of faba bean proteins concentrates by dry fractionation (~140%) is within the same range of soy isolates (137%), but lower than that of pea

TABLE 4 Amino acid profiles of faba bean protein isolates and concentrates obtained from dry fractionation, acid and alkaline extractions expressed as g/100 g protein (N×6.25)

Amino Acid	Protein isolates (alkaline extraction)	Protein isolates (acid extraction)	Proteins concentrates (dry fractionation)	FAO standard (adult)
Essential amino acids				
Histidine	2.48	2.49	2.39	1.6
Isoleucine	4.81	4.25	3.73	1.3
Leucine	8.34	8.09	7.10	1.9
Lysine	5.83	6.51	6.34	1.6
Methionine	0.67	0.54	0.60	1.7
Phenylalanine	4.95	4.68	4.13	1.9
Threonine	4.11	3.30	3.54	0.9
Tryptophan	0.3	0.74	0.69	
Valine	5.93	4.59	4.14	1.3
Nonessential amino acids				
Aspartic acid	11.51	11.18	10.30	
Glutamic acid	15.13	17.96	16.25	
Alanine	5.58	3.94	3.85	
Arginine	7.81	10.09	10.48	
Glycine	7.16	4.02	3.81	
Proline	5.52	4.45	4.24	
Serine	6.54	5.36	4.87	
Cysteine	1.21	0.62	0.77	
Tyrosine	2.96	3.74	3.05	
ΣNEAA	63.42	61.36	57.62	
ΣAA	100.84	96.55	90.28	
ΣEAA/ΣAA	37.11	36.45	36.18	36
References	Eckert et al. (2019); Singhal et al. (2016)	Vogelsang-O'Dwyer et al. (2020)	Coda et al. (2015); Vogelsang-O'Dwyer et al. (2020)	WHO/FAO/UNU (2007)

Note: ΣEAA sum of all essential amino acids, ΣNEAA sum of all nonessential amino acids, ΣAA sum of all amino acids, ΣEAA/ΣAA: the essential amino acid content to the total amino acid content ratio.

TABLE 5 Technofunctional properties of faba bean protein isolates and concentrates obtained from dry fractionation acid and alkaline extractions (Eckert et al., 2019; Johnston et al., 2015; Karaca et al., 2011; Martinez et al., 2016; Vogelsang-O'Dwyer et al., 2020)

Technofunctional properties	Protein isolates (alkaline extraction)	Protein isolates (acid extraction)	Proteins concentrates (dry fractionation)
Solubility at pH = 7, 22°C	24.7	32–52	82–88
Foam capacity (%) at pH 7°C and 22°C	77	15–30	140
Foam stability (%) at pH 7°C and 22°C	64	90	73–85
Emulsion capacity (g oil/g protein), pH= 7	513	487	
Emulsion stability (%)	92	11	83
Oil holding capacity (g/g)	1.2	0.87	1.2
Water holding capacities (g/g)	0.5	-	0.5

(208%) and lentil proteins (414%) (Jarpa-Parra et al., 2015; Lam et al., 2017; Martinez et al., 2016). Vogelsang-O'Dwyer et al. (2020) reported that the foaming capacity of dry fractioned faba bean proteins was higher than that of acid extracted, whereas foam stability was similar for both ingredients (Vogelsang-O'Dwyer et al., 2020). The foam stability of the faba bean protein concentrates (73%) was similar to that of the soy protein concentrates (70%) and higher than that of faba bean isolates (64%) and pea (63%) protein concentrates (Martinez et al., 2016).

4.3.3 | Emulsification properties

Several studies reported the good emulsion capacities of faba bean proteins at different pH values (3.0, 5.0, and 8.0) (Felix et al., 2018, 2019a; Gumus et al., 2017; Yang et al., 2018). The emulsion stability of dry fractionated faba bean proteins (~83%) was found significantly lower than that faba bean/pea (92%) and soy (89%) (Martinez et al., 2016). The oil-holding capacity of dry fractionated and alkaline extracted faba bean proteins was found similar (1.2 g/g) and within the same range of other plant proteins (pea [1.1 g/g] and soy [1.3 g/g]) (Martinez et al., 2016). Water hydration capacity of dry fractionated faba bean protein (0.5 g/g) and alkaline extracted (0.9 g/g) was reported very low compared with soy (5.0 g/g) (Martinez et al., 2016).

4.3.4 | Gelling properties

Gelling ability of dry-fractionated proteins was found higher compared with acid extracted proteins due to the presence of carbohydrates. Langton et al. (2020) reported that the extraction method did not affect gelation properties because pH was more determinant. Gels formed at pH 7 by alkaline-extracted proteins had a more dense and fine network structure compared with gels formed at pH 5 (Langton et al., 2020).

5 | POSTPROCESSING OF FABA BEAN PROTEINS

5.1 | Biological treatments

As summarized in Table 6, enzymatic modification (hydrolysis or crosslinking) is a mild, safe and low-cost processing applied for improving the biological, nutritional, and technological properties of faba bean proteins (isolates and concentrates). Proteolytic treatments induce the breakdown of the primary sequence of the proteins resulting in protein molecular weight reduction. Protein hydrolysis has advantages at three levels: biological, nutritional, and functional. First, these reactions can improve the biological properties of protein hydrolysates through the increase in the release of bioactive peptides with health beneficial effects. For instance, pepsin

TABLE 6 Strategies for improving faba bean proteins

Posttreatment	Pros	Cons	References
Enzymatic hydrolysis	– Mild processing conditions, easy control of reaction, and minimal by-products formation – Improve functional properties (solubility, foaming capacity, oil holding capacity and emulsifying capacity) – Produce bioactive amino acids and peptides.	– Formation of peptides associated with off-flavor	Eckert et al. (2019)
Enzymatic crosslinking	– Improve lipid oxidative stability in emulsion.	– Reduce solubility, emulsifying activity and physical stability of emulsion. – Prolonged treatment accelerate protein oxidation	Liu et al. (2019b); Nivala et al. (2017)
Enzymatic crosslinking-heat treatment/ acidification	– Improve solubility, gelling, and emulsifying properties – Increase water holding capacity	– Form heterogeneous gel	Nivala et al. (2020).
Fermentation	– Decrease vicine and convicine contents, trypsin inhibitor activity and condensed tannins – Increase the amount of free amino acids – Increase essential amino acids and γ-aminobutyric acid – Enhance the in vitro protein digestibility – Lower the hydrolysis index	– Difficult standardization of the process	Coda et al. (2015)

treatment of a faba bean protein extract significantly increased the antioxidant properties along with angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory activities (Ali, 2019; Felix et al., 2019a; Samaei et al., 2020). Proteolysis may also modify the nutritional properties by removing or reducing anti-nutritional compounds such as phytic acid, vicine, and convicine (Rosa-Sibakov et al., 2018). A third advantage is the improvement of the functional properties of faba proteins (Liu et al., 2019a; Samaei et al., 2020). At enzymatic treatment at neutral pH with pepsin, trypsin, flavourzyme, alcalase or neutrase significantly increased protein solubility (from 24.4% to 88.8% at pH 7% and 81.0% at pH 5), foaming (from 31.2% to 122.2% at pH 5 and 66.7% to 131.2% at pH 7) and oil-holding capacities (from 6.12 to 8.21 g/g) (Eckert et al., 2019; Samaei et al., 2020). Eckert et al. (2019) found that specific fractions [I (Mw > 10 kDa) and II (Mw: 5–10 kDa)] separated by ultrafiltration from hydrolyzed faba bean protein isolates, could improve emulsifying, foaming, and oil holding capacities (Eckert et al., 2019).

Additionally, Alcalase hydrolysis of a faba bean protein isolate increased the physical and oxidative stability of oil/water emulsions and markedly reduced lipid oxidation during storage (Liu et al., 2019a). A potential drawback of hydrolysis could be the formation of peptides associated with the development of off-flavors (Nivala et al., 2017). Nevertheless, the appropriate selection of the proteolytic enzymes and reaction conditions are crucial factors for determining the right degree of hydrolysis (Eckert et al., 2019; Liu et al., 2019a; Samaei et al., 2020).

Enzymatic crosslinking is also a biological option for modifying textural and structural properties of faba bean proteins through the formation of inter- and intramolecular covalent crosslinks. Nivala et al. (2017) investigated enzymatic crosslinking of faba bean proteins by transglutaminase (EC 2.3.2.13) and tyrosinase (EC 1.14.18.1). Results showed that transglutaminase reduced the particle size of proteins and improved colloidal stability and foaming properties. However, the crosslinking caused a reduction in surface hydrophobicity leading to a reduction in solubility (Nivala et al., 2017, 2020). Similarly, tyrosinase reduced the solubility of protein despite its limited crosslinking ability compared with transglutaminase (Nivala et al., 2017). In a recent study, transglutaminase treatments (60 min) of faba bean isolates increased emulsifying activity and physical stability of emulsion and improved lipid oxidative stability in emulsion, whereas prolonged treatments (>120 min) induced excessive surface hydrophobicity and accelerated lipid oxidation in emulsion (Liu et al., 2019b). Thus, these studies showed that crosslinking treatment was not efficient in enhancing solubility and emulsifying properties of the faba bean proteins (Liu et al., 2019b; Nivala et al., 2017). Recently, Nivala et al. (2020) went further investigating different combination of treatments (heating ad acidification) together with transglutaminase. They found that heating (90°C for 5 or 30 min) followed by transglutaminase treatment improved emulsifying properties (increase in surface hydrophobicity) compared with native proteins and cross-linked proteins. The water holding capacity of acid- and TG-induced gels (>98%) was higher than that of native protein-based gel (93%). However, the gel microstructures showed mainly heterogeneously-sized protein aggregates regardless of the treatment (Nivala et al., 2020).

Lactic acid fermentation of flour has been proposed by Jakubczyk et al. (2019) to generate hydrolysates where bioactive peptides with antioxidant, antihypertensive, antimicrobial, and anticarcinogenic activities were identified (Jakubczyk et al., 2019). Fermentation also ensured the decrease of vicine and convicine contents by more than 91% and significantly reduced trypsin inhibitor activity and condensed tannins (by more than 40% in dry processed proteins). Fermentation of faba bean concentrates with lactic bacteria increased the amount of free amino acids, especially those essential and γ-aminobutyric acid, as well as it enhanced in vitro protein digestibility and significantly lowered the hydrolysis index (Coda et al., 2015).

5.2 | Physical treatments

Heat treatments enable structural modifications including the unfolding of the native tertiary structure of the protein, exposure of hydrophobic and sulfhydryl groups leading to the formation of aggregates which could affect protein solubility, emulsion activity, and foaming properties depending on the type of thermal processing, protein concentration, heating temperature and pH (Alavi et al., 2021). Dry heating (75–175°C) of faba bean protein concentrate (dry fractionated) increased water-holding capacity and reduced solubility due to partial denaturation of protein and exposure hydrophobic sites (Bühler et al., 2020). The impact of heating under alkaline pH (11.0) irreversibly disrupted the original aggregates of faba bean isolates, along with the formation of smaller aggregates with higher surface hydrophobicity. The heating under alkaline pH (11) significantly improved the protein solubility at acid and neutral pH values (3.0, 6.0, and 7.0) to more than 90% (Alavi et al., 2021). Regarding emulsifying capacity, both microwave and conventional thermal treatments considerably increased the oxidative stability of emulsions prepared with water-soluble protein extracts. The extent of lipid and protein oxidation was more pronounced in microwaved emulsions compared with those conventionally treated (Gürbüz et al., 2018). This indicates a more continuous propagation of lipid oxidation mechanism due to microwave treatment. The advantage of microwave heating relies on the modification of protein complexes in a fast way, yet the scarce uniformity of heat distribution can make the upscaling more complicated than conventional heating processing (Espinosa et al., 2020). High-pressure homogenization

increased solubility (from 35% to 99% at neutral pH) and foaming capacity (from 91% to 260%) and reduced protein emulsifying properties. These changes can be attributed to the dissociation of large insoluble protein aggregates (>1 μm) into soluble supramolecular aggregates and the increase in surface hydrophobicity (Yang et al., 2018). The use of high-intensity ultrasound improved the foaming properties of faba bean protein isolates, which showed smaller bubble diameter, higher stability, and yield stress as well as lower liquid drainage compared with nontreated samples (Martínez-Velasco et al., 2018). High-intensity ultrasound reduced the particle size of protein dispersions and increased water solubility due to a larger interaction area between protein and water molecules. Overall, these innovative processes can be used strategically to modulate protein aggregation and meet the desired protein functionality properties (Yang et al., 2018).

5.3 | Chemical treatments

Protein functionality can be enhanced through protein–carbohydrate conjugation (Alavi et al., 2021). A recent study reported that chitosan increased the physical stability and oxidative stability of faba bean isolates-containing emulsion due to chitosan's ability to improve the formation of a layer-by-layer interfacial structure. This suggests that the complex chitosan-faba bean protein could be used as a natural emulsifier to design a food matrix with enhanced oxidative stability (Liu et al., 2020). Faba bean protein isolate-maltodextrin conjugate produced stable emulsions and foams and resulted more stable when this complex is heat treated at pH 11.0 (Alavi et al., 2021). Heated conjugates also improved gel-strengthening ability and stability at different pH values (Y. Xu et al., 2018). This can be attributed to the intermolecular protein–dextran interactions formed through the Maillard reaction (Y. Xu et al., 2018; Zhao et al., 2021). Exopolysaccharides were also reported as good texture modifiers of faba bean protein concentrate contributing to the formation of continuous and dense gels (Y. Xu et al., 2019).

6 | APPLICATIONS OF FABA BEAN PROTEIN IN FOOD FORMULATION

Even though multiple publications investigated in the last 5 years the functional and compositional properties of faba bean proteins and how these can be improved, studies focusing on their application in food/drinks formulations are still scarce. In a recent study, bread was reformulated by using alternative plant protein sources including faba bean protein concentrates (61.25% protein) to replace 15% of wheat flour (Hoehnel et al., 2019). The rheological evaluation showed that faba bean protein formed a medium gluten network stronger than that created by pea proteins. Bread formulated with faba bean concentrate had a specific volume of 2.26 ml/g, higher than those observed in samples including lupine (1.98 ml/g) and pea (2.00 ml/g) proteins, but not significantly different from the control (2.55 ml/g). Firmness was similar to that of the control (20.11 N) for both pieces of bread formulated with faba bean (20.11 N) and pea (16.68 N) proteins. This suggests that bread enriched with protein can be developed by using faba bean protein concentrates without hindering the technological quality of the final product (Hoehnel et al., 2019).

Conventional durum wheat pasta enriched with 25% of faba protein concentrate or isolate showed low overall acceptability due to the high firmness which penalizes texture ratings. However, pleasantness and tastiness were not different from the control (Chan et al., 2019). This addition increased protein content and reduced postprandial glycemia and appetite. Pasta enriched with faba bean protein concentrates showed higher resistant starch and dietary fiber compared with those made with isolates and gave lower in vivo glucose response due to the protein–starch network reducing the accessibility of amylase enzymes (Chan et al., 2019).

Infant formulas were reformulated by Le Roux et al. (2020) with a partial substitution (50%) of dairy proteins with faba bean proteins. Products containing faba bean concentrates showed a higher hydrolysis degree (73%) than those containing only whey proteins (50%). Furthermore, PDCAAS of faba bean (76%) formulas was statistically similar to that of formulas containing only whey protein (75%), and higher than that of formulas with pea proteins (67%). So, the authors concluded that faba bean proteins could be a good candidate for partial substitution of whey proteins in infant formulas, but robust in vivo studies are required to confirm such assumptions.

Samaei et al. (2020) included faba bean protein hydrolysates in apple juice formulation; this addition did not generally induce any significant impact on the sensory perception of these products if compared with the controls, except in some cases a bit of sour, bitter or salty taste and increased turbidity (Samaei et al., 2020). Likewise, hydrolyzed faba bean protein was found as an efficient ingredient to reduce egg yolk powder in low-fat mayonnaise formulations (Ouraji et al., 2020). The formulations containing equal compositions of faba bean protein and egg yolk powder (0.375%) and the mix made with 0.5% faba bean protein and 0.25% egg yolk powder were substituted for the conventional formulation.

7 | CONCLUSION

Innovation in animal-free food products is boosting manufacturers to diversify their plant-based protein portfolio as this market is no longer limited to vegan

consumers. In the realm of alternative proteins, faba bean is emerging as an affordable high protein source. Faba bean protein extracts can be obtained by combining different pretreatment, extraction, and functionalization processes, resulting in a broad range of ingredients (protein concentrates and isolates) showing very different compositional and techno-functional characteristics. Protein extraction yield is still a priority, but postextraction technologies are gaining interest due to the possibility to modulate the quality of proteins at nutritional, functional, sensory, and biological levels. This can further increase interest in dry fractionated concentrates as alternatives to protein isolates. Even though faba bean proteins demonstrated interesting characteristics (e.g., the ratio of essential amino acid content to the total amino acid content > 36% as recommended by FAO), functionality is still a limiting factor. Protein posttreatments through biological treatments can enable a dual objective to enhance functionality and bioactivity. Lactic bacteria fermentation is especially promising at the lab level but more research is required to identify the appropriate conditions and to control proteolysis degree for upscaling. Faba bean proteins application in food products compared with animal proteins (e.g., egg white protein and milk proteins) or other more "mature" plant proteins (e.g., soy proteins). Nevertheless, faba bean proteins are increasingly being recognized due to the raising demand for alternative proteins for formulating meat analogs and plant-based milk alternatives.

AUTHOR CONTRIBUTIONS

Fatma Boukid: Conceptualization, investigation, methodology, writing—original draft, writing—review & editing. **Massimo Castellari:** Conceptualization, project administration, writing—review & editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

None declared.

ORCID

Fatma Boukid https://orcid.org/0000-0003-4857-6351

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