

Fava bean (Vicia faba L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color

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Full Title: Fava bean (*Vicia faba L.*) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color

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ABSTRACT: The food industry, along with the consumers is interested in plant-based diet because of its health benefits and environmental sustainability. *Vicia faba* L. (*V. faba*) is a promising source of pulse proteins for the human diet and can yield potential nutritional and functional ingredients *viz.* flours, concentrates and isolates which are relevant for industrial food applications. Different processes produce and functionalize *Vicia faba* ingredients relevant for industrial food applications, along with various alternatives within each unit operation used in their production. Processing modifies functional properties of the ingredients, which can occur by: (i) changing in overall nutritional composition after processing steps, and/or (ii) modifying the structure and conformation of protein and of other components present in the ingredients. Furthermore, *V. faba* limitations due to off-flavor, color, and anti-nutritional factors (ANF) are influenced by ingredient production and processing which play a significant role in their consumer acceptability in foods. This review attempts to elucidate the influence of different ways of processing on the functional, sensory and safety aspects of *V. faba* L. ingredients, highlighting the need for further research to better understand how the food industry could improve their utilization in the market.

1. INTRODUCTION

Interest in plant based nutrition has been steadily growing within the last years, along with the rising concern within groups of consumers, scientists and organizations regarding health and nutritional aspects of sustainable diets (Calles, del Castello, Baratelli, Xipsiti, & Navarro, 2016; Lynch et al., 2018). Plant-based diets containing legumes, whole grains, vegetables, fruits, nuts and seeds are associated with the prevention and management of diseases such as obesity, type 2 diabetes, hypertension, hyperlipidemia and cancer (Tuso, Ismail, Ha, & Bartolotto, 2013; Dinu, Abbate, Gensini, Casini, & Sofi, 2017; McMacken & Shah, 2017). Indeed, such diets trigger mechanisms that promote insulin resistance, a healthy body weight and food microbiome interactions while decreasing the intake of saturated fats, advanced glycation end products, nitrosamines and heme iron (Pasiakos, Agarwal, Lieberman, & Fulgoni, 2015; Mariotti, 2017; McMacken & Shah, 2017; Lynch et al., 2018; Satija & Hu, 2018). In addition to dietary benefits, the production of plant proteins requires less energy and resources when compared to that of animal proteins (Figure 1). For instance, if the consumption of meat continues to be at the same rate, phosphorus reserves could get completely depleted by the use of fertilizers within the next 50-100 years (Lynch et al., 2018).

Fava bean (*V. faba*), also known as faba bean, field bean, horse bean or broad bean, belongs to the *Fabaceae* family and is cultivated as a staple dietary food in cultures from North Africa and Middle East (Multari, Stewart, & Russell, 2015; O'Sullivan & Angra, 2016). This pulse crop can usually grow without irrigation, especially in regions of cold and rainy seasons. The most commonly grown genotypes of *V. faba* are: (a) *V. faba* var. *major*, with large seeds, (b) *V. faba* var. *equine,* with medium-sized seeds and (c) *V. faba* var. *minor,* with small seeds (Karkanis et al., 2018; Etemadi, Hashemi, Barker, Zandvakili, & Liu, 2019).

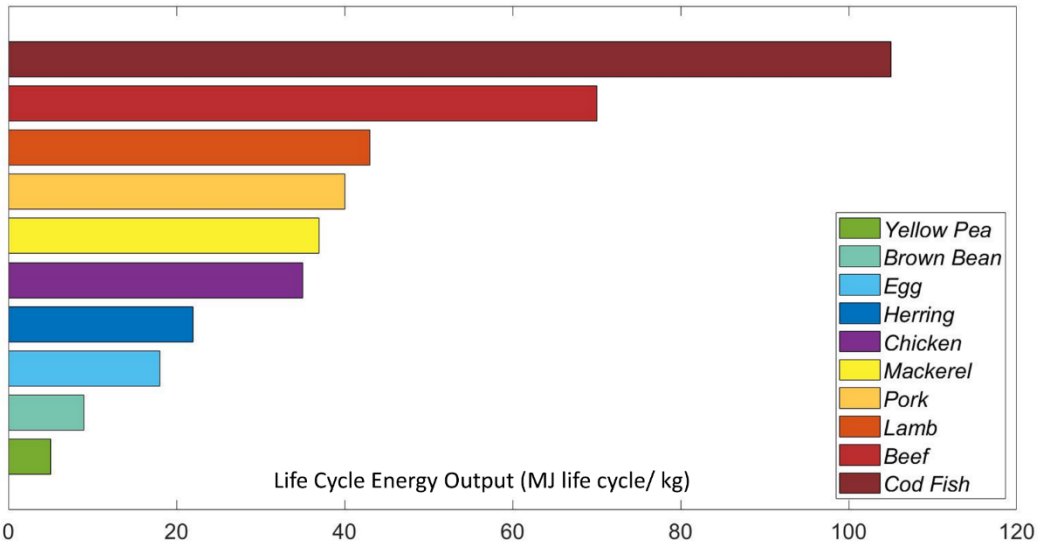


Figure 1 – Sustainability of Plant-Based Sources**:** Life cycle energy outputs for ready to eat proteinaceous foods represented in mega-joules life cycle per kilogram of that food category (Carlsson-Kanyama, Ekström, & Shanahan, 2003)

V. faba is a sustainable protein source with a great potential in nutritional and functional properties (Multari et al., 2015). Given the vast sphere of knowledge available in the literature on the nutritional potential of *V. faba* and its impact by processing, we stress in this review rather on the potential of *V. faba* proteins which determine functional properties of ingredients (e.g. flours, concentrates, isolates) that are relevant for industrial food applications. We draw particular attention to the impact of production and functionalization of such ingredients on their functional properties. Further attention is given to flavor and color, which play a key role in the acceptability of *V. faba* and its ingredients, as well as anti-nutritional factors (ANF) that are specific to *V. faba* and are determinant to safety of *V. faba* and its ingredients.

2. V. FABA: A POTENTIAL PROTEIN SOURCE FOR HUMAN CONSUMPTION

2.1. Agronomy

V. faba is a cool-season grain legume crop, which germinates at soil temperatures as low as 12.5 °C. The crop fixates nitrogen (up to 100-200 $kg{\cdot}N{\cdot}ha^{-1}$), solubilizes insoluble phosphorus and increases microbial activity in the soil, thus improving soil properties such as organic matter content, bulk density, porosity and field capacity. In the last two decades, dry *V. faba* global production has increased from 3.7 to 4.9 million tons. In 2018 (latest year reported), China was the greatest producer of dry *V. faba*, followed by Ethiopia and United Kingdom (Figure 2). A noteworthy agronomic benefit of *V. faba* is its high yield per harvest area. In 2018, for instance, *V. faba* crops had the lowest requirement in harvest area when compared to other pulse crops for a similar or higher yield (Figure 2). While the yield of green pea (*Pisum sativum*) matches that of fava bean, the area of its harvest is five times as much compared to that of *V. faba* (Figure 2).

2.2. Nutrition

Pulses, including *V. faba*, are considered to be a major source of proteins, fibers, vitamins, minerals and compounds possessing antioxidant and anti-carcinogenic properties (Mudryj, Yu, & Aukema, 2014). *V. faba* is nutritionally beneficial owing to its high protein-to-carbohydrate ratio when compared to other pulses (Figure 3), as well as its amino acid profile compared to the adults' requirements for essential amino acids (Table 1). While cereals are rich in cysteine and methionine and limiting in lysine, pulses are rich in lysine and poor in cysteine and methionine, and their dietary intake along with cereals in suitable quantities fulfills the daily requirement of essential amino acids (Asif, Rooney, Ali, & Riaz, 2013; Polak, Phillips, & Campbell, 2015). *V. faba* seeds contain 23-41 %

proteins on dry weight basis (Hove, King, & Hill, 1978; Sjödin, 1982; Patterson, Maskus, & Bassett, 2005). About 80 % by weight of the total seed proteins constitute enzymatically inactive seed storage proteins present in seed cotyledons supplying nutrients to help the seed germinate into a seedling (Shewry, Napier, & Tatham, 1995; Liu et al., 2017). The storage proteins exist as protein bodies that surround larger starch granules inside individual cells within the microstructure of the cotyledon (Figure 4). In particular, nutritional quality of *V. faba* proteins has been studied extensively in the literature (Elsheikh, El Tinay, & Fadul, 1999; Borowska, Giczewska, & Zadernowski, 2003; Vioque, Alaiz, & Girón-Calle, 2012; Millar, Gallagher, Burke, McCarthy, & Barry-Ryan, 2019).

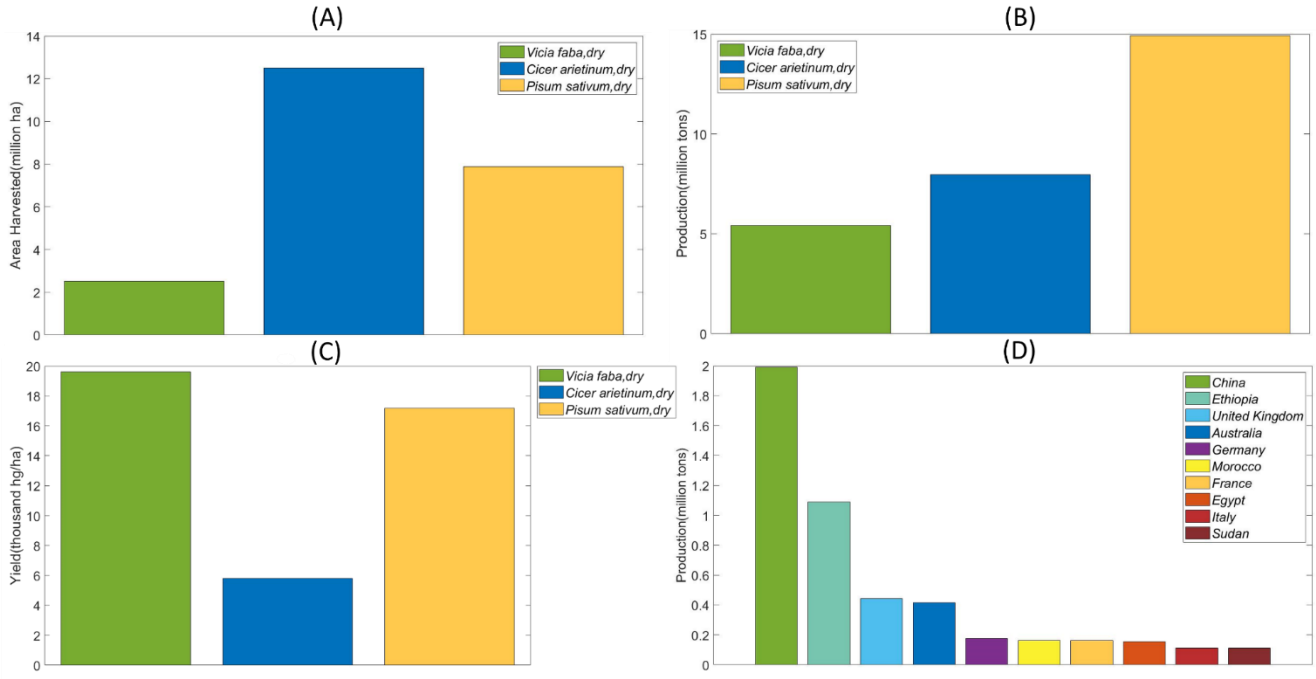


Figure 2 – Agronomic Benefit of V. faba: 2017 statistics of some key pulses including fava bean (Vicia faba), pea (Pisum sativum) and chickpea (Cicer arietinum) on the basis of area utilized for crop harvest (A), crop production (B), and dry crop yield (C) along with V. faba production in top producers in the world (D) (FAO/WHO, 2018).

Table 1 – Relative Amino Acid Levels in *V. faba* seeds and globulins

Amino Acids	Whole Seed[1*]	Whole Seed[2¥]	Legumin[3*]	Vicilin[3*]	Adult Daily

					Requirement[4¥]
Tyrosine	3.5	3.67 - 4.27	2.61	2.59	
Tryptophan	nd	nd	nd	nd	3.8
Phenylalanine	4.5	3.58 - 5.25	3.56	5.20	
Methionine	0.9	0.79 - 1.10	0.59	0.31	2.2
Cysteine	nd	1.10 - 1.42	0.80	0.31	
Lysine	7.1	5.80 - 8.56	4.57	7.13	4.5
Histidine	2.8	2.70 - 4.15	2.44	1.95	1.5
Threonine	4.2	3.76 - 4.39	4.28	3.27	2.3
Valine	5.1	3.75 - 5.64	4.91	4.90	3.9
Isoleucine	4.5	3.29 - 4.64	3.98	5.12	3.0
Leucine	8.4	6.60 - 8.27	7.84	9.21	5.9
Arginine	9.8	8.80 - 12.10	7.95	5.59	
Glycine	5.1	4.15 - 4.93	7.40	5.00	
Alanine	6.6	3.43 - 3.53	6.10	4.87	
Proline	4.7	4.42 - 6.29			
Serine	6.1	4.68 - 6.29	6.50	6.59	
Glutamic acid	14.9	14.20 - 15.89	16.40	15.30	
Aspartic acid	12.0	9.67 - 10.98	10.6	11.6	

Note: Tryptophan was not determined due to analytical challenges and low quantities. In any case, legumin monomer before post translational modification consists of four tryptophan residues whereas vicilin monomer pro-polypeptide has none (Bassuner, van Hai, Jung, Saalbach, & Müntz, 1987; Weschke, Bäumlein, & Wobus, 1987; Heim, Bäumlein, & Wobus, 1994).

*- all the values have been reported as % of total amino acid residues

¥ - all the values have been reported as g amino acid /100g protein

nd - not determined

[1] = Hove et al., 1978, [2] = Makkar et al., 1997, [3] = Jackson, Boutler, & Thurman, 1969, [4] = FAO/WHO, 2007

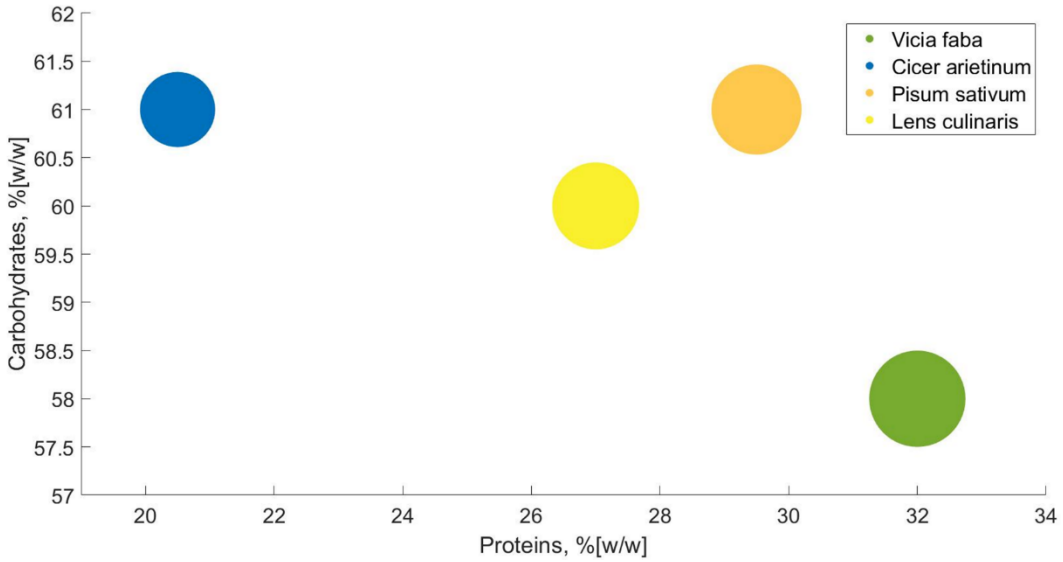


Figure 3 – Nutritional Significance of *V. faba*: Bubble plot of proteins %[w/w] dry weight as a function of carbohydrates %[w/w] dry weight indicating a higher protein to carbohydrate ratio in *Vicia faba* (Hove et al., 1978; Sjödin, 1982; Patterson et al., 2005) compared to *Cicer arietinum* (Bramsnaes & Olsen, 1979; FAO/WHO, 1991; Patterson et al., 2005; Boye et al., 2010), *Pisum sativum* (Hove et al., 1978; Patterson et al., 2005) and *Lens culinaris* (Bhatty, Slinkard, & Sosulski, 1976; FAO/WHO, 1991; Patterson et al., 2005). Dietary intake of higher proportion of proteins compared to carbohydrates has favorable health benefits (Bahadoran, Mirmiran, Hosseini-Esfahabni, Sadeghi, & Azizi, 2013).

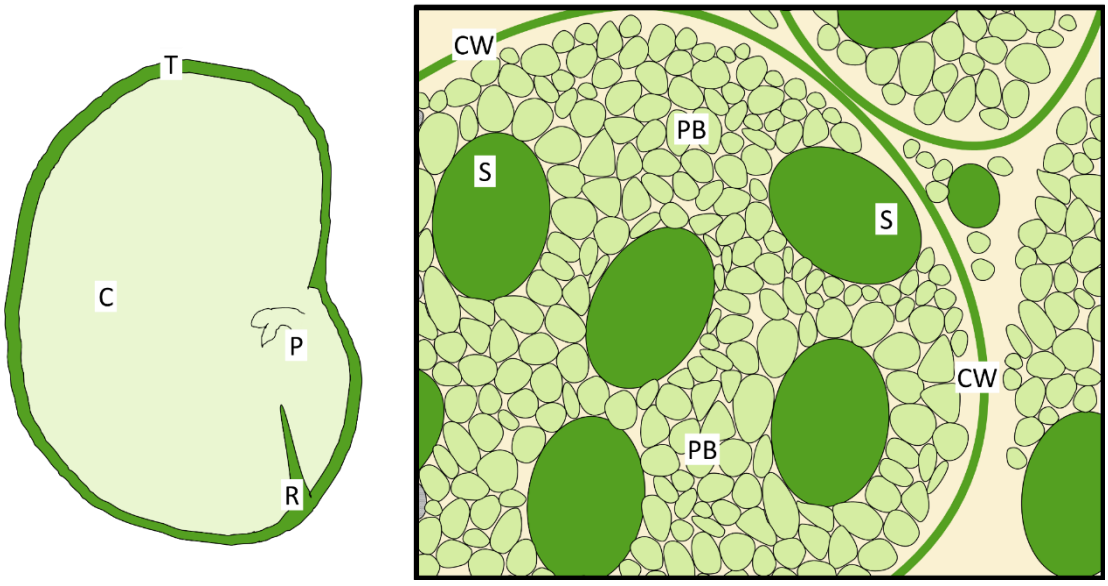


Figure 4 – Protein Localization in *V. faba* Seeds: Illustration of a seed of *V. faba* L. *(Left)* consisting of testa or the seed coat (T), cotyledon (C) containing food reserves mainly starch and protein bodies, plumule or the embryonic shoot (P) and radicle or the embryonic root (R); along with the illustration of the microstructure of the cotyledon comprising of large starch granules (S) of size ranging between 18-23 µm surrounded by smaller protein bodies of size 1-10 µm, together embedded with a cell wall (CW) structure (McEwen, Dronzek, & Bushuk, 1974).

2.3. Functional Properties

Functional properties are a result of physico-chemical phenomena occurring during ingredient or food product storage, processing and consumption. They are driven mainly by protein properties: their hydration in fluids, their surface activity (hydrophobicity, charge distribution) and their structure (primary, secondary, tertiary and quaternary), which together contribute to solubility, wettability, aggregation, interfacial adsorption in colloids (foams and emulsions) and rheological characteristics (viscosity, elasticity, adhesiveness and gelation). Since food products exhibit a multicomponent character, their functionalities should be considered as a result of the interaction of proteins with other constituents, including macro-constituents (lipids, polysaccharides) and micro-constituents (phenolic compounds, phytic acid, saponins, etc.) (Schwenke, 2001; Alu'datt et al., 2013; Mirmoghtadaie, Shojaee Aliabadi, & Hosseini, 2016). These properties can be modified during ingredient production (due to change in composition and the use of process conditions) or by ingredient post-processing (due to the use of process conditions). We henceforth refer to modification of functional properties of ingredients as 'functionalization'.

Functional food ingredients from *V. faba* are potential foaming, emulsifying and gelling agents which can be used for producing dairy and meat alternatives (J. Boye, Zare, & Pletch, 2010; Multari et al., 2015; Singhal, Karaca, Tyler, & Nickerson, 2016). *V. faba* proteins have superior functional properties when compared to animal proteins, and even in comparison to other pulses sources (Sosulski & McCurdy, 1987; Raikos, Neacsu, Russell, & Duthie, 2014). These functional properties of *V. faba* ingredients depend on bean variety (Hood, Cramer, Medrano, & Xu, 2012), protein structural conformation (Saio & Monma, 1993; Husband, Wilde, Clark, Rawel, & Muschiolik, 1994; Lam & Nickerson, 2013; Liu et al., 2017; Felix, Romero, Carrera-Sanchez, & Guerrero, 2019), interactions

with other macromolecules (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014; Assatory, Vitelli, Rajabzadeh, & Legge, 2019) and processing (Sosulski & McCurdy, 1987; Cepeda, Villarán, & Aranguiz, 1998; Luo & Xie, 2013; J. Jiang, Wang, & Xiong, 2018; J. Yang, Liu, Zeng, & Chen, 2018). The effect of processing on functional properties are discussed more in depth in further sections.

2.4. Protein Diversity

V. faba proteins comprise many types of proteins that can be classified based on their solubility in different solvents, namely albumins, globulins, glutelins and prolamins (Shewry et al., 1995).

Globulin Proteins. Amongst seed storage proteins, approximately 85 % by weight consist of salt-soluble proteins called globulins which are rich in aspartic acid, glutamic acid, leucine and arginine (Derbyshire, Wright, & Boulter, 1976; Müntz, Horstmann, & Schlesier, 1999). Globulins are classified based on their sedimentation coefficients ($S_{20.w}$) into 7S proteins (vicilin and convicilin in fava bean, chickpea, green pea and lentil; conglycinin in soybean (*Glycine max)* and β-conglutin in lupin (*Lupinus spp*.)) and 11S proteins (legumin in fava bean, chickpea, green pea and lentil; glycinin in soybean and α-conglutin in lupin), each type being conserved in all other species of legumes (Danielsson, 1949; Shewry et al., 1995; O'Kane, Happe, Vereijken, Gruppen, & Van Boekel, 2004; Singhal et al., 2016). *V. faba* shares a similar amino acid profile of 11S and 7S globulins as compared to other pulses (Table 1). Prolamins are alcohol-soluble proteins devoid of lysine and tryptophan, but rich in leucine, proline and glutamic acid (Multari et al., 2015). They can be solubilized in alcohol/water mixtures (ethanol/water 60/40 or 70/30 [v/v], or propan-1-ol/water 50/50 [v/v]) and contain high levels of glutamine and proline (Shewry et al., 1995). Glutelins are soluble in sodium hydroxide and show an amino acid profile similar to those of prolamines, but with higher levels of

glycine, methionine and histidine (Multari et al., 2015). Albumins contain higher amounts of sulfur-containing amino acids than globulins or other seed proteins (El Fiel, El Tinay, & Elsheikh, 2002).

In *V. faba*, legumin (11S globulin) is a hexameric holoprotein, whereas vicilin (7S globulin) exists as trimers, both made of polymorphic subunits encoded by multigene families. With isoelectric points at pH 4.8 and pH 5.5 respectively, legumin and vicilin can be separated using isoelectric precipitation. In mature fava beans, legumin accounts for 55 % of the total seed protein. Major subunits of fava legumin are of two types: A and B. Legumin A contains methionine residues, whereas type B is methionine-free. The subunits of legumin exhibit heterogeneity in electrophoresis and in ion-exchange chromatography. Four major 60 kDa subunits have been isolated from legumin using ion exchange chromatography in 6 M urea. Two other legumin subunits with 75 kDa and 80 kDa have also been identified (Figure 5). All these subunits comprise α-chains (MW 40 kDa) and β-chains (MW 20 kDa) that are linked by a disulfide bridge. This disulfide bond is formed before the post-translational processing of the αβ precursor chains, and therefore legumin A α-chain would always be exclusively linked to the legumin A β-chain. The formation of the hexameric state is a statistical mixture of different subunits (minor and major subunits) that arrange between themselves to form a functional legumin (Figure 6) and thus possess different chromatographic profiles with heterogeneous molecular weights (Horstmann & Müntz, 1986; Horstmann, Schlesier, Otto, Kostka, & Müntz, 1993; Müntz et al., 1999).

Vicilin represents about 30 % of the storage proteins and convicilin corresponds to 3.2 % of the total seed protein content in *V. faba*. Vicilin and convicilin polypeptides comprise MW 50 kDa and MW 70 kDa subunits, respectively. These subunits are both cysteine-free and are not linked by disulfide

bridges. At pH values below 3 or above 11, vicilin dissociates into two 3S molecules (Bassuner, Hail, Jung, Saalbach, & Muntz, 1987; Sáenz de Miera, Ramos, & Pérez de la Vega, 2008).

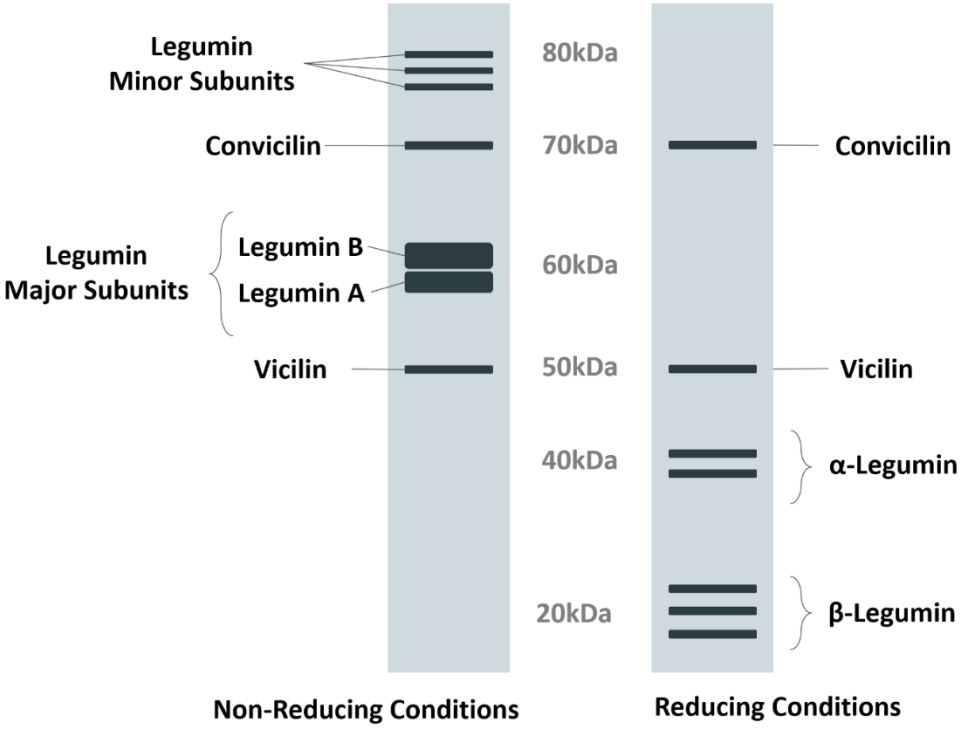


Figure 5 – Subunit Heterogeneity of *V. faba* Globulins: Representation of subunit heterogeneity in the globulins of *V. faba* through 1-dimensional polyacrylamide gel electrophoresis in non-reducing and reducing conditions (Bassuner, Hail, et al., 1987; Sáenz de Miera et al., 2008).

Non-Globulin Proteins. Seed albumins in *V. faba* are primarily metabolic proteins that may or may not have enzymatic functions, including protease inhibitors (*e.g.* trypsin inhibitor), lectins (*e.g.* phytolectin), albumin-2 (PA2), defensins 1 and 2 and Bowman-Birk inhibitors (BBI) (Warsame, O'Sullivan, & Tosi, 2018; Li et al., 2019). Enzymes in the seed regulate the synthesis, transport and storage of starch and proteins during every stage of seed development. Transcripts coding certain enzymes involved in carbohydrate metabolism have been identified in *V. faba* seeds, *viz*. sucrose phosphate synthase (118 kDa), ADP glucose pyrophosphorylase (MW 200-240 kDa), invertase (MW

64 kDa) and glucan phosphorylase (MW 110 kDa) (Weber, Heim, Borisjuk, & Wobus, 1995; Buchner, Borisjuk, & Wobus, 1996; Heim, Weber, & Wobus, 1996). Sucrose-binding proteins (SBPs), which are homologous to vicilin, have also been found in *V. faba* seeds (Müntz et al., 1999). Active transport systems, *viz*. sucrose carriers (*VfSUT1*) and hexose carriers (*VfSTP1*), help in the transport of sugars in different parts of the bean (Weber, Borisjuk, Heim, Sauer, & Wobus, 1997). Another enzyme, phosphoenolpyruvate carboxylase (PEPCase), is found in developing cotyledons that synthesize organic acids which are essential for amino acid synthesis (Golombek, Heim, Horstmann, Wobus, & Weber, 1999). Proton-coupled amino acid and peptide transporters in the cotyledons, such as amino acid permeases (*VfAAP1, VfAAP3* and *VfAAP4*) and peptide transporters (*VfPTR1* and *VfPTR2*), mobilize nitrogen during seed development (Miranda et al., 2001, 2003). Bowman-Birk type serine proteinase inhibitor (MW 7 kDa) has been isolated and characterized in *V. faba* (Ye, Ng, & Rao, 2001). The aquaporin family, which includes tonoplast intrinsic protein (*Vf*TIP1, *Vf*TIP2, *Vf*TIP3), plasma membrane intrinsic proteins (*Vf*PIP2) and nodulin26-like intrinsic protein (*Vf*NIP1), ensures no loss of seed viability by transporting water during seed drying (Novikova et al., 2014). A recent study characterized certain *V. faba* proteins, including elongation factor Tu (43 kDa), citrate synthase (47 kDa), GroEL chaperonins (97 kDa and 52 kDa), phosphate ABC transporter periplasmic substrate-binding protein (36 kDa), electron transfer flavoprotein subunit alpha (31 kDa), alkyl hydroperoxide reductase C22 subunit (21 kDa), motA/TolQ/ExbB proton channel family protein (27 kDa), htlv-1 Gb21 ectodomain maltose-binding protein chimera (49 kDa) and putative sucrose-binding protein (47 kDa) (Liu et al., 2017).

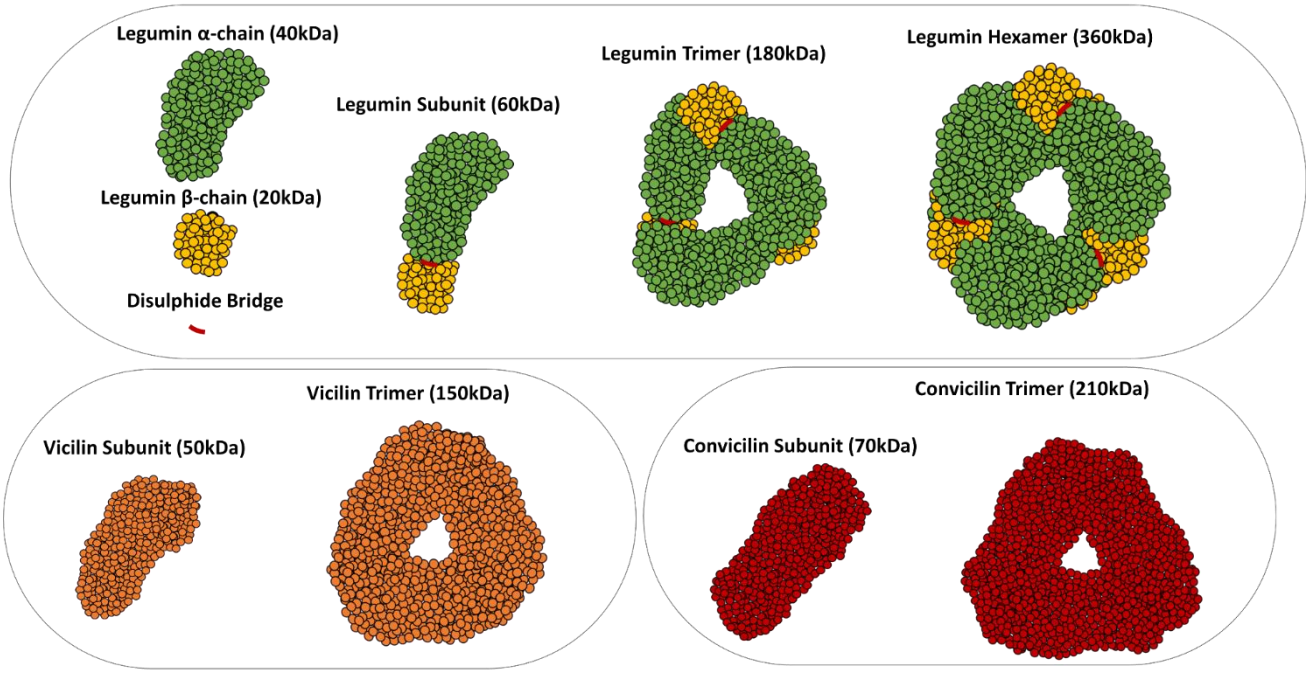


Figure 6 – Native Subunit Arrangement of *V. faba* Globulins: Legumin (MW 360 kDa) forming a hexamer whereas vicilin (MW 150 kDa) and convicilin (MW 210 kDa) forming trimers in their native quaternary conformations. This is hand-drawn from homology modelled protein sequences of *V. faba* to illustrate the protein subunits arrangement. X-ray crystallographic data is yet to be obtained for *V. faba* proteins (Bienert et al., 2017; Waterhouse et al., 2018).

3. *V. FABA*: LIMITATIONS FOR HUMAN CONSUMPTION

Despite the aforementioned potential of *V. faba* in agronomy, nutritional and functional properties, several aspects might hamper its utilization as a source of proteins for the human diet. Certain key safety (anti-nutritional) and sensory (flavor and color) limitations are discussed below. The effect of processing on these aspects are considered in further sections.

3.1. Anti-Nutritional Factors

Concerning protein digestibility and safety, *V. faba* proteins seem to be affected by the presence of ANF, which reduce the bioavailability of proteins and minerals.

Vicine & Convicine. Vicine (2,6-diamino-4,5-hydroxypyramidine-5-[β-D-glucopyranoside]) and convicine (2,4,5-trihydroxy-6-aminopyramidine-5-[β-D-glucopyranoside]) are pyramidine glycosides which are found in the genus *Vicia* and popular ANF associated with *V. faba* beans and ingredients. Dried, dehulled *V. faba* contains 0.73 %[w/w] vicine and 0.30 %[w/w] convicine (Olsen & Andersen, 1978). Hydrolysis of β-glucosidic bonds transforms them into their respective aglycones *viz.* divicine (2,6-diamino-4,5-hydroxypyramidine) from vicine and isouramil (6-amino-2,4,5-trihydroxypyramidine) from convicine. The reaction occurs either by β-glucosidase during the development of the seeds, or by microbial β-glucosidase during consumption and digestion in the large intestine and cecum (Rizzello et al., 2016). These aglycones cause favism, a fatal disease characterized by hemolytic anemia which is common in the Middle East and the Mediterranean basin (Jamalian, 1978; Duc, 1997; Reading et al., 2016). Favism is a life-threatening disease for sensitive individuals having red blood cells (RBCs) with low-activity variants of glucose 6-phosphate dehydrogenase (G6PD). G6PD protects cells from oxidative stress by producing reduced nicotinamide adenine dinucleotide phosphate (NADPH) and regenerating reduced glutathione in the hexose monophosphate shunt. Oxidative stress and resulting phagocytosis are a result of G6PD deficiency. Thus, *V. faba* ingestion causes acute hemolysis as the aglycones produced from vicine and convicine foster oxidative damage in G6PD deficient individuals (Rizzello et al., 2016).

Other Anti-Nutritional Factors. *V. faba* also comprises other factors commonly found in pulses including saponins, glycosides, tannins, alkaloids, phytic acid conjugates and lectins that either reduce digestibility of seeds and/or favor development of certain pathologies (Gupta, 1987; Gupta, Gangoliya, & Singh, 2015). Lectins, a class of glycoproteins, constitute about 2-10 % of total proteins in legume seeds (Gupta, 1987). They reversibly bind to specific sugars and glycoproteins on gut

cellular surface and interfere with the digestion and absorption of nutrients, along with favoring development of food allergies (Vasconcelos & Oliveira, 2004). *V. faba* lectins called favins contain two chains, an α-chain (5.6 kDa) and a β-chain (20 kDa), which are linked to carbohydrate moieties (Hemperly, Hopp, Becker, & Cunningham, 1979). Saponins, on the other hand, could induce erythrocyte hemolysis, enzyme inhibition and affect cholesterol levels, nutrient absorption and growth (Cheeke, 1971; Barakat & Reim, 2015; Multari et al., 2015). Relationship between foaming property of saponins and bloating has been established in rumens (Mangan, 1959). Tannins are water soluble polyphenols which form precipitates with proteins and metal ions, thereby protecting plants against pathogens and rotting by depriving the organisms from metal ions and proteins (Mila, Scalbert, & Expert, 1996). Phytic acids are main storage forms of phosphorus in many plant tissues. They bind with proteins, minerals and starches, forming insoluble complexes and reducing their bioavailability (Bohn, Meyer, & Rasmussen, 2008; Multari et al., 2015).

3.2. Flavor

Consumer acceptance of pulse-based products is hampered by the presence of undesirable flavors (Roland, Pouvreau, Curran, Van De Velde, & De Kok, 2017). Flavor perception is the result of a multimodal combination of stimuli that arise mainly from the interaction of (i) non-volatile taste molecules and (ii) volatile odorant compounds with sapid and/ or olfactory receptors on the tongue and/ or in the nasal cavity (Roland et al., 2017). The flavor of a plant is primarily dependent on its genetic and structural characteristics (*i.e.* the availability and distribution of enzymes and flavor precursors) and both environmental and cultivation aspects (Reineccius & Heath, 2006). Flavor-contributing volatile and non-volatile compounds are either inherent to the grain or produced during the food supply chain including harvesting, processing, and storage (Roland et al., 2017).

Volatile Odorant Compounds. Odorant compounds are organic molecules comprising alcohols, aldehydes, ketones, carboxylic acids, terpenes, sulfur-containing compounds, methoxypyrazines and aromatic hydrocarbons (Murray, Shipton, Whitfield, & Last, 1976; Jeleń & Gracka, 2016; Singh, 2017). Their typical low molecular mass (<300 Da) carbonic chains result in strong hydrophobicity, as well as the ability of volatilizing into the gas phase and reaching olfactory receptors in the nasal cavity (Jeleń & Gracka, 2016; Wang & Arntfield, 2017; Roland et al., 2017). While a minor number of odorant compounds are present in the natural state of legume grains, as is the case for the highly odorant 3-alkyl-2-methoxypyrazines (mainly isobutyl, isopropyl and *sec*-butyl) (Murray & Whitfield, 1975; Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998), the majority of odor-active volatiles arise from the degradation of non-volatile precursors such as lipids, amino acids, carbohydrates and carotenoids during harvesting, post-harvest processing and storage (MacLeod, Ames, & Betz, 1988; Reineccius & Heath, 2006; Roland et al., 2017).

Lipid oxidation *via* enzymatic and/or non-enzymatic pathways is deemed the primary source of flavor-related molecules in pulse ingredients (Sessa & Rackis, 1977; MacLeod et al., 1988; Reineccius & Heath, 2006). Enzymatic lipid oxidation involves the action of lipoxygenase (LOX) on free or esterified fatty acids, whereas non-enzymatic lipid oxidation comprises free-radical chain reactions that occur in the presence of molecular oxygen and initiators such as light, photosensitizers (*e.g.* chlorophylls), metallic ions (mainly Fe^{2+} and Cu^{2+}) and/or temperature (Eriksson, 1975; Sessa & Rackis, 1977; Lee, Min, Choe, & Min, 2003; Belitz, Grosch, & Schieberle, 2009; Damodaran & Arora, 2013). *V. faba* contains roughly 1.3-3.2 g/100g of lipids, amongst which 30.7-56.0 % correspond to linoleic acid ($C_{18:2}$, *n*-6), a polyunsaturated fatty acid (PUFA) that is highly susceptible to undergo

oxidative reactions (Duc, Marget, Esnault, Le Guen, & Bastianelli, 1999; Caprioli et al., 2016; Kan et al., 2018; Akkad, Kharraz, Han, House, & Curtis, 2019).

The volatile compounds reported in *V. faba* consist mainly of aldehydes and alcohols, which represent over 60 % of the total composition in volatiles detected in the seeds (Oomah, Razafindrainibe, & Drover, 2014; Akkad et al., 2019). Alcohols and aldehydes are shown to be responsible for the green, grassy and beany notes associated with *V. faba* beans (MacLeod et al., 1988; Mishra, Tripathi, Gupta, & Variyar, 2017; Ong & Liu, 2018). Other compounds that potentially play a role in *V. faba* flavor on account of their low odor thresholds are 3-isopropyl-2-methoxypyrazine (0.004 ppb in water), a naturally-occurring methoxypyrazine associated with pea-like and earthy aromas (Murray & Whitfield, 1975; Czerny et al., 2008), 2-pentylfuran (6 ppb in water), a furan which can impart green, beany and earthy notes (Buttery, Seifert, Guadagni, & Ling, 1971; Sessa & Rackis, 1977; Oomah et al., 2014; Szczygiel, Harte, Strasburg, & Cho, 2017), and limonene (10 ppb in water), a terpenoid characterized by citrus, green and fruity odors (Buttery et al., 1971; Mishra et al., 2017; Akkad et al., 2019). A detailed listing of the volatile compounds detected in the seeds of *V. faba* and their theoretical odors is presented on Table 2.

Table 2 – Volatile Compounds in *V. faba* Seeds

Compounds	Theoretical odor[3]	CAS Registry
Alcohols		
1-heptanol[1,2]	leafy, coconut, herbal, strawberry, chemical, musty, sweet, woody, violet, green	111-70-6
(E,E)-3,5-octadien-2-ol[2]	nd	126869-35-0
1-hexanol[1,2]	oil, alcoholic, ethereal, resin, fusel, sweet, fruity, flower, green	111-27-3
1-nonanol[1,2]	dusty, rose, fat, floral, green, clean, wet, orange, fresh, bitter, oily	143-08-8

1-octanol[1,2]	burnt, orange, rose, waxy, chemical, metal, aldehydic, mushroom, green	111-87-5
1-octen-3-ol[1,2]	raw, fishy, oily, earthy, fungal, chicken, mushroom, green	3391-86-4
1-pentanol[1,2]	oil, balsamic, vanilla, fusel, sweet, balsam	71-41-0
1-propanol[2]	alcoholic, fermented, alcohol, musty, fusel, pungent, peanut	109-78-4
2,3-butandiol[2]	nd	344750-80-7
2-butanol[2]	oily, sweet, wine, apricot	05-11-17
2-ethyl-1-hexanol[1]	mild, oily, sweet, slightly floral	104-76-7
2-methylbutanol[2]	roasted, onion, malt, wine, fruity	137-32-6
2-octen-1-ol[1]	oily, nutty, fatty	18409-17-1
2-pentanol[2]	waxy, stale creamy, chicken fatty	6032-29-7
2-phenylethanol[2]	lilac, rose, rose water, honey, rose flower, floral, spice, bitter, rose	60-12-8
2-phenylpropan-2-ol[1]	Hyacinth	617-94-7
2-propanol[2]	Alcoholic	67-63-0
3-methylbutanol[2]	oil, alcoholic, burnt, whiskey, malt, banana, fusel, fruity	123-51-3
3-octanol[2]	citrus, nut, moss, herbal, earthy, woody, melon, minty, mushroom, spicy	589-98-0
4-ethylcyclohexanol[2]	nd	19781-62-5
benzyl alcohol[2]	berry, balsamic, rose, floral, walnut, sweet, cherry, flower, grapefruit	100-51-6
ethanol[2]	alcoholic, ethereal, medical, sweet	1725-82-2
Aldehydes		
(E)-2-heptenal[1]	soap, vegetable, fat, fresh, fatty, pungent, almond, green	18829-55-5
(E)-2-nonenal[2]	fatty, orange peels	18829-56-6
(E)-2-octenal[1,2]	fatty, walnut, fruity, leaf, green	2548-87-0
2-methylbutanal[2]	cocoa, coffee	96-17-3
2-methylpropanal[2]	nd	78-84-2
3-methylbutanal[2]	peach, sour, chocolate, ethereal, malt, fatty, aldehydic	590-86-3
benzaldehyde[1,2]	cherry, almond, sweet, burnt sugar, sharp, strong, bitter	100-52-7
decanal[1,2]	citrus, soap, orange peel, tallow, waxy, floral, sweet, aldehydic	112-31-2
heptanal[1,2]	citrus, ozone, fat, herbal, fresh, wine, rancid, fatty, aldehydic, green	111-71-7

hexanal[1,2]	leafy, grass, sweaty, tallow, fat, fresh, fatty, fruity, aldehydic, green	66-25-2
nonanal[1,2]	citrus, lime, orange peel, rose, fat, green, fishy, waxy, fatty, grapefruit	124-19-6
octanal[1,2]	lemon, citrus, soap, orange peel, fat, waxy, fatty, aldehydic, green	124-13-0
pentanal[1]	bready, fermented, berry, malt, pungent, fruity, nutty, almond	110-62-3
phenyl acetaldehyde[2]	hyacinth, honey, clover, sweet, hawthorne, cocoa, grapefruit, green, peanut, floral, bitter	122-78-1
p-isopropylbenzaldehyde[2]	spicy, acid, herbal, sharp, oily, cumin, green	27246-91-9
Alkanes		
2,4-dimethyldodecane[2]	nd	6117-99-3
2,6-dimethyldecane[2]	nd	13150-81-7
3,7-dimethylnonane[2]	nd	17302-32-8
3-methyltridecane[2]	nd	6418-41-3
decane[2]	nd	124-18-5
dodecane[1,2]	nd	112-40-3
heptane[1,2]	ethereal, sweet	142-82-5
hexane[2]	nd	110-54-3
nonadecane[2]	nd	629-92-5
nonane[1,2]	nd	111-84-2
octane[1,2]	Gasoline	111-65-9
pentadecane[2]	nd	629-62-9
pentane[2]	nd	109-66-0
tetradecane[2]	nd	90622-46-1
tridecane[1,2]	nd	629-50-5
undecane[1]	nd	1120-21-4
Alkenes		
(E)-3-ethyl-2-methyl-1,3-hexadiene[1,2]	nd	61142-36-7
Aromatic hydrocarbons		
1,2,3-trimethylbenzene[2]	nd	526-73-8
ethylbenzene[1,2]	nd	100-41-4
isopropylbenzene[1]	nd	98-82-8
p-isopropyltoluene[2]	Citrus	13816-33-6
p-xylene[1]	nd	106-42-3
styrene[1]	balsam, gasoline, floral, sweet, plastic	100-42-5
toluene[1,2]	paint, sweet	108-88-3
Esters		
2-ethylhexyl acetate[2]	nd	103-09-3
methyl 3-methylbutanoate[2]	strong, pineapple, apple, fruity	556-24-1
δ-caprolactone[2]	coconut, cream, chocolate	502-44-3
ϒ-caprolactone[2]	coconut, tobacco, coumarin, herbal, sweet	502-44-3

Furans		
2-ethylfuran[1]	earthy, sweet, burnt, malty	3208-16-0
2-pentylfuran[1,2]	butter, green bean, vegetable, earthy, beany, fruity, metallic, green	3777-69-3
4-Methyl-4-vinyldihydro-2(3H)-furanone[2]	nd	nd
dihydro-2(3H)-furanone[2]	caramel, oily, fatty, sweet, creamy	96-48-0
Ketones		
(E,E)-3,5-octadien-2-one[1,2]	fat, fatty, fruit, fruity, grassy, mushroom, green	30086-02-3
(E,Z)-3,5-octadien-2-one[1]	nd	4173-41-5
2,3-octanedione[1]	"warmed-over"	585-25-1
2-butanone[1,2]	ethereal, ether, fruity, acetone, camphor	78-93-3
2-heptanone[1,2]	coconut, soap, herbal, sweet, woody, fruity, spicy, cinnamon	110-43-0
2-nonanone[2]	soap, herbal, fresh, fishy, hot milk, earthy, sweet, soapy, weedy, green	821-55-6
2-octanone[2]	natural, earthy, gasoline, weedy, herbal, woody, bitter, soap	111-13-7
3-hydroxy-2-butanone[2]	butter, cream, milky, fatty, creamy, sweet, dairy, buttery	513-86-0
3-octanone[2]	butter, herbal, resin, fresh, mushroom, sweet, lavender, herb	106-68-3
3-octen-2-one[1]	crushed bug, nut, herbal, earthy, hay, sweet, blueberry, mushroom, spicy	1669-44-9
6-methyl-5-hepten-2-one[1,2]	pepper, apple, mushroom, citrus, musty, rubber, nutty, green, hazelnut, bitter, lemongrass	110-93-0
acetone[1,2]	solvent, apple, pear, ethereal	107-87-9
acetophenone[2]	mimosa, hawthorn, sweet, acacia, almond, pungent, chemical, flower, bitter, must	98-86-2
Organic acids		
2-methylbutanoic acid[2]	sour, sweat, acid, strawberry, roquefort cheese, pungent, cheese	116-53-0
3-methylbutanoic acid[2]	sour, sweat, acid, stinky, sweaty, animal, rancid, tropical, feet, cheese	503-74-2
acetic acid[2]	sour, pungent, sharp, vinegar	64-19-7
hexanoic acid[2]	fatty, sour, sweat, cheese	142-62-1
Terpenes		
D-limonene[2]	mint, lemon, citrus, orange, fresh, sweet	5989-27-5

NOTE: nd – non-determined, [1] = Oomah et al., 2014; [2] = Akkad et al., 2019; [3] = Garg et al., 2017

Non-Volatile Taste Compounds. Non-volatile sapid molecules in legumes are developed essentially during the growing stage. Taste originates from an initial dissolution of non-volatile compounds in the saliva and the stimulation of specialized receptor cells on the tongue and throughout the oral cavity (Reineccius & Heath, 2006; Thomas-Danguin, Barba, Salles, & Guichard, 2016; Roland et al., 2017). Pulses are mainly associated with bitter and astringent tastes, which could be related to the inherent presence of sapid glycosylated compounds such as saponins, isoflavones, flavonols and phenolic acids (MacLeod et al., 1988; Damodaran & Arora, 2013; Roland et al., 2017; Ong & Liu, 2018). Saponins are non-volatile triterpene glycosides composed of non-polar aglycone backbones with one or more sugar moieties (Liener, 1994; Barakat & Reim, 2015). They are distributed in the cells of a wide variety of plants, being particularly noteworthy in pulses, in which they exert foaming properties in aqueous solutions and can impart bitter or metallic tastes and astringency (Liener, 1994). Amongst the saponins identified in *V. faba* seeds are soyasapogenol B (0.020 $mg \cdot g^{-1}$), soyasaponin Bb (0.040 $mg \cdot g^{-1}$), soyasaponin βg and azukisaponin IV (Ayet et al., 1996; Ha et al., 2014; Barakat & Reim, 2015). Soyasaponin βg differs from the Bb type in that it contains a DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) moiety attached to its C_{22}, imparting enhanced bitterness (Heng et al., 2006).

Polyphenols are also known to impart astringent sensations in the mouth by forming insoluble precipitates with salivary proteins (Drewnowski & Gomez-Carneros, 2000; Troszyńska, Amarowicz, Lamparski, Wołejszo, & Baryłko-Pikielna, 2006). Certain phenolic compounds have additionally been reported to trigger bitter taste receptors, as is the case for the aglycones of quercetin and kaempferol, which are the major flavonols accounted for in pulses (Roland et al., 2017). The main phenolic compounds detected in *V. faba* are flavonols (glycosylated derivates of quercetin,

kaempferol, apigenin and myricetin), flavanols (catechin compounds), flavanonols, flavanones, isoflavones (genistein and daidzein), proanthocyanidins (prodelphynidins and procyanidins) and phenolic acids (caffeic, ferulic, p-coumaric, synaptic, fukiic and protocatechuic acids). They are mostly located in the cotyledons of the *V. faba* seeds (Kaufman, Duke, Brielmann, Boik, & Hoyt, 2007; Baginsky et al., 2013; Turco, Ferretti, & Bacchetti, 2016; Abu-Reidah, Arráez-Román, Warad, Fernández-Gutiérrez, & Segura-Carretero, 2017).

3.3. Color

Pigmented pulses including fava bean, chickpea, lentil and common bean (*Phaseolus vulgaris)* are rich sources of phenolic compounds. Seed coats contain tannins as the main phenolic compounds with antioxidant activity (Pereira, Valentão, Pereira, & Andrade, 2009). In *V. faba*, tannins constitute 72-82 % of the total phenolics content in the hull of colored beans. 96 % of the seed tannins are comprised of proanthocyanidins (Nasar-Abbas et al., 2008, 2009). Proanthocyanidins in white- and brown- colored *V. faba* are 0 % and 6 % respectively in seed hulls (Cansfield, Marquardt, & Campbell, 1980). Single recessive genes (*tan tan*) in *V. faba* have been identified responsible for the absence of tannins. Furthermore, zero-tannin lines are devoid of anthocyanin pigments in their flower petals and dark seed coats as a result of blocked flavonoid biosynthetic pathways (Crofts, Evans, & McVetty, 1980; Nasar-Abbas et al., 2008).

Seed color is thus an important factor as for instance, seeds with dark brown testa color are associated with poor acceptability of the seeds in the market. Depending on storage and post-harvest processing of the seeds, the color of freshly-harvested beige seed testa can develop into brown or dark brown color. Temperature, seed moisture content, light and oxygen can cause

discoloration of *V. faba* and other beans (Nasar-Abbas et al., 2008, 2009). The effect of processing on *V. faba* color is discussed in further sections.

4. PROCESSING OF *V. FABA* FOR FOOD APPLICATIONS

Industrially relevant ingredients from *V. faba* - flours, concentrates and isolates, have been extensively studied in the literature. They can be prepared using a combination of various processing methods, along with alternatives to the classically utilized unit operations during the processing and extraction of protein-rich ingredients (Figure 7). These operations can be divided into ingredient production (including size reduction and protein extraction) followed by ingredient functionalization (occuring either during or after ingredient production) and finally ingredient application in foods.

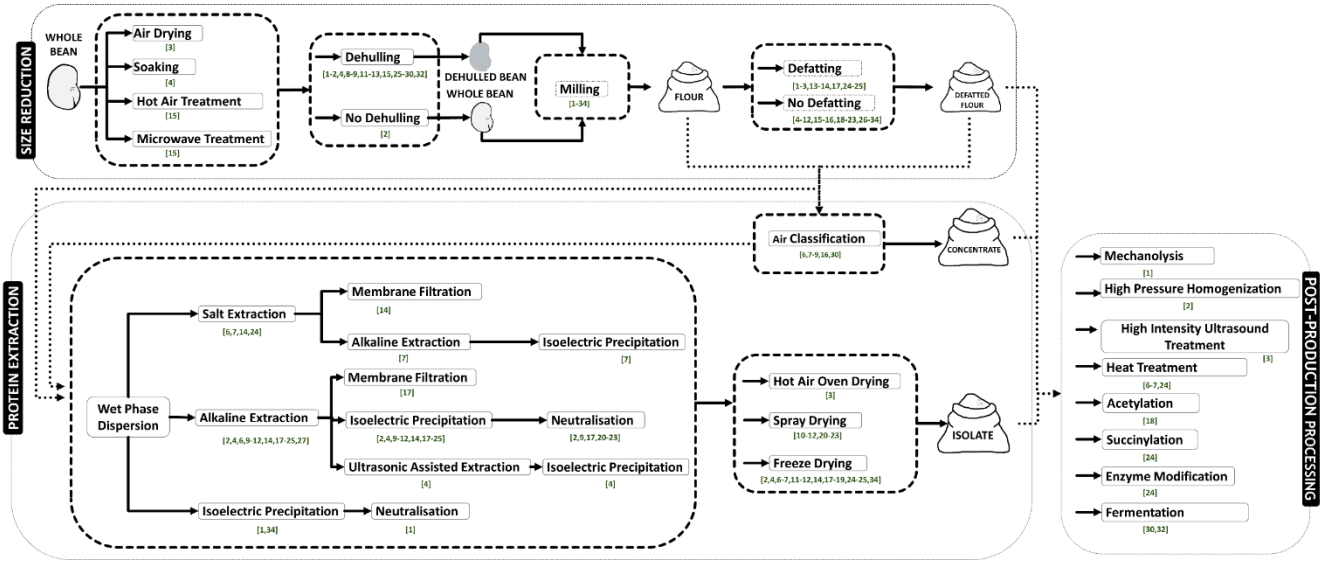


Figure 7 – Production and Processing of *V. faba* Ingredients: Illustration of various methods studied in literature for the production and post-processing of ingredients from *V. faba*. 1 = (Husband et al., 1994); 2 = (Yang et al., 2018), 3 = (Martínez-Velasco et al., 2018); 4 = (Wang et al., 2010); 5 = (Jaddou et al., 1985); 6 = (Arntfield et al., 1985); 7 = (Arntfield & Murray, 1981); 8 = (Sosulski, Hoover, & Tyler, 1985); 9 = (Sosulski & McCurdy, 1987); 10 = (Hartmann & Schmandke, 1988); 11 = (Otegui et al., 1997); 12 = (Cepeda et al., 1998); 13 = (Arogundade et al., 2006); 14 = (Karaca et al., 2011); 15 = (Gürbüz et al., 2018); 16 = (Felix et al., 2018); 17 = (Makri et al., 2005); 18 = (Schmandke et al., 1981); 19 = (Schneider, Schultz, & Schmandke, 1985); 20 = (Schwenke et al., 1991); 21 = (Schwenke, Dudek, Mothes, Raab, & Seifert, 1993); 22 = (Krause, Mothes, & Schwenke, 1996); 23 = (Schwenke, Knopfe, Seifert, Görnitz, & Zirwer, 2001); 24 = (Schwenke et al., 1983); 25 = (Eckert et al., 2019); 26 = (Abo-Bakr, 1987); 27 = (Youssef, 1988); 28 = (Giménez et al., 2012); 29 = (Laleg et al.,

2016); 30 = (Rosa-Sibakov et al., 2016); 31 = (Laleg et al., 2017); 32 = (Rizzello et al., 2017); 33 = (Millar et al., 2017); 34 = (Alu'datt et al., 2017).

4.1. Ingredient Production

Size Reduction. Before *V. faba* beans are milled, they may be air dried (Martínez, Ganesan, Pilosof, & Harte, 2011), soaked (Wang, Li, Ahmed, & Song, 2010), hot-air or microwave treated (Gürbüz et al., 2018). Dehulling of the *V. faba* seeds is an optional step during processing. The pre-treated beans are milled to produce flours, which are optionally defatted, reducing up to 50 % [w/w] of total lipids in the *V. faba* flours (Figure 7). Industrially, a defatting step is discouraged due to the use of solvents including isopropanol and supercritical carbon dioxide (Husband et al., 1994), hexane (Karaca, Low, & Nickerson, 2011) or petroleum ether (Arogundade, Tshay, Shumey, & Manazie, 2006).

Protein Extraction. Protein extraction from pulse flours yields either protein concentrates or protein isolates. Concentrates are prepared using dry or wet processing methods, whereas isolates derive solely from wet processes, all of which are based on unique protein properties of solubility, density, charge or size. By definition, protein concentrates contain 65-90 %[w/w] proteins that are water- or alcohol- soluble, along with sugars, fibers and flavor components (FAO/WHO, 1989; Lusas & Rhee, 1995). Isolates, on the other hand, contain at least 90 % proteins and are devoid of fibers (FAO/WHO, 1989; Lusas & Rhee, 1995). *V. faba* protein concentrates are typically produced using air classification, which separates beans into fractions based on different particle sizes (Andersson, Andersson, & Åman, 2000). Air currents fed into a classifying chamber separate flour based on centrifugal and gravitational forces as a function of size and density, which then generates two main fractions: a fine protein-rich and a coarse starch-rich fraction (Lundgren, 2011). Electrostatic separation is another dry extraction method relying on differences in dielectric properties of particles instead of their size and density. Based on the types and magnitudes of charges, an electric

field can separate protein-rich and carbohydrate-rich particles (Tabtabaei, Vitelli, Rajabzadeh, & Legge, 2017; Assatory et al., 2019). Another process removes sugars using an aqueous ethanol solution, resulting in protein-rich wet flakes that are desolvated and further dried to produce concentrates (Patent No. US4265925A, 1981). Flours and concentrates are then dispersed in a wet phase for the preparation of *V. faba* protein isolates.

V. faba protein isolates are produced with the help of alkaline extraction, isoelectric precipitation, ultrafiltration and salt extraction (Figure 7). Solubilized flours or concentrates can first be adjusted to an alkaline pH (pH 9-11) to remove insolubilized fibers and starch, then proteins are precipitated at their isoelectric point (pH 4-5), washed and reconstituted at neutral pH (pH 6.8) and further dried to yield powders (Schwenke, Anders, Junker, & Schneider, 1991; Krause, Buchheim, & Schwenke, 1996). Protein isolation has other alternatives that are based on conditions other than changing the pH. Membrane-based protein separation is one alternative to acid leaching process; it encompasses micro-filtration (0.1-5.0 μm), ultra-filtration (0.01-0.1 μm), nano-filtration (0.001 μm) and reverse osmosis, which separate and extract components based on molecular sizes (Koros, Ma, & Shimidzu, 1996). Membrane processing provides many advantages over wet processing which modifies and denatures proteins based on pH and heat. Proteins of smaller sizes can be removed using such processes as well, along with phytates and lysinoalanine (Applewhite, 1989)**.** Salt extraction techniques also allow to extract proteins, especially globulins. Among salts, both ammonium sulfate and sodium chloride are commonly used in lab-scale pulse protein extraction. Industrially, proteins are clarified in sodium chloride solution (0.3-0.5 M) at neutral pH to remove insoluble material and precipitated by dilution or by dialysis to lower the ionic strength (Patent No. US4169090, 1979). Removal of salts promotes formation of self-aggregated, non-covalent protein micelles, called the

protein micellar mass (PMM) that grow in size and amount (Parades-López, Ordorica-Falomir, & Olivares-Vázquez, 1991; Sun & Arntfield, 2010). The 7S trimers and 11S *V. faba* hexamers are dissociated using 0.3-0.6 M salt solutions at slightly acidic pH as a soluble fraction, then concentrated and stripped off the salt to 0.2 M to allow formation and precipitation of the PMM. Subsequently, PMM-protein concentration is increased by either ultra-filtration and/or centrifugation and neutralization followed by a drying method (Patent No. US4208323A, 1979; Arntfield, Murray, & Ismond, 1986; Muranyi, Otto, Pickardt, Koehler, & Schweiggert-Weisz, 2013).

Isolate production steps require wet phase dispersion, with addition of salts and/or acids, and hence are followed by neutralization and drying techniques to obtain food-grade powders that are stored and utilized as ingredients for food applications. Drying improves ingredient preservation and transport properties (Fellows, 2009) . Isolates produced by wet extraction are industrially spray-dried, freeze-dried or vacuum dried (Patent No. US4208323A, 1979). For *V. faba* isolates however, only spray-drying and freeze-drying have been noted in literature (Figure 7).

In general, wet processing also consumes large amounts of water and energy and generates acidic by-products, thus raising a concern from a sustainable point of view (Mondor et al., 2009; Schutyser & van der Goot, 2011). Considering the disadvantages of wet extraction, dry extraction processes *viz.* air classification and electrostatic separation, which do not use chemical reagents, can serve as promising alternatives that preserve native structural and functional properties of the components (Tabtabaei et al., 2017; Assatory et al., 2019).

4.2. Ingredient Functionalization

During Ingredient Production. Functional modifications during changes in composition due to processing have been established for *V. faba* flours, concentrates and isolates (Table 3). *V. faba* ingredients show a decrease in aqueous solubility with increasing degree of protein extraction, accompanied by an increase in oil and water holding capacities and oil emulsification capacity (Table 4). It must be noted that, in addition to the degree of protein extraction, there is also an influence of process conditions employed during the protein extraction and prior to extracting proteins, during pre-processing of the beans and flours on resultant ingredient properties. Alternatives to remove fat from *V. faba* have been explored, among which isopropanol defatting increases the presence of α-helices, possibly due to traces of solvent present. Supercritical carbon dioxide assisted defatting does not leave any trace of solvent but still reveals higher levels of α-helix, indicating possible protein modifications in both defatted flours. The latter improves foaming property by 200 % (Husband et al., 1994).

Table 3 – Proximate Composition of *V. faba* Flours, Concentrates and Isolates

Nutrients	Flour	Concentrate	Isolate
Crude Protein	21 – 35	53 – 71	80 – 95
Crude Fat	1 – 2	2 – 5	1 – 2
Crude Fiber	2 – 10	2 - 3	0 – 31
Carbohydrates	35 – 58	8 – 30	2 – 4
Total Ash	2 – 4	5 – 6	3 – 4

NOTE: All values are reported as % [w/w] dry weight basis (Bhatty & Christison, 1984; Sosulski & McCurdy, 1987; Otegui et al., 1997; Vioque et al., 2012; Alu'datt et al., 2017; Coda et al., 2017; Felix, Lopez-Osorio, Romero, & Guerrero, 2018).

Ultrafiltration to isolate *V. faba* proteins yields 94 %[w/w] of proteins (Vose, 1980), which results in foaming and emulsifying properties (Table 4) that are comparable to those from isoelectric precipitation, which yields 91 %[w/w] of proteins (Vose, 1980). On the other hand, *V. faba* isolates from isoelectric precipitation containing 84 %[w/w] proteins have superior emulsifying property and protein solubility when compared to salt extracted isolate with similar protein content, i.e. 82 %[w/w] proteins (Karaca et al., 2011), highlighting the role of process conditions in modifying functionalities.

Table 4 – Effect of Processing on *V. faba* Ingredient Functionalities

Study	Samples	Protein Solubility	Foaming Property	Emulsion Property	Water Holding Property	Oil Holding Property	Gelling Property
Degree of Protein Extraction[1]	Flour	C	C	C	C	C	C
	Concentrate	-	nd	+	+	+	nd
	Isolate	- -	nd	+	+ + + +	+ + + + +	nd
Defatting[2]	Non-Defatted	C	C	C	C	C	C
	Isopropanol Defatted Isolate	nd	+	nd	nd	nd	nd

	Supercritical Carbon dioxide Defatted Isolate	nd	+ + + +	nd	nd	nd	nd
Drying[3,4]	Freeze Dried Isolate	C	C	C	C	C	C
	Spray Dried Isolate	nd	=	+ +	+	nd	nd
Salt Extraction[5]	Acid-Base Extracted Isolate	C	C	C	C	C	C
	Salt Extracted Isolate	-	nd	-	nd	nd	nd
Membrane Extraction[6]	Acid-Base Extracted Isolate	C	C	C	C	C	C
	Ultrafiltration	nd	+	=	nd	nd	nd
Mechanolysis[2]	Untreated Protein Isolate	C	C	C	C	C	C
	Mechanolyzed Isolate	nd	+ + + + +	nd	nd	nd	nd
High Pressure Homogenization[7]	Untreated Protein Isolate	C	C	C	C	C	C
	103MPa Treated Isolate	+ + + +	nd	-	nd	nd	nd
	207MPa Treated Isolate	+ + + +	nd	-	nd	nd	nd
High Intensity Ultrasound Treatment[8]	Untreated Protein Isolate	C	C	C	C	C	C
	High Intensity Ultrasound Treated Isolate	+ +	+ +	nd	nd	nd	nd
Acetylation[9]	Untreated Protein Isolate	C	C	C	C	C	C
	16 % Acetylated Isolate	nd	nd	+	nd	nd	nd
	42 % Acetylated Isolate	nd	nd	+	nd	nd	nd
	78 % Acetylated Isolate	nd	nd	+	nd	nd	nd
	97 % Acetylated Isolate	nd	nd	+ +	nd	nd	nd
Succinylation[10]	Untreated Isolate	C	C	C	C	C	C
	60 % Succinylated Isolate	nd	+	nd	nd	nd	nd

	83 % Succinylated Isolate	nd	+ +	nd	nd	nd	nd
	95 % Succinylated Isolate	nd	+ + + + + +	nd	nd	nd	nd
***Enzyme Hydrolysis*[11]**	Non-Hydrolyzed Protein Isolate	C	C	C	C	C	C
	Pepsin Hydrolyzed	+ + + + +	+	-	nd	+	nd
	Trypsin Hydrolyzed	+ + + +	+	-	nd	+ + + +	nd
	Flavourzyme Hydrolyzed Isolate	+ + + +	+	- -	nd	=	nd
	Neutrase Hydrolyzed Isolate	+ + + +	+	-	nd	-	nd

NOTE: nd – non-determined, C – Control.
0 % Change in Property – ''='';
0-50 % Increase / Decrease in Property – ''+ / -'';
50-100 % Increase / Decrease in Property – ''+ + / - -'';
100-150 % Increase / Decrease in Property – ''+ + + / - - -'';
150-200 % Increase / Decrease in Property – ''+ + + + / - - - -'';
200-250 % Increase / Decrease in Property – ''+ + + + + / - - - - -'';
250-300 % Increase / Decrease in Property – ''+ + + + + + / - - - - - -'';
All % changes are calculated with respect to the control
[1] = Sosulski & McCurdy, 1987; [2] = Husband et al., 1994; [3] = Otegui et al., 1997; [4] = Cepeda et al., 1998; [5] = Karaca et al., 2011; [6] = Makri, Papalamprou, & Doxastakis, 2005; [7] = Yang, Liu, Zeng, & Chen, 2018; [8] = (Martínez-Velasco et al., 2018); [9] = Schmandke et al., 1981; [10] = Schwenke, Rauschal, & Robowsky, 1983; [11] = Eckert et al., 2019

Processing conditions influence protein properties which then reflects in the ingredient functionalities. During pre-processing of beans, size reduction, protein extraction and ingredient preparation, the proteins, along with starch, lipids and other constituents, are exposed to changes in temperature, pH, pressure and salt concentrations (Schwenke, 2001). In *V. faba*, effects of process conditions on protein isolates have been established using thermal denaturation curves. The PMM isolated using sodium chloride and considered as 'native' gave an enthalpy change (ΔH) of 4.39 cal·g^{-1}. 10 %[w/w] aqueous dispersion of this isolate, denatured for 30 minutes at 80 °C (ΔH = 2.59 cal·g^{-1}), 90 °C (ΔH = 0.99 cal·g^{-1}) and 95 °C (ΔH = 0 cal·g^{-1}), showed the effect of temperature in heat

treatment towards the extent of denaturation in *V. faba* globulins. A decrease in enthalpy change was hypothesized to be due to the increase in stability of hydrophobic interactions that unfold during denaturation of proteins (Arntfield, Murray, & Ismond, 1985). A similar effect was presented in a heat treatment of 10 minutes at 75°C, 80°C and 95°C, where complete denaturation was observed in DSC thermograms for 95°C treated protein concentrate and protein isolate from *V. faba* (Arntfield & Murray, 1981). Along with temperature, the effect of pH during protein isolation has been studied. Alkaline extraction at pH 12 followed by isoelectric precipitation at pH 4.5 and freeze-drying yielded isolates with $\Delta H = 0$ cal·g^{-1}, whereas pH 8 alkaline-extracted isolate gave a thermogram with $\Delta H = 1.59$ cal·g^{-1}. When heat treated (95°C for 30 minutes), the latter showed complete denaturation with $\Delta H = 0$ cal·g^{-1}. Isolation using sodium chloride is minimally invasive in the case of *V. faba* proteins ($\Delta H = 4.39$ cal·g^{-1}) when compared to the pH-based protein isolation ($\Delta H = 0$-1.59 cal·g^{-1}) (Arntfield & Murray, 1981; Arntfield et al., 1985, 1986). The effect of pressure on V. *faba* proteins has not been studied to a great extent. *V. faba* protein isolate, under high pressure homogenization of above 103 MPa presented enhanced foaming properties (Table 4).

In sum, the extraction of *V. faba* proteins through wet processing, which is based on salts, pH and/or drying, leads to protein denaturation and either loss or gain in functional property. Alternatives to downstream processing to remove water have been compared, in which the *V. faba* protein isolates produced by spray drying are superior to freeze drying in foaming and emulsifying properties (Figure 7; Table 4).

After Ingredient Production. Ingredients formed by conventional processing methods have been additionally treated by 'post-production processing' techniques, including physical, chemical, enzymatic or fermentative processes to assess changes in their functional properties and to evaluate

their scope in food applications. Broadly speaking, chemical treatment is the most efficient treatment for functionalizing *V. faba* ingredients when compared to physical treatment. Protein solubility is better improved by high pressure homogenization and enzyme hydrolysis than by high intensity ultrasound (HIUS) treatment. Mechanolysis and succinylation improve foaming properties to the greatest extent compared to enzyme hydrolysis or HUIS treatment. Only acetylation enhances emulsifying properties while high pressure homogenization and enzyme hydrolysis both impair emulsification (Table 4).

In addition to the type of post-production processing, the extent of processing also has an effect on functional properties. Increase of the extent of succinylation and acetylation increases foaming and emulsifying properties, respectively. Conversely, higher pressure during homogenization does not further impact functional properties (Yang, Liu, Zeng, & Chen, 2018). Interestingly, the type of enzyme during enzyme hydrolysis also has varying effects on functionalities. For instance, oil binding property can be greatly or subtly enhanced by trypsin and pepsin, respectively, while remaining unmodified by flavourzyme or being impaired by neutrase (Table 4). Enzymatic hydrolysis of purified legumin from *V. faba* enhances emulsion properties, increases creaming stability and decreases surface tension of leguminT (trypsin-hydrolyzed) (Schwenke, Staatz, Dudek, Krause, & Noack, 1995). Lastly, alcalase treatment of *V. faba* protein isolate can yield tripeptides with potential pre-biotic properties (Xiao, Liu, Rizwan-ur-Rehman, Kang, & Wang, 2015).

As shown in Table 4., there exists a wide range of post-production processing possibilities yet to be determined for all functionalities for *V. faba* ingredients. In addition to this, industrial relevance of post-processing also needs to be considered while studying novel or new methodologies.

4.3. Ingredient Food Application

With regard to the utilization of *V. faba* ingredients, the United States of America contributes the most to vegan/dairy-free market, followed by the United Kingdom and Canada. Amongst these products, *V. faba* ingredients that are rich in proteins have been utilized mainly in dairy and meat alternatives (Mintel, 2019).

The environmental and nutritional benefits, along with functional properties of *V. faba* as ingredients are yet to be completely translated into real time use in food applications. During the most recent decade, foods with vegan/dairy-free claims using *V. faba* flours accounted for 2.40 % of the products with similar claims that used legume and pulse flours as ingredients. During the same period, foods with vegan/dairy-free claims using *V. faba* proteins (concentrates and isolates) constituted only 0.45 % of total foods using plant-proteins as ingredients (Mintel, 2019). In the literature, *V. faba* ingredients have a great potential in food applications, notably in partially or completely substituting traditionally used ingredients in foods, *viz.* pastas, crackers, mayonnaise, sausages and meatball analogs. However, research insights are less on the application potential of *V. faba* ingredients. Only flours find a place in research, being primarily used to replace meat, wheat flour, semolina and eggs (Table 5). The application potential of isolates and concentrates along with nutritional, sensory and safety specifications of all ingredients during their utilization is yet to be understood. Regardless of the potential in functional properties stated in the previous sections, current market and research preference on *V. faba* applications is remarkably low. This low utilization could be related to various factors, including the limitation of *V. faba* as ingredients with regard to sensory and safety aspects. Flavor and color contributors present in *V. faba* seeds are also subjected to changes during production and processing of ingredients – either moving towards, or

away from their consumer acceptability. Moreover, amount of ANFs which are one of the determinants of food safety in ingredients are influenced by process conditions too. Despite sensory and safety limitations of *V. faba,* there might be other factors accounting for low utilization of *V. faba* ingredients that we are yet to decipher. Insights on these aspects might throw light on some of the reasons behind the gap between the potential and present state of *V. faba* ingredients (Nasar-Abbas et al., 2009; Boye, Zare, & Pletch, 2010; Pechey & Monsivais, 2016).

Table 5 – Replacement of Traditional Ingredients by *V. faba* Ingredients in Food Applications

Ingredient	Post-Processing	Application
Flour	-	20 %[w/w] meat replacement in sausage[1]
Protein micellar mass (PMM)	-	20 %[w/w] in snack food and meatball analog[2]
Flour	-	Up to 30 %[w/w] wheat flour replacement in noodles[3]
Flour	-	100 %[w/w] in pasta[4]
Flour	1. Air classification 2. Fermentation(Lactic acid bacteria, 30°C, 48h), freeze drying, milling	100 %[w/w] semolina replacement in pasta[5]
Flour	-	Up to 100 %[w/w] wheat flour replacement in pasta[6]
Flour	Dough preparation, fermentation (*Lactobacillus plantarum*)	30 %[w/w] semolina replacement in pasta[7]
Flour	-	40 %[w/w] wheat flour replacement in crackers[8]
Protein Isolate	-	3 %[w/w] in mayonnaise[9]

Reports on the utilization potential of *V. faba* ingredients, with higher emphasis on the replacement of traditional ingredients (meat, wheat flour, egg, semolina) by *V. faba* ingredients.
1 Abo-Bakr, 1987; 2 = Youssef, 1988; 3 = Giménez et al., 2012; 4 = Laleg, Cassan, Barron, Prabhasankar, & Micard, 2016; 5 = Rosa-Sibakov et al., 2016; 6 = Laleg et al., 2017; 7 = Rizzello et al., 2017; 8 = Millar et al., 2017; 9 = Alu'datt et al., 2017.

5. IMPACT OF *V. FABA* PROCESSING ON ITS INGREDIENT VALUE

5.1. Effect on Anti-Nutritional Factors

Vicine & Convicine. The glycoside precursors of favism can be reduced by processing techniques. They are generally unstable in acidic medium and degrade into their aglycones at higher temperatures. Convicine is more readily hydrolyzed than vicine. After a week at 30 °C, convicine reduces to 22 % and 96 % in 0.1 N and 1.0 N hydrochloric acid whereas vicine degrades to 17 % and 83 %, respectively (Marquardt, Muduuli, & Frohlich, 1983)**.** Ingredients from pre-processed beans have varying glycoside contents depending on the type of bean processing. Dehulling of seeds increases vicine and convicine to 58 %[w/w] and 25 %[w/w], respectively (Olsen & Andersen, 1978). Alternatively, bean roasting and cooking decreases the glycoside contents. Bean roasting at 120°C for 10 min, which decreases 2-6 %[w/w] vicine and 0-10 %[w/w] convicine, is less effective than bean cooking at 121°C for 20 min, which eliminates 12-40 %[w/w] vicine and 17-60 %[w/w] convicine. Gamma irradiation removes up to 38 % of glycoside content (Jaddou, Mhaisen, Al-Adamy, & Naji, 1985). Hydrogen peroxide seed treatment lowers vicine by 91-93 %[w/w] and bean soaking and germination lowers vicine by 86 %[w/w]. Both these treatments completely remove convicine (Jamalian, 1999). In addition to bean processing, selection of young, ripe or older seeds impacts the final ingredient glycoside content due to the changes in the level of β-glucosidase enzyme. Young and old seeds are low in enzyme activity while ripe seeds have the highest enzyme activity (Rizzello et al., 2016). The extent of glycoside removal also depends greatly on the bean variety (Cardador-Martínez et al., 2012).

Protein extraction, depending on wet or dry process, impacts the glycoside concentrations. Wet protein extraction method leaches the glycosides out, leaving behind isolates with 42 %[w/w] vicine

and 9 %[w/w] convicine removal when compared to the whole seeds. Conversely, air classification (dry method) increases vicine and convicine concentrations by 53 % and 56 % respectively (Olsen & Andersen, 1978).

Ingredient post-processing, including fermentation of *V. faba* flour by *Lactobacillus plantarum* can remove more than 95 % of glucosides (Rizzello et al., 2016). Apart from fermentation, enzymatic processes eliminate upto 90 % glycosides. Microbial β-glucosidases from *Aspergillus oryzae*, *Fusarium graminearum* and lactic acid bacteria have been reported for *V. faba* flours (McKay, 1992). Frying of *V. faba* flour dough for bean cake application removes 56 % vicine and 34 % convicine (Hussein, Motawei, Nassib, Khalil, & Marquardt, 1986).

Other Anti-Nutritional Factors. Bean pre-processing effect on other *V. faba* ANF has been most extensively studied. Dehulling and soaking beans prior to processing reduces upto 11 % phytic acid, 59 % overall tannins (Luo & Xie, 2013) and 26-29 % saponins (Sharma & Sehgal, 1992) in *V. faba* beans. Trypsin inhibitors activity increases by dehulling as they are mainly located in the seed cotyledons (Luo & Xie, 2013). Heat treatment along with soaking further reduces ANF. Maximum reduction of all ANF is observed in dehulled beans that are soaked and autoclaved. For instance, autoclaving of dehulled and soaked beans reduces up to 66 % overall tannin content (Sharma & Sehgal, 1992; Luo & Xie, 2013). Favins are thermosensitive and hence heat treatment lowers their levels to a great extent. There are also some lectins that are partially heat stable and survive the passage through the gut, causing digestive disorders and diseases by their interaction with the gut epithelium (Pusztai, 1998). Despite this property, lectins still remain in the seeds after dehulling and soaking. Even germination fails to remove lectins from the seeds (Sharma & Sehgal, 1992). During ingredient production and protein concentration, wet process leaches out upto 46 % phytic acid, 91

% tannins and even 2.5 % trypsin inhibitors (Otegui et al., 1997). ANF that are flavor and color contributors are discussed in their respective sections too.

5.2. Effect on Flavor

Volatile odorant compounds. Numerous studies have been carried out in recent years to better understand the formation and evolution of odorant volatile compounds in pulse ingredients during different stages of protein extraction (Azarnia, Boye, Warkentin, Malcolmson, et al., 2011) or due to thermal treatments (Ma, Boye, Azarnia, & Simpson, 2016; Mishra et al., 2017; Chigwedere et al., 2019) and storage (Berger, Küchler, Maaßen, Busch-Stockfisch, & Steinhart, 2007; Azarnia, Boye, Warkentin, & Malcolmson, 2011).

Enzyme-mediated degradations begin during harvesting or in early stages of processing that disrupt the physical barriers separating enzymes (*e.g.* lipases, lipoxygenases, lyases and dehydrogenases) from their respective substrates (*e.g.* esterified or free fatty acids, amino acids and glucosides). This enables the degradation of the substrates into highly odorant volatile compounds (MacLeod et al., 1988; Siegmund, 2015; Ong & Liu, 2018).

Additional formation of odor-active compounds can be induced by temperature and pH variations during processing and storage of the ingredients, usually involving the degradation and rearrangement of amino-acids and carbohydrates *via* the Strecker degradation and the Maillard reaction (MacLeod et al., 1988; Reineccius & Heath, 2006; Belitz et al., 2009).

Dehulling and milling reportedly boost the formation of aldehydes in *V. faba* by increasing the total surface area and prompting the exposition of lipids to air (Akkad et al., 2019). *V. faba* flour exhibits high LOX activity (0.219-0.330 $mmol \cdot min^{-1} g^{-1}$), which could increase the potential for oxidative

reactions and consequent formation of deleterious flavor notes in food preparations with high amounts of lipids (Yang, Piironen, & Lampi, 2017). *V. faba* seeds have been reported to contain two type-II isoenzymes: broad bean lipoxygenase-1 (BBL-1), which produces ketodienes and both 9- and 13- hydroperoxides, and broad bean lipoxygenase-2 (BBL-2), originating predominantly 13-hydroperoxides (Eskin & Henderson, 1974; Clemente, Olías, & Olías, 2000). Both isoenzymes have shown oxidizing activity towards linoleic acid, methyl linoleate and trilinolein (Eskin & Henderson, 1974; Abbas, Siddiqi, & Toama, 1979; Chang & McCurdy, 2013). Several treatments have been proposed to inactivate the enzyme in *V. faba* seeds and thus control LOX-catalyzed lipid oxidation, including microwave heating at 950 W for 1.5 min (Z. Q. Jiang et al., 2016), blanching at 70 °C for 2 min (Al-Obaidy & Siddiqi, 1981) and heat treatment at 75 °C for 2 min (Al-Obaidy & Siddiqi, 1981) or at 70 °C for 15 min (Eskin & Henderson, 1974).

Non-volatile taste compounds. The overall saponin content of *V. faba* seeds (13.70-39.30 $mg \cdot g^{-1}$) (Makkar, Becker, Abel, & Pawelzik, 1997; Barakat & Reim, 2015) has been shown to decrease significantly upon soaking, dehulling, cooking and/or germination (Sharma & Sehgal, 1992; Barakat & Reim, 2015). Amongst saponins, soyasaponin βg can readily convert into soyasaponin Bb by releasing its DDMP moiety in the form of maltol either enzymatically or when exposed to temperatures above 30 °C, slightly acidic pH values and highly polar solvents (Ruiz et al., 1996; Daveby, Åman, Betz, & Musser, 1998; Heng et al., 2006). Polyphenols have been shown to decrease upon boiling and autoclaving by leaching into the cooking broth (Siah, Wood, Agboola, Konczak, & Blanchard, 2014; Turco et al., 2016).

5.3. Effect on Color

The color of *V. faba* ingredients also have an impact in the acceptability of the beans as ingredients in foods (Nasar-Abbas et al., 2009).

Proanthocyanidins in *V. faba* get oxidized through phenolic reactions and, by this way, can also lead to the development of a dark coloration (Beninger & Hosfield, 2003). It has been shown that high tannin varieties of *V. faba* beans, containing more proanthocyanidins, darken more in the presence of air than low tannin varieties, whereas white-seeded varieties present no darkening in an oxygen-rich environment (Crofts et al., 1980; Nasar-Abbas et al., 2008).

In addition to the constituents responsible for color of *V. faba* ingredients, the degree of protein extraction is also an important factor for ingredient color. Dehulled and milled seeds from *V. faba* yield flours that are creamy-yellow in color, with air classification increasing the yellow coloration. Air classified concentrates show lighter color than the isolates produced by acid- or alkali- extraction, isoelectric precipitation and freeze drying (Sosulski & McCurdy, 1987). Freeze drying of dehulled, milled, alkaline extracted and isoelectrically precipitated proteins contribute to dark coloration while the spray drying process have no darkening effect (Cepeda et al., 1998).

Several reactions can be hypothesized to form colors in ingredients. Browning reactions take place during production, processing and storage of foods. Browning reactions emerge through several ways in foods, including phenolic compounds' oxidation, Maillard reaction, ascorbic acid oxidation, lipid oxidation and caramelization (Corzo-Martínez, Corzo, Villamiel, & del Castillo, 2012). Maillard reaction in particular plays a role during high thermal conditions, and is popularly known to occur by reaction between amino acid and/or peptides along with carbohydrates. Amino acids are destroyed

as a result of this reaction, losing nutritional significance and leading to formation of toxic and anti-nutritional end products that compromise food safety (MacLeod et al., 1988; Mottram, 1993). These phenomena are yet to be studied in *V. faba* ingredients to assess their acceptability and safety.

6. CONCLUSIONS

V. faba is an agronomically sustainable plant-based source, and its proteins have a great potential in nutritional and functional properties for food applications. There are various approaches for producing and processing industrially relevant ingredients that further favor or impair their functional properties based on the type of processing techniques. Protein denaturation has primarily been studied in the context of functional property of *V. faba*, but there exists a need to explore the interactions of lipids, polysaccharides as well as polyphenols and phytic acids that could modify functional properties too. Despite promising studies on functional aspects, insights on food applications and consumers' recognition and appreciation in the food market for *V. faba* ingredients are low. Moreover, *V. faba* ANF, flavor and color properties certainly a limit the acceptability and safety of its ingredients in foods. Although changes in ANF, flavor and color are likely to occur during production and processing of ingredients, more insight is needed on the ingredients' complete functional, nutritional, anti-nutritional, and organoleptic profile to increase their food market acceptability. Care must be taken in assessing these limitations as there might be different other factors playing a role in food market availability, some of which include bioavailability of essential amino acids, availability of raw material for human consumption, socio-economic limitations. A great deal of knowledge still remains unexplored. For instance, is *V. faba* concentrate produced from a dry protein extraction technique and further post-processed functionally significant for applications, nutritionally safe and sensorially pleasing for consumers and industrially relevant for the food

market? As we cannot answer this now, we still have a long way to go in our scientific understanding to improve the use of *V. faba* as 'sustainable' ingredients in the food market.

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8. AUTHOR CONTRIBUTIONS

Siddharth Sharan planned, wrote, revised and edited the manuscript. Gabriela Zanghelini wrote and revised the sections concerning flavor aspects of *Vicia faba*. Daniel Bonerz and Julian Aschoff developed the idea for the review and revised the manuscript. Jens Zotzel, Anne Saint-Eve and Marie-Noëlle Maillard developed the outline and the plan of the manuscript, revised and edited the manuscript.

9. CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

10. DATA AVAILABILITY

The data will be shared (after acceptance of the manuscript) in Zenodo data repository supported by the European Commission (Framework Programme 7/ Horizon 2020).

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