# Faba beans protein as an unconventional protein source for the food industry: Processing influence on nutritional, techno-functionality, and bioactivity

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# Faba Beans Protein as an Unconventional Protein Source for the Food Industry; Processing Influence on Nutritional, Techno-functionality, and Bioactivity.

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## ABSTRACT

The nutrition and food industries are investigating unconventional protein sources because of the expanding demand for plant proteins and increased knowledge of the health and nutritional benefits of alternative proteins. Proteins from faba bean are high and outperform other pulse proteins in terms of nutritional, and functionalities. Raw faba beans contains numerous allergenic compounds hindering the potential for utilization in various foods. Processing faba beans by extracting of valuable compounds such as proteins enhances the applicability in different food systems and ensuring safety during consumption. Major proteins identified are globulins and non-globulin fractions with no adverse amino acids. Faba beans proteins are easy to extract however presence of pyrimidine glycoside may raise safety concerns. Faba bean proteins have useful functionalities for food applications but their solubility are minimal due to their compact protein structure. Further, different thermal and non-thermal techniques have been aimed at improving functionality and reduce allergenic proteins. The goal of this review is to

provide a comprehensive summary on current investigation on faba bean proteins. Suggestions for improving the faba bean's utilization are also provided to aid in its development.

**KEYWORDS:** Functionality, bioactivity, faba bean protein, peptides, nutrition, Processing, Pyrimidine glycosides

## 1. INTRODUCTION

Historically, the main source of protein in the human diet has been animal proteins. Diets based on animals, however, are raising more and more environmental sustainability issues (Badjona, Adubofuor, Amoah, & Diako, 2019). The production of animal meat, including cattle, shrimp, lamb, and pigs, is linked to the greatest percentage of greenhouse gas emissions per 100 g of protein, according to a new investigation (Poore & Nemecek, 2018). Alternative protein sources can cut land usage requirements and save 8 Gt CO2 eq year, according to a University of Oxford analysis (Collett, callaghan, Mason, & Godfray, 2021).

Faba bean (V. faba) **(Fig. 1**), also known as horse or broad bean is a member of the Fabaceae family grown as a staple meal in Middle Eastern and North African societies (Multari, Stewart, & Russell, 2015a). Due to its high protein content (approximately 30%), ease of growing, and superior nitrogen-fixing ability, FB has become more popular as a plant-based source of protein (Eckert et al., 2019; Liu, C., Damodaran, & Heinonen, 2019). According to their sedimentation coefficient, globulins, which make up 70–80% of the storage protein in faba bean seeds, may be divided into two classes: the 7S vicilin-type globulins and the 11S legumin-type globulins (El Fiel, El Tinay, & Elsheikh, 2002).

However, like other plant proteins, faba bean protein (**Fig. 1 d**) is currently only used in small amounts in food products due to its low solubility and limiting functionality when compared to animal proteins like egg white protein and milk proteins (Yang, Liu, Zeng, & Chen, 2018).

To improve safety and functionality of FBP, wet and dry fractionation methods are employed to isolate the components of proteins (Badjona, Bradshaw, Millman, Howarth, & Dubey, 2023b). The wet fractionation technique involves the removal of non-protein fractions and an improvement in purity by the use of organic solvents, acidic solutions, and alkaline solutions; nevertheless, this process frequently results in significant protein denaturation and requires a lot of water and energy. On the other side, dry fractionation, a softer process that often produces lower protein purities while maintaining the functions of protein, entails fine grinding, separation, and air classification. Utilizing the advantages of both methods or utilising cutting-edge processing technologies like microwaves, ohmic heating, ultrasound, enzymatic procedures, or high-pressure processing, both methodologies attempt to increase the quality of the extracted proteins through hybrid approaches (Sá, Laurindo, Moreno, & Carciofi, 2022).

Due to the nutritional benefits of FBP, there has been increasing research in this area on health benefits derived from bioactive peptides as well as structural and functional properties (Badjona, Bradshaw, Millman, Howarth, & Dubey, 2023a). Extraction and purification of proteins result in changes in nutritional (amino acid composition), physicochemical(surface charge, surface hydrophobicity), and functional properties such as WAC, OHC and solubility which ultimately affect final products when incorporated to foods since proteins impart superior functional characteristics. Besides, these functional and

structural properties are important indicators for developing functional foods, ingredients, and novel food products hence it is reasonable to expect that there will be an increasing utilization of faba bean derived ingredients in various food applications in the future (Paul, Kumar, Kumar, & Sharma, 2020; Paximada, Howarth, & Dubey, 2021).

This article provides a comprehensive summary of the chemical composition and structural characteristics of faba bean proteins as well as antinutrients specific to faba bean proteins. Processing of faba bean protein extraction and functional properties are discussed as well as their potential application in food matrices. Further attention is given to the potential of faba bean bioactive peptides preparation due to their health benefits. Faba bean proteins' physicochemical characteristics have been discussed as well. Attention is also drawn to the recent progress in the modification of faba bean proteins on their functional properties.

## 2. Chemical composition of faba bean protein

Faba beans are regarded as a nutritious food source of fats, carbohydrate, proteins, proteins, dietary fibre, vitamins, and minerals (Adamidou, Nengas, Grigorakis, Nikolopoulou, & Jauncey, 2011; Mayer Labba, Frøkiær, & Sandberg, 2021). The main nutrient in FBS, protein, has attracted a lot of research and interest globally. The chemical composition of FB flour, concentrate as well as isolate with other plant-based proteins is shown in **Table.1**. Despite the high protein content in faba bean flour, this overall protein content is insufficient to stabilise food product or applied in specialized food systems (Day, 2013).

Hence, protein concentrate, and isolate are typical obtained either through wet extraction processes or dry fractionation and as a result, there is a significant increase in the protein content of the final flour. The amount of protein in concentrates and isolates is depends

on the quantity of protein in the original raw material, the type of protein, and the method used to extract these proteins.

The protein content of FB flour was found to be 26% with a high percentage of carbohydrate accounting for 58.79 (**Table.1**) (Kumar, Sadiq, & Anal, 2021), however following protein extraction, proteins levels increased to approximately 60 and 90% for concentrate and isolate respectively with low amount of carbohydrates (Felix, Lopez-Osorio, Romero, & Guerrero, 2018; Vogelsang-O'Dwyer et al., 2020). Interestingly, protein extraction process led to relatively high percentage of fat and ash in concentrate and isolate. The high content of ash may be due to the use of acidic and basic solutions used in extraction processes for pH modification. However, some authors have reported less than 0.1% fat content in FBI (Vioque, Alaiz, & Girón-Calle, 2012).

Differences in nutritional composition of concentrate or isolate may be attributed to seed cultivar, pre-processing methods used and variation in extraction process. The protein content of FBI did not differ from soy protein isolate but was higher compared to whey protein isolate and chickpea isolate (Johnston, Nickerson, & Low, 2014a; Keivaninahr, Gadkari, Zoroufchi Benis, Tulbek, & Ghosh, 2021; Vogelsang-O'dwyer et al., 2020). According to this data FBC and FBI represent an alternate source of high protein alternatives to be used for various application in the food industry, pharmaceutical industry, and other emerging food industries such as targeted nutrition.

## 3. Faba bean proteins; extraction and functionalities

Seed storage proteins comprise a major source of dietary protein in legumes (Shewry & Halford, 2002). However, 80% of these proteins represent enzymatically inactive forms

stored in the cotyledon for seed germination into a seedling (Liu, Y., Wu, Hou, Li, Sha, & Tian, 2017). Large starch granules are enclosed by storage proteins in individual cells within the cotyledon microstructure. Depending on their solubility in various solvents, the proteins in faba beans are divided into four categories: albumins, glutelins, globulins, and prolamins (Shewry, Napier, & Tatham, 1995).

*3.1 Faba bean protein fractions*

The protein subunit is of vital importance since its examination can reveal the composition and corresponding functionality of seed storage proteins. Additionally, this helps in attaining breeding objectives for the improvement of protein quality in faba beans as well as studies on protein nutrition. A 2017 study examined the composition of seed storage proteins in FB seeds (Liu, Y. et al., 2017). Six specific protein subunits consisting of 97, 96, 94, 47, 42, and 38 kDa were discovered from a total of 16 proteins identified by combining liquid chromatography-electron spray ionization coupled with tandem mass spectroscopy. Following hydrolysis of each protein (1-10 peptide fragments per protein), the protein fragments were composed of about 8-23 amino acids. Legumin (47 and 42 kDa), putative sucrose binding protein (47 kDa), and convicine in the 64 kDa subunit were recognised as distinct proteins that had already been discovered in faba beans. Examining the variety of faba bean proteins will assist breeders in their selection attempts to create new genotypes in light of nutritional needs and protein intake from faba beans.

*3.2 Globulins*

Albumin and globulin are among the primary storage proteins in faba beans. Based on their sedimentation coefficients (S20.w), globulins are divided into 7S proteins and 11S proteins. 7S proteins consist of vicin and convicine (v-c) while 11S proteins are mainly of legumin (Singhal, 2016a). Using electrophoresis and ion-exchange chromatography the subunits of

legumin have been shown to be heterogeneous; it is composed of four major 60 kDa subunits following isolation with ion-exchange chromatography in 6M urea. There are also known legumin subunits of 75 and 80 kDa. These subunits are formed via a disulphide bridge and are formed before post-translational processing of the α-β precursor chains, hence legumin A α-chain is exclusively linked to the legumin Aβ-chain (Saenz de Miera, Ramos, & Perez de la Vega, 2008).

Globulins tend to dominate faba bean storage proteins and thus serve as the main supply of amino acids (Liu, Y. et al., 2017). **Figure 1** shows the presence of several protein fractions (corresponding to different bands) in faba bean. Analysis of thermal properties shows that the denaturation temperature of purified 7S proteins in faba bean to be 84℃ while 11S globulin exhibit denatured at 95 ℃ indicating that thermal property was due to both 7S and 11S proteins.

The 11S globulin proteins are hexameric holoproteins, whereas vicin(7S) is a trimer composed of polymorphic subunits encoded by multiple gene families. Multiple genes encode legumin subunits of type A (contains methionine) and type B (absence of methionine). In the literature, only a few genes encoding type-A, type-B and legumin polypeptide (LeB3) have been described (Bäumlein, Nagy†, Villarroel, Inzé, & Wobus, 1992; Fuchs & Schubert, 1995; Horstmann, Schlesier, Otto, Kostka, & Muntz, 1993).

Isoelectric precipitation can be used to isolate these proteins since v-c has an isoelectric point of 4.8 and 5.5, respectively. About 55% of the total protein in mature faba beans is made up of the protein legumin. Legumin A and B are the two main subunits of faba bean legumin. Legumin A has methionine rich residues while the B form lacks methionine. Vicine consists of 3% of seed storage proteins while convicine represents up to 3.2% of the total protein content. Polypeptide fractions of vicin and convicine contain 50 and 70 subunits, respectively.

Both polypeptide chains lack cysteine and are not linked via disulphide bridges as compared to legumin proteins. Vicin dissociates into 3S subunits at pH levels below 3 and above 11 (Saenz de Miera et al., 2008).

*3.3 non-globulin proteins*

Additionally, faba bean seed albumins are mostly metabolic proteins with potential enzymatic activity which include lectins, protease inhibitors, defensins, albumin-2 as well as Bowman-Brik inhibitor (Li et al., 2019) (Waterhouse et al., 2018). Albumin fraction has substantial amounts of sulphur-containing acid compared to other seed proteins (El Fiel et al., 2002).

Another group of proteins in faba beans is prolamins. These proteins are lysine and tryptophan-free alcohol-soluble proteins that are nevertheless abundant in proline, glutamic acid, and leucine (Multari, Stewart, & Russell, 2015b). They are also soluble in ethanol/water mixtures and propan-1-ol/water solutions (Shewry et al., 1995). However, glutelin proteins tend to have a higher solubility in sodium hydroxide with a similar amino acid profile to that of prolamins. This protein contains high levels of glycine, histidine as well as methionine (Multari et al., 2015b).

## 4. FABA BEAN PROTEIN EXTRACTION

*4.1 Faba bean protein concentrate*

Faba bean concentrate (FBC) is prepared following dehulling and subsequent milling of beans into particulate flour size. The defatting process may be omitted in some cases since faba beans contain a low amount of fat. Faba bean concentrate has been processed in varied conditions in other to optimize protein yield. Protein-rich flour obtained containing up to 65% of protein (N

x 6.25) has been achieved (Vogelsang-O'Dwyer et al., 2020). Faba bean protein concentrate generated by densification showed a protein content of 56% which has been demonstrated to be eco-friendly with promising techno-functional properties (Felix et al., 2018).

To maximum protein yield, some researchers have employed enzymatic assisted extraction using different enzymes such as pepsin and pancreatic enzymes to improve protein yield and solubility, which was shown to improve extractability by 10-15% (Abdel-Aal, Shehata, El-Mahdy, & Youssef, 1986). To maximize the yield of faba bean concentrate, some researchers obtained concentrate using isoelectric precipitation. Alkaline extraction was carried out at pH 9.0 proceeded by isoelectric precipitation at pH 4.0 which generated a yield ranging from 73.2 to 75.6% (Otegui et al., 1997).

*4.2 Faba bean protein isolate*

Protein isolates from faba bean in the most commercially purified form contain protein content > 90%. Protein isolates from plant-based material can be produced using varying methods such as salt extraction with subsequent micellization, basic, neutral, or acidic extraction followed by precipitation at isoelectric point (Eckert et al., 2019; Vogelsang-O'Dwyer et al., 2020). Faba bean isolate is produced from dehulled and fat-free faba bean through removal of nonprotein constituents. Defatting prior to isolation of protein is necessary to improve extraction by limiting lipid-protein interaction.

The most common techniques for isolating protein from legumes are isoelectric precipitation and salt extraction. The extraction method used has a significant effect on functional properties as the extraction process affect the physicochemical properties of proteins such as globulin, legumin and vicilin. Abdel-Aal et al. (Abdel-Aal et al., 1986) studied the

impact of various extraction techniques on the functionality and extractability of protein isolate from faba beans. Protein isolate was obtained using Alkaline/isoelectric precipitation, precipitation by ionic strength and salt extraction.

Depending on the extraction method and conditions employed, functional property and purity of isolate generated may vary considerably. Optimisation of extraction conditions in terms of temperature, pH, solvent ratio, extraction time, centrifugation time and drying conditions is a prerequisite to obtain desired protein isolate. Alkaline/isoelectric precipitation has been shown to reduce favism induced by aglycones vicine and convicine in protein isolates by 99% as compared to the raw flour (Vioque et al., 2012).

By using isoelectric precipitation (Keivaninahr et al., 2021) produced faba bean isolate by isoelectric precipitation although their yield was 87% w/w lower than that of (Vioque et al., 2012). FPI was also produced by Karaca et al. (Karaca, Low, & Nickerson, 2011) using alkaline/isoelectric precipitation and salt extraction. Alkaline extraction was carried out at pH 9.5 due to the proteins high solubility at high pH followed by isoelectric precipitation at 4.50 using 0.1 M HCL, followed by centrifugation and freeze-drying. Salt extraction was conducted using potassium sulphate salt followed by dialysis and then freeze-dried.

Isolate generated by isoelectric precipitation generated a higher concentration (84.1%) compared to salt extraction (81.4%). Based on physicochemical properties, it was observed that extraction method plays a key role in structural/conformational changes (Karaca, Low, & Nickerson, 2011). Extremely alkaline or acidic pH is not employed, compared to alkaline/isoelectric precipitation which may affect subunit composition hence the observed difference in physicochemical properties.

Based on SDS-PAGE composition of soluble and insoluble fractions of faba bean isolate and concentrate similar band distribution with fewer variations for molecular (MW <72) For higher molecular weight bands (>95kDa), both soluble and insoluble fractions were found, although the soluble fraction of isolates included a spectrum of polypeptides up to 250kDa while the insoluble fraction displayed a prominent band at about 110kDa. The main difference was observed in the intensity of the band which was high in isolate than in concentrate due to the high protein content of isolate (Keivaninahr et al., 2021).

One key advantage of obtaining protein isolate is the reduction of antinutrients such as glycoside vicine and convicine and other antinutrients. After protein isolation, residual vicine and convicine content was less than 1 % (Vioque et al., 2012).

## 5. Nutritional, digestibility, and amino acid distribution

The nutritional requirement of individuals and animals is not merely based on protein content but specific quantities of essential amino acids. The amino acid profile of faba bean isolate is comparable to other pulses with limiting sulphur-containing amino acids that can be supplemented by incorporation of grains or cereals. Protein soluble extract at pH 4 was found to be deficient in tryptophan, isoleucine, and leucine but not in sulphur-containing amino acids. This is due to the presence of albumins which are soluble at this pH and contain sulphur-containing amino acids (Vioque et al., 2012).

There were 497 amino acids in conviciline, and there was a total of 3 positively charged residues (Cys + Met). Additionally, 46 leucine and 62 glutamic acids accounted for up 12.5 % and 9.3 %, respectively, of the total amino acids. Legumin A contained 482 amino acids and a total number of positively charged residue (Cys + Met) of 8. Protein efficiency ratio (PER) of protein isolate obtained from alkaline/isoelectric precipitation was found to be higher than 2

(low-quality protein has a value lower than 1.5). This value is calculated using the concentration of tyrosine, methionine, leucine, and Histidine. Furthermore, the theoretical biological value of protein isolate was found to be 47 (Vioque et al., 2012).

Amino acid levels from faba bean protein rich fraction (FBC) and isolate (FBI) were similar except in essential amino acids where FBI was slightly higher than FBC. The amino acid requirement was above the recommended levels (WHO,2007) except for sulphur-containing amino acids (SAA), which were low. The limiting sulphur-containing AA as a fraction of WHO adult requirement showed amino acid scores of 0.62 and 0.53 for faba bean concentrate and isolate respectively (Vogelsang-O'Dwyer et al., 2020). Based on a total protein requirement of 66 g/kg body weight, the EAA are equivalent to those in other high-quality proteins and sufficient for adults, according to the WHO and FAO recommendation. When the AA composition of whole faba beans is contrasted to protein product, the impact of protein content can be seen, as shown in **Table 2**.

The protein digestibility of FBC and FBI was examined by Vogelsang-O'Dwyer et al. from short-term to long-term exposure (Vogelsang-O'Dwyer et al., 2020). Overall protein digestibility was determined to be 5-6% for pepsin, 22-26% for short-term, 25-30% for mid-term, and 33-39% for long-term. investigated protein digestibility of FBC and FBI. Pepsin digestibility was found to be 5-6%, whereas overall protein digestibility values ranged from 22-26% (short-term), 25-30% (mid-term), and long-term (33-39%). Between FBC and FBI, pepsin digestibility and overall protein digestibility were higher in FBI. This result indicates that aqueous isolation of proteins is useful in improving protein digestibility which may be ascribed to the reduction of enzyme inhibitors (e.g., trypsin inhibitor) and less amount of dietary fibre and cell wall interferences. Currently there is paucity of information on the digestibility for faba bean concentrate and isolate extracted using different processing methods.

The relative protein digestibility of optimized ultrasound treatment was observed reduce protein digestibility compared to native FBI (Martínez-Velasco et al., 2018).

## 6. Functional properties

The value and applicability of food ingredients depends on the complex interactions and behaviour of its structure, physiochemical properties as well as extent and nature of the environmental conditions in which these are associated is known as functional properties (Kaur & Singh, 2006; Siddiq, Nasir, Ravi, Dolan, & Butt, 2009). Functional properties are necessary to evaluate and perhaps forecast the behaviour of novel proteins, lipids, fibres, and carbohydrates in certain food system.

Through complex interactions with other molecular components, food ingredients serve several non-nutritive roles that change the behaviour of food systems as a whole. These non-nutritive functions (functionality) play crucial roles in the preparation, storage, sensory qualities, and general food quality. Functional properties of interest include water and oil holding capacity, emulsification, foaming ability, and gelation which are useful properties that facilitate their incorporation into different food systems (Kaur & Singh, 2005) . Prerequisite for the development of alternative foods from plants requires understanding and controlling protein functionality. In this section the functional properties of FBC and FBI is discussed and compared with other protein sources as shown in **Table 3**.

### *6.1 Water binding*

The extent to which protein material or flour can bound and retain water is extremely important in various food product development. This functionality is useful in maintaining and predicting product quality, shelf stability and organoleptic properties such as mouthfeel and texture. Water holding capacity may be influenced by intrinsic factors such as protein conformation, amino

299 acid sequence, surface hydrophobicity as well as extrinsic factors such as temperature, pH, and
300 ionic strength (Moure, Sineiro, Domínguez, & Parajó, 2006a; Paredes-Lopez,
301 Ordorica-Falomir, & Olivares-Vazquez, 1991). The study reported by Raikos et al.
302 (Raikos, Neacsu, Russell, & Duthie, 2014) showed that faba bean flour (1.7 g/g) showed
303 a stronger WHC compared to buckwheat (0.9 g/g), green (1.3 g/g) and pea (1.5 g/g) flours as
304 shown in **Table 3**.

305 WHC of FBPI at pH 2 and 7 was higher compared to its concentrate and deflavoured
306 forms (Keivaninahr et al., 2021). High WAC of protein isolates is due to their high protein
307 and less amount of non-protein components as well as exposure of polar amino acid residues.
308 WHC of proteins may be influenced by processing conditions employed during protein
309 extraction. Overall WHC of FBC was 1.25 $gg^{-1}$ which is less than that of soy protein
310 concentrate (3.53g/g) (Bühler, Dekkers, Bruins, & Goot, 2020a). The study reported by
311 Hall & Moraru, (Hall & Moraru, 2021) showed that FBC had a lower WHC compared to
312 lupin and pea protein concentrate. The high amount of proteins in isolates as well as the low
313 amount of starch has been attributed to contributing factor to higher WHC (Pelgrom,
314 Vissers, Boom, & Schutyser, 2013).

315 The role of water binding properties in various food formulations is extremely critical
316 in emerging topic such reducing fat content in meat products. In these cases, adding water
317 holding compounds such as faba bean proteins may prove useful in maintain and improving
318 sensorial and texture properties.

*6.2 Gelation*

Gelation is a desirable functionality in food formulations such as puddings, jellies and several desert and meat applications. Since many food applications have pH levels between 5-7, understanding how protein gels react in this range is crucial. A measure of a protein's capacity to form a gel is called the least gelation concentration (LGC). A low LGC indicates a high gelling capacity (Raikos et al., 2014).

Faba bean protein isolates, which include globular proteins, often result in one of two types of gels, depending on the charge the original protein. For instance, for whey protein, when repulsion is high, fine-stranded gels develop, however as the isoelectric point is reached, a network of colloidal particles forms (Langton & Hermansson, 1992). Gel formation of faba bean flours occurred at a concentration range of 100-140 g/L. Faba bean flour formed firm gels than lupin and hemp flours at pH 4 and 7 (Raikos et al., 2014). Due to variation in proteins, lipid and carbohydrate content between these plant-based proteins, the relative interactions of proteins, polysaccharides, and lipids may have an impact on gelation (Sathe, Deshpande, & Salunkhwe, 1982). Carbohydrate have been shown to reduce the thermodynamic affinity of proteins to water molecules thereby magnifying interaction between proteins molecules and consequently enhancing gelling capacity (Yemisi A. Adebowale & Kayode O. Adebowale, 2008).

PH shifts also greatly affect the gelling ability of proteins through alteration of charge distribution among amino acid residues and this can improve or inhibit interactions between proteins (Raikos, Campbell, & Euston, 2007). Langton et al. (Langton et al., 2020) investigated the LGC for alkaline protein isolate and soaked protein at pH 5 and 7, with and

without sodium chloride. They observed that proper gels were produced at 13% concentration while soaked protein extract showed a low LGC. They suggested a high protein concentration of 15% for the formation of hydrogels. Gel produced from alkaline protein extract at pH 7 without sodium chloride showed a dense and finer networks structure while gels at pH 5 showed a particulate structure. At pH 7, however, the G and Young modulus were low. They observed that extraction method and addition of salt had less influence on microstructure and rheological properties. At pH 5, however, adding 2% NaCl caused the microstructure of the gel to separate into a coarser and finer network.

*6.3 Solubility*

Protein solubility is a key parameter for application of protein ingredients in functional foods. It is a determining factor of the organoleptic properties of developed foods and influences functional properties such as emulsification, gelling and foaming capacity of developed food products (Morr, 1990). For proteins to remain soluble in an aqueous medium, the balance between protein and water interactions is a determining factor and that of surface charge. Solubilisation of proteins can be achieved when charged particles undergo repulsion thereby restricting protein-protein interactions and promoting strong interactions between polar groups of proteins with water molecules (Karaca et al., 2011; Singhal, 2016b).

The pH-dependent solubility profile of FBC and FBI displays a typical curve-like feature as shown in **Fig 4.b** with an IP (where net charge is zero) at about 4.5 for FBI and FBC which corresponds to least protein solubility. Both FBI and FBC showed similar pattern, however FPI showed a lower protein solubility compared to FBC. Observed differences was not due to surface charge property as both showed comparable results. Hence differences could be attributed to several reason such as the extraction method employed, and the drying used in

preparing the isolate. Protein solubility of faba bean isolate at neutral pH has been indicated to vary from 24 to 85% (Fernandez-Quintela, Macarulla, Barrio, & Martinez, 1997; Johnston, Nickerson, & Low, 2014b).

The solubility profile of FBI indicated that the least solubility was at pH 4-5 whiles the peak solubility occurred at pH 10-11 (Eckert et al., 2019), which undoubtedly corresponds to the isoelectric point hence absence of surface charge facilitates aggregation and precipitation of proteins (Kramer, Shende, Motl, Pace, & Scholtz, 2012). At neutral pH, FBI showed poor solubility (24.7%) (Eckert et al., 2019). Protein denaturation and aggregation during alkaline conditions primarily at pH 10-11, may be accountable for the low solubility of FBI at pH 7. The poor solubility of FBP at neutral pH minimizes their physicochemical and functionalities for food applications hence the need for modification using various processing techniques such as pH shift which will be discussed in later sections.

The protein solubility profile of faba ban flour is pH dependent. Solubility levels increased over pH range from 4 to 10. pH 4 is close to the isoelectric point of most proteins (Raikos et al., 2014) hence protein-protein interaction occurs due to less molecular repulsion which result in precipitation and aggregation of proteins thus lower protein solubility at pH 4. However, protein solubility was observed to increase above the isoelectric point which could be attributed to ionic hydration, high negative charge as well as electrostatic repulsion (Lawal, 2004; Moure, Sineiro, Domínguez, & Parajó, 2006b). The protein extraction method can greatly impact solubility as was evidenced by (Karaca et al., 2011), who observed that the overall solubility of FBI prepared for IEP was superior to salt extraction.

*6.4 Foaming properties*

The foaming ability of flours is extensively employed in baked and confectionery products such as cakes, toppings, and mousses. A proteins capacity to readily adsorb to the air-water interface determines their foaming potential while foam stability relies on multilayer properties and surrounding film of air bubbles to ensure resistance against coalescence and drainage (Sreerama, Sashikala, Pratape, & Singh, 2012).

Despite the high foaming ability of FBF at ph 4 and 10(5.7%), stability of the foam was found to be low (2.7%). The molecular flexibility of proteins tends to facilitate foam formation however maintaining the stability of foams depends on intermolecular interactions at the air-water (Raikos et al., 2014). FBI showed a low foaming capacity of 31.2 % at pH 5 and 66.7 % at pH 7 (Eckert et al., 2019) which was less than other protein sources such as adzuki bean protein isolate and moringa protein isolate (as shown in **Table 3**.) as well as pea (167.4-243.7%) and lentil (403-425%) isolates (Jarpa-Parra et al., 2014; Lam, Warkentin, Tyler, & Nickerson, 2017). Low solubility of FBI has been reported to be responsible for its poor FC (Vioque et al., 2012). FPI foams had multimodal size distribution, distorted polyhedral shape, and larger mean bubbles (d1,0 = 363.5 m) with less defined and thinner lamellae with foaming activity of 145.8 %. After 30 minutes, foam coarsening became apparent, and bubble size increased noticeably (d1,0 = 482.5 m) (Martínez-Velasco et al., 2018).

Nivala et al. (Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura, 2017) observed a poor foaming property for FPI compared to oat protein despite the high solubility of FPI at neutral pH. Foam expansion (FE) of FBC was observed to be 244% with a foam liquid

expansion of 10% (Hall & Moraru, 2021). A high FE indicates a higher tendency to incorporate air into the foam through protein adsorption. A similar study by Yang et al. (Yang et al., 2018) showed that faba bean protein isolate showed a foaming capacity of 91.1% with corresponding foam stability of about 100%. The difference in foam property could be attributed to the extraction method employed and the variety of cultivars used. At 0.1-1% protein concentration, the foaming capacity of FBC and FBI were observed to be similar at neutral pH with further increases in concentration up to 3.3 % having minimal impact on FC.

In general, the FC of FBC was greater compared to FBI. This agrees with the high solubility profile of FBC in **Fig 6** (Vogelsang-O'Dwyer et al., 2020). Since, intrinsic factors such as solubility, protein concentration, and surface hydrophobicity also affect foaming properties, thus the observed differences in foam properties (Malomo, He, & Aluko, 2014).

*6.5 Oil binding*

Oil binding also referred to as fat absorption capacity is a crucial attribute for food products such as meat, mayonnaise, and dairy-based products (Escamilla-Silva, Guzmán-Maldonado, Cano-Medinal, & González-Alatorre, 2003). Through hydrophobic interactions of the aliphatic side chains of fatty acids and the nonpolar area of certain amino acids, OHC reflects protein-lipid interactions (Abugoch, Romero, Tapia, Silva, & Rivera, 2008).

OHC capacity of faba bean protein was observed to be 6.12 g/g (Eckert et al., 2019). (Keivaninahr et al., 2021) observed that FBPI had a higher OHC than concentrate and unflavoured samples. FBPI has a superior OHC(5g/g) compared to other protein isolate

(Eckert et al., 2019; Jain, Subramanian, Manohar, & Radha, 2019; Nunes, Favaro, Miranda, & Neves, 2017) such as moringa seed protein, soy protein isolates and others (**Table.3**), indicating their possibility to be used in the food systems to develop meat analogues and applied in baking. Oil holding capacity involves trapping of oil in protein structure and is hence mostly influenced by protein conformation, concentration, hydrophobicity, surface properties and protein size. Vogelsang-O'Dwyer et al. (Vogelsang-O'Dwyer et al., 2020), reported values of 124 and 87 g/100g for FBC and FBI, respectively. OHC of faba bean isolates also has been shown high compared to faba bean flour (Vioque et al., 2012) possibly due to unfolding and exposure of hydrophobic groups during protein extraction.

Overall, the OHC of faba bean protein is better compared to lupin protein hydrolysates, maize and soy concentrate which have OHC in the range of 2.6–4.7 g/g of protein (Hassane Lqari, Justo Pedroche, Julio Girón-Calle, Javier Vioque, & Francisco Millán, 2005; Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015; Wasswa, Tang, Gu, & Yuan, 2007).

*6.6 Emulsification properties*

The emulsion activity Index is an indication of the interfacial area stabilized per unit weight of protein of a diluted emulsion over a defined time (Pearce & Kinsella, 1978). Emulsifying ability of faba bean flours was found to be low at pH 4(12.5 $m^2/g$) but improved at alkaline pH (pH 7 and pH 10; 23.5 and 38.2 $m^2/g$ respectively). Lowest emulsifying ability and stability was observed at pH 4 compared to pH 7 and 10 (Raikos et al., 2014).

Proteins capacity to migrate and adsorb at the interface depends on protein solubility. The partial unfolding of globular proteins, which exposes hydrophobic and hydrophilic regions

and increases surface activity at the interface, may be the reason for the improved emulsifying capabilities at alkaline pH (Nir, Feldman, Aserin, & Garti, 1994). Faba bean protein isolate showed EAI and ESI values of 36.4 $m^2/g$ and 48.1 min respectively (Eckert et al., 2019). Low EAI values of FBI compared to pea, lentil, and chickpea has been reported by Karaca et al. (Karaca et al., 2011) and this could be due to the low solubility of faba bean protein as well as its compact structure. FBC was reported to have an EAI of 6 $m^2$ /g with an EAI of 2111 min lower than pea and lupin concentrate (Hall & Moraru, 2021). According to Yang et al. (Yang et al., 2018) the emulsifying activity index of FBI was shown to be 27 $m^2/g$ with an emulsion stability of 40 min.

FBI and FBC stabilised emulsions at pH 2 showed smaller particle size compared to pea protein and whey protein isolate which indicate the advantage of faba bean proteins over other proteins under specific emulsification condition. FBI emulsion at pH 7 showed a large particle size 25.8 mm compared to pea protein (8.6 mm). FBI stabilised emulsions had large particle size compared to its concentrates and deflavoured samples despite high protein content of isolate (Keivaninahr et al., 2021). Large particle size may be due to protein unfolding during isolate production resulting in lower solubility which affect smaller emulsion droplet formation and aggregation of oil and protein.

Confocal images (**Fig 5**) of all faba beans stabilised emulsion showed spherical oil droplets (red colours) and aggregates of proteins in the continuous phase in Fig 7. The particle size of FB stabilised emulsions at pH 2 were generally smaller compared to pea protein and whey protein isolate indicating superior property of faba proteins. However, at pH 2 the emulsion droplet size was higher compared to pH 7 and was ascribed to the small interfacial tension at pH 7. By contrast FBP isolate stabilized emulsion formed larger particles compared

to concentrate despite their high protein content probably due to extraction method which caused lower solubility and resulted in oil droplet aggregation (Keivaninahr et al., 2021). Further studies on functionality of faba bean globulins and albumins will provide useful information understanding faba beans proteins functionality and improving its application.

*6.7 Interfacial properties*

The adsorption of protein at interfaces generally involves three main steps. First protein migrates from bulk phase to interface. Thereafter, proteins adsorb at the interface resulting in structural changes. Finally, interfacial protein network is formed through intermolecular interactions and multilayer structures (Macritchie, 1989). (Keivaninahr et al., 2021) indicated that FBC and FBPI showed a lower interfacial tension compared to pure oil/water emulsion indicative of emulsifying ability. FBC and FBI showed an IT value of about 14 mN/m at pH 2 while pH 7 showed lowers values of about 7 mN/m. Interfacial tension of 42 mN/m for 0.25% FBP isolate has been stated by Karaca et al. (Karaca et al., 2011) against flaxseed oil at pH 7.

According to Johnston et al. (Johnston et al., 2014a) incorporation of FPI into canola oil-water interface was able to reduce the interfacial tension by a magnitude of ~6.1 mN/m. The force (or energy) required to drive a probe through an interface, such as a du Nöuy ring, is measured by interfacial tension. If this tension is reduced, smaller emulsion droplets will form, creating an easier-to-control emulsion (Damodaran, 2005; Karaca et al., 2011). Differences in interfacial tension could be attributed to protein concentration and the source, pH, purity of oil and analytical methods used as well as protein composition.

*6.8 Thermal properties*

Proteins in their natural environment are either folded into secondary, tertiary, or quaternary structures through hydrogen bonds, hydrophobic as well as electrostatic interactions. The thermal stability of proteins during processing plays a key role in the functionality and hence their applicability in food systems. Denaturation of proteins generally depends on amino acid sequence, and processing method used in extraction. Purified proteins are rarely encountered in various food matrices. In the case of faba bean isolate the dominant structural proteins are usually legumin and vicilin as well as other minor non-protein compounds as shown in **Fig 4.A**.

Protein denaturation is often an irreversible process, and it may be observed using differential scanning calorimetry (Ricci et al., 2018). FBC exhibits a typical protein denaturation temperature of $T_{onset}$ ~89°C and $T_{peak}$ ~94°C when analysed at a concentration of 15 g protein/100g) at a heating rate of 2°C/min (Hall & Moraru, 2021). Several components have been demonstrated to influence the thermal stability of FBI, for instance Arntfield et al. (Arntfield, Murray, & Ismond, 1986) showed that water content significantly affects the denaturation temperature. FBPI exhibited two typical endothermic peaks with a $T_d$ at 90$^o$C and 100$^o$C in 0.5M NaCl. These two peaks correspond to both Legumin ($T_{d=}$ 100 $^o$C) and vicilin ($T_{d=}$ 90 $^o$C) forms of proteins . PH effect was demonstrated to cause a reduction in $T_d$ and enthalpy of reaction when the pH was shifted below 2.5 and above 11.5 (Arntfield et al., 1986).

A much lower denaturation temperature was observed for FBI obtained from alkaline-isoelectric extraction ($T_d$ =85$^o$C) compared to micellized FBI ($T_d$ =90$^o$C) (Arntfield et al., 1986). This can be explained by the differences in the extraction method employed, as

micellization represents a milder extraction method that has a minimal impact on affecting the native structure of proteins compared to alkaline-isoelectric precipitation which involves strong acid or bases that disrupt intermolecular bonds. As reported by Kimura et al. (Kimura et al., 2008), the 11S fraction of faba bean protein showed an endothermic peak with a denaturation temperature $T_d$ of 95.4°C while the 7S fraction showed a $T_d$ value of 83.8°C. The $T_d$ for FPI was also reported to be 94°C with $T_{onset}$ around 83°C (Nivala et al., 2017).

## 7. Structural modification for improvement of functionality

### *7.1 Thermal treatment*

Exposure to more hydrophobic amino acid residues is often associated with better emulsifying activity of oil-water emulsion. Heat treatment at 95°C for 15 min significantly improved emulsifying activity index (ESI) and foam stability (FS) of FBC. The improvement in ESI and FS may be attributed to increased surface hydrophobicity following heat treatment (Hall & Moraru, 2021).

Nonetheless, emulsification properties of proteins are affected by several aspects such as surface hydrophobicity and charge, protein conformation state and molecular flexibility, ionic strength, protein concentration as well presence of non-protein components (Manoi & Rizvi, 2009). Heat treatment of 10% algae O/W emulsion stabilized by FBP at pH 7 showed an increase in droplet size at 90°C (Gumus, Decker, & McClements, 2017). Faba bean protein isolate and concentrate upon heating at 90°C for 30 mins showed a reduction in particle size due to loss of large oil droplets (Keivaninahr et al., 2021). A pronounced increase in surface hydrophobicity was observed in colloidal FPI after heat treatment (90°C, 5 or 30 min)

from 181 to 504 RFU (Nivala, Nordlund, Kruus, & Ercili-Cura, 2021). Increment in surface hydrophobicity may be attributed to partial denaturation of proteins which expose buried hydrophobic amino acid regions. As a result, it would be reasonable to assume that increasing surface hydrophobicity would increase EA since hydrophobicity is one of the primary factors influencing protein adsorption at oil/water interfaces.

Nivala et al. (Nivala et al., 2021) indicated that heat treatment showed minimal improvement in EAI of FPI from 25 to 27 $m^2$ /g. Various heat treatment has been employed in various research to reduce or eliminate antinutritional factors in pulses. Heat treatment (95℃ for 15min) showed a drastic reduction in trypsin inhibitor activity than untreated FBC. Trypsin inhibitor activity was lowered by ~78% in heat-treated FBC compared to the untreated control . Heating (75 to 175 ℃) of FBC applied to improve its water holding capacity. Heating FBC at 75 and 100 ℃ did not show any notable change in WHC however an elevated temperature of 150 and 175℃ showed a drastic improvement in the WHC (Bühler, Dekkers, Bruins, & Goot, 2020b). Improvement in WHC was attributed to an increased hydrophobicity of insoluble protein fraction of FBC, indicating that heating exposed buried hydrophobic regions by denaturation.

*7.2 Enzymatic treatment*

Enzymatic modification of proteins has been employed in the food application due to their exceptional nutritive, bioactive, and functionalities. Faba bean hydrolysates are of importance to researchers and industrial applications due to their health benefits and specific ability to modify functional properties.

Hydrolysis of faba bean isolate was conducted using various enzymes under specific temperature and pH conditions. The highest degree of hydrolysis (DH) was observed for pepsin treatment (9.5-16.9%) followed by flavourzyme (6.8-12.2%) whiles the least degree of hydrolysis was observed in trypsin (6.4-9.9%) and neutrase (2.1-6.4%). After enzymatic treatment, the solubility at neutral pH for pepsin, trypsin, flavorzyme, and neutrase hydrolysates increased from 24.44 to 88.8, 82.7, 72.9, and 63.1%, respectively. This could be attributed to reduced molecular weight and surface hydrophobicity compared to untreated FBI. Based on the amino acid profile of hydrolysates there was an increment in negatively charged glutamic acid than in intact protein which can bind water and improve solubility (Eckert et al., 2019).

Faba bean protein has surface charges of 25 mV at pH 7 and 15 mV at pH 5, respectively. Because more ionisable amino and carboxyl groups are exposed as a result of protein unfolding and hydrolysis, the hydrolysates have a greater negative net charge at neutral pH (Achouri, Zhang, & Shiying, 1998). After enzymatic hydrolysis, faba bean isolates showed an increased improvement in FC. Pepsin treatment showed an FC of 122.2% at pH 5 and 131 at neutral pH (Eckert et al., 2019). Higher FC of pepsin hydrolysates may be due to increased solubility arising from smaller size peptides generated which can easily migrate to the air-water interface (Taheri, Anvar, , & , 2013). Foaming stability was improved after hydrolysis as FS value was close to 100% was observed for neutrase treatment (60min), pepsin, trypsin and flavourzyme at pH 7 (Eckert et al., 2019).

Following transglutaminase (TG) treatment (1000 nkat/g protein) there was a decrease in surface hydrophobicity from 181 to 162 RFU. However, a combined heat treatment (90°C, 5 or 30 min) and TG treatment (1000 nkat/g protein) led to a significant increase in surface

583 hydrophobicity from 181 to 435 RFU (Nivala et al., 2021). Enzymatic crosslinking with
584 TG lead to a reduction in surface hydrophobicity due to intermolecular and intermolecular
585 crosslinking (Ercili-Cura et al., 2015) indicating that TG reduced binding of hydrophobic
586 regions. Up to 31 $m^2/g$ improvement in EAI after TG treatment of native FPI was observed
587 (Nivala et al., 2021). A 70% decrease in solubility for FBP has been observed by Nivala et
588 al.(Nivala et al., 2017) following crosslinking with TG. The effect of microbial
589 transglutaminase cross-linking with FBPI was investigated by (Liu, C. et al., 2019) to
590 improve the physical and oxidative stability of the O/W emulsion. MTG treatment increased
591 the surface charge by 8% as well as increased emulsion particle size by 19-135%. The
592 emulsion's emulsifying activity and physical stability were decreased as a result of the MGT
593 treatment's rise in surface hydrophobicity after 120 and 240 minutes. Faba bean legumin
594 following cross-linking by dimethylsuberimidate showed an increase in surface hydrophobicity
595 while foaming and emulsification properties were negatively impacted (Krause, Dudek, &
596 Schwenke, 2000).

597 *7.3 Ultrasound treatment*

598 Novel technologies such as high-intensity ultrasound treatment in food applications especially
599 biopolymer modification have been increasing (Arzeni et al., 2012). Functional properties
600 such as gelation, emulsification and formability have shown improvement following High-
601 intensity ultrasound treatment. Such improvement in functionalities has been attributed to
602 several factors such as thermal effect, cavitation, shear stress, agitation as well as turbulence
603 which cause physicochemical changes in protein or other molecules (Güzey, Gülseren,
604 Bruce, & Weiss, 2006).

Sonicated faba bean isolate (SFBI) solubility ranged from 25.25 to 44.33 % and while NFPI was 19.87 %. High amplitude and shorter times showed higher solubility (Martínez-Velasco et al., 2018). The high solubility of ultrasound treatment over untreated protein isolate results from the small particle size of SFB enabling proteins to have a larger contact area (Liu, S. et al., 2016). OFPI and NFPI both showed a reduction in surface tension over time at the air interface indicating strong surface-active properties which can be observed during the first seconds. However, OFPI showed a greater decrease in surface tension compared to NFPI which indicates that ultrasound treatment had a greater effect in improving adsorption (Martínez-Velasco et al., 2018). Improvement in surface tension in OFPI is attributed to a reduction in net ζ-potential which results in electrostatic repulsion hence promoting increased adsorption rate (Martínez-Velasco et al., 2018) and was attributed to the smaller particle size of protein molecules creating higher surface activity and mobility at the interface.

*7.4 PH shift*

Different foods vary in their acidity levels which are impacted by processing conditions and raw materials used. Several foods such as mayonnaise and salad dressing with a pH of 4.5 or less rely on acidification in other to produce desired products. Modification of protein conformation using pH shift based on alkaline or acidic pH is used in food processing to improve techno-functional properties. Alkaline shift treatment is an approach used in the modification of proteins and their corresponding functionality. Usually, protein solutions are exposed to extremely high or low pH and adjusted back to neutral. In alkaline shifting, the protein solution is subjected to a pH adjustment that is very alkaline before being neutralised. At high pH beyond the isoelectric point, protein unfolding occurs exposing buried hydrophobic regions. Conformational changes at this point are not reversible by shifting the pH back to 7.0

hence a molten globule structure is formed which is highly flexible (Jiang, Wang, & Xiong, 2018; Tian et al., 2020).

Ultrasound treatment combined with controlled alkaline treatment was studied by Alavi et al. (Alavi, Chen, & Emam-Djomeh, 2021) to improve the functional properties of faba bean protein isolate (FBI). The ultrasound treatment aided alkaline shifting resulted in the dissociation of large FBI aggregates into smaller units with an increment in surface hydrophobicity. Furthermore, there was an improvement in FBPI solubility from 12.2 to 40.4 % to more than 95% at pH 3 and 7. Also, the foaming capacity showed a significant increase from 93 % to 306-386% and stability from 10 s to 473-974s. Improvement in protein solubility was attributed to a reduction in particle size, breakdown of non-covalent interactions (mechanical forces from ultrasound treatment) and weakening of hydrogen bonding. However, improved foaming was attributed to small particle size, high solubility, and increased surface hydrophobicity (decreased interfacial tension to enable the protein to easily adsorb at the air-water interface).

Sharan et al. (Sharan et al., 2021) found that pH application during utilization and ingredient modification at different pH has an important influence on faba bean concentrate during ingredient processing and application as shown in **Fig 5**. Principal component analysis showed that functionalities such as foaming are mostly influenced by pH used during processing while on the other hand pH modification of FBC greatly influenced emulsion properties. As evidence in the PCA, differences arising from pH during utilisation is from the first to third quadrant with foaming properties along the second principal component while the emulsification properties are in the first principal component. Foaming and emulsification properties was strongly influenced by zeta potential and nitrogen solubility, thus the evidence that modification of physiochemical properties affecting protein functionality. The relationship

between process condition, variations in protein and non-protein components, and their impact on emulsion and foam characteristics is clearly seen in **Fig. 6**.

## 8. Faba bean protein bioactivity and allergenicity

Bioactive peptides are short-chain amino acid sequences released from precursor protein via enzymatic digestion that can interact and modify specific sites thereby conferring several physiological benefits beyond normal nutrition (López-Barrios et al., 2014; Möller et al., 2008). Faba bean derived peptides, using controlled hydrolysis, have been studied in various research works and have been summarized in **Table. 4**.

Inhibition of angiotensin converting enzyme (ACE), anticarcinogenic, antioxidant, hypocholestrolemic effect, antimicrobial activity, tyrosinase inhibitory activity and serum glucose regulation has been evidenced in faba bean peptides. Bioactive peptides (BPs) are generated during gastrointestinal digestion; however, in vitro methods employ gastrointestinal enzymes such as trypsin, pepsin, and pancreatin (Felix, Cermeño, & FitzGerald, 2021; Jakubczyk et al., 2019a; Karkouch et al., 2017a; León-Espinosa et al., 2016; Samaei et al., 2020a). (León-Espinosa et al., 2016) subjected FBC to enzymatic hydrolysis in a sequential order first with trypsin followed by chymotrypsin and pancreatin. Among the enzymes used, trypsin showed the highest antioxidant activity in comparison with the other enzymes for hydrolysates obtained. Mice fed FBH displayed a decrease in atherogenic markers induced by HCD (High Density lipoprotein Cholesterol) which indicate the presence of bioactive peptides. An interesting observation indicated that reduction in atherogenic markers was achieved at a low dose (10 mg/kg).

A similar work by Ashraf et al. (Ashraf et al., 2020) involved exposure of FBI to sequential in vitro-gastrointestinal digestion using pepsin and trypsin with and without heat

675 treatment. Hydrolysates produced from heated treated FBI showed a higher degree of
676 hydrolysis compared to unheated FBI. Size exclusion chromatography of the hydrolysates
677 showed peptides fractions ranging from 500-1000 Da with a high concentration of lower
678 fraction (1-3 kDa). Peptides obtained from the study showed excellent scavenging activity
679 using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as well as the potential to reduce $Fe^{3+}$ to
680 $Fe^{2+}$. To evaluate the cholesterol lowering activity, an in-vitro cholesterol micelle model was
681 employed. There was a noticeable increase in the inhibition of cholesterol solubilization into
682 micelles which was attributed to the presence of high concentration of hydrophobic amino acid
683 and aromatic side chains (Ajibola, Fashakin, Fagbemi, & Aluko, 2013; Karkouch et al.,
684 2017a).

685 Karkouch et al. (Karkouch et al., 2017b) isolated and identified several peptide
686 sequences from FBH using strong cation exchange chromatograph followed by LC-MS/MS
687 with orbitrap hybrid mass spectrometer. The following seven peptides, designated P1 through
688 P7, were discovered:
689 GGQHQQEEESEEQK(P1),ENIAQPAR(P2),IINPEGQEEEEEEEEEK(P3),GPLVHPQSQS
690 QSN(P4), LSPGDVLVIPAGYPVAIK(P5), VESEAGLTETWNPNHPELR(P6), and
691 EEYDEEKEQGEEEIR(P7). Among these peptides, five were found to possess antioxidant
692 activity with P6 having the highest radical scavenging ability. This was ascribed to the presence
693 of aromatic amino acid residue (Trptophan) as well as Valine at the N-terminal (Li et al., 2011).
694 Peptide P5 LSPGDVLVIPAGYPVAIK exhibited ferrous chelating ability while P7, P6 AND
695 P1 demonstrated inhibition of *P. aeruginosa* biofilm formation.

696 8.1 *Allergic reaction to faba bean pyrimidine glycosides*

697 Despite the numerous advantages of faba bean seeds, their production and utilization have
698 historically been constrained because they contain the pyrimidine glycosides vicine and

convicine, which are present at roughly 1% dry matter in the cotyledons of most FBS (Purves, Zhang, Khazaei, & Vandenberg, 2017).

Degradation of β-glycosidic linkages leads to the transformation of vicine and convicine into their corresponding aglycones respectively divicine and isouramil. Hydrolysis occurs either through enzymatic action (β-glucosidase) during seed germination or by microbial action in the intestine (Rizzello et al., 2016). These generated aglycones lead to a condition called favism characterised by haemolytic anaemia (Duc, 1997; Reading et al., 2016). This condition is prevalent in people with deficiency in glucose-6-phosphate dehydrogenase(G6PD). G6PD's function is to defend against oxidative stress in cells by creating reduced nicotinamide adenine dinucleotide and replenish reduced glutathione hence reduction in their activity leads to oxidative stress resulting in in a condition haemolytic anaemia (Rizzello et al., 2016).

*8.2 Technologies used to reduce allergic proteins.*

Vicine and convicine are heat stable, however their concentrations can be lowered substantial using different processing methods. Pre-processing techniques such as soaking, roasting, boiling, microwaving, fermentation, irradiation, and frying can reduce the content of vicine and convicine in faba beans (Cardador-Martínez et al., 2012; Hussein, Motawei, Nassib, Khalil, & Marquadt, 1986; Rizzello et al., 2016).

In addition, alkaline extraction followed by isoelectric precipitation can also reduce the content of vicine and convicine content, however this method may be costly and require high amount of intensive energy. FBPI produced showed a ratio of vicine to protein to be approximately 0.034 to 100 w/w, indicating 96-99% lower vicine content (Johns & Hertzler,

2021) compared to ratio of vicine to protein in whole faba beans (Goyoaga et al., 2008; Hegazy & Marquardt, 1983) . The method of production of FBPI caused a substantial reduction (96-99 %) in convicine content, in each step of the extraction process, the aqueous medium dissolve alkaloids and hence can further be separated from the protein following centrifugation. Currently breeding has been targeted as an approach to reduce the content of v-c and this could represent possibly the best solution.

## 9. Emerging technologies for modification

Several other emerging technologies have enormous potential to improve the techno-functional properties of proteins. High-pressure processing (Bouaouina, Desrumaux, Loisel, & Legrand, 2006; Hall & Moraru, 2021; Yang et al., 2018). Other strategies include High hydrostatic pressure, irradiation, filtration, supercritical carbon dioxide, plasma technology, electric fields, and ultrasonication are all gaining popularity. More research is needed in this area to understand processing conditions and their influence on functionality on faba bean ingredients.

## 10. Conclusion

The food and nutraceutical industries are increasingly turning to faba beans as a source of protein-rich material. The demand for faba bean protein is projected to grow drastically due to increasing consumer interest in products from natural sources. Faba bean proteins functionalities and bioactivities have been proven by a myriad of research to be a viable source of protein and can be successfully incorporated into myriad food products. The functionalities and physicochemical characteristics of FBP was reviewed. In addition, FBP and its bioactivities have also been discussed. This review provides a steppingstone for the production and commercialization of faba bean protein. More studies are needed to investigate the

structural-functionality relationship of FB isolates, particularly its subunit and the impact of processing conditions. Despite being nutritional, native faba bean protein's poor solubility restricts its use in food systems for specialised purposes. To improve faba beans protein solubility and diversify its application, structure-modifying technologies must be thoroughly investigated using emerging technologies.

## Declaration of competing interest

The authors declare no conflict of interest.

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## Abbreviations

DP Degree of polymerization

$\Delta H$ Enthalpy

TDF Total dietary fibre

GAE Gallic acid equivalent

TPC Total phenolic content

SDS-PAGE Sodium dodecyl-sulphate polyacrylamide gel electrophoresis

FBC Faba bean concentrate

764 FBI Faba bean isolate

765 Cys Cysteine

766 Met Methionine

767 BV Biological Value

768 PER Protein efficiency ratio

769 SAA sulphur-containing amino acids

770 EAA essential amino acids

771 BPs Bioactive peptides

772 FBH Faba bean hydrolysate

773 ACE Angiotensin I-converting enzyme

774 G6PD Glucose-6-phosphate dehydrogenase

775 RFO Raffinose family oligosaccharide

776 TIU Trypsin inhibiting unit

777 ANS as 8-Anilinonaphthalene-1-sulfonic acid

778 $T_d$ Denaturation temperature

779 WHC Water holding capacity

780 IEP Isoelectric precipitation

781 FC Foaming capacity

782 OBC Oil binding capacity

783 EAI emulsifying activity Index

784 ESI emulsifying stability Index

785 DPPH 2,2-diphenyl-1-picrylhydrazyl

786 ORAC oxygen radical-absorbance capacity

787 MTG microbial transglutaminase

788 GABA γ-aminobutyric acid

789 WHO World Health Organization

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**Fig 1.** Faba beans tree and parts. Faba bean tree **A**; **B** fresh seed; dried seeds **C** and protein extract **D**.

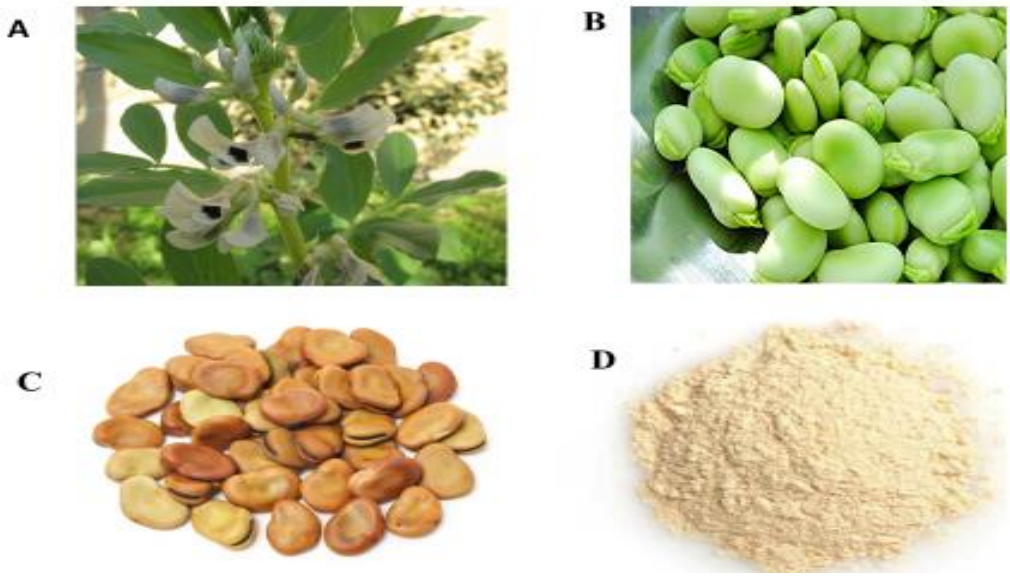


**Fig 2:** Faba bean protein SDS-PAGE analysis at various pH levels (2.5, 5.0 and 8.0)(Felix et al., 2018)

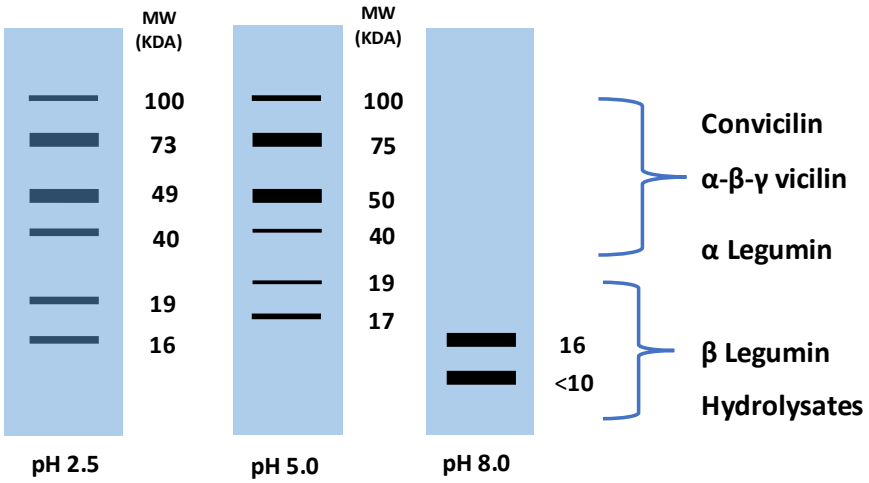


**Fig 3.** (A) Faba bean proteins after being homogenised under high pressure and at 22°C exhibit intrinsic fluorescence spectra. Intrinsic fluorescence spectra from 320 to 340 nm are shown in the inset image (Yang et al., 2018) ; (B) FTIR spectra of amide regions of native FBPI and sonicated Faba bean protein isolate (Martínez-Velasco et al., 2018).

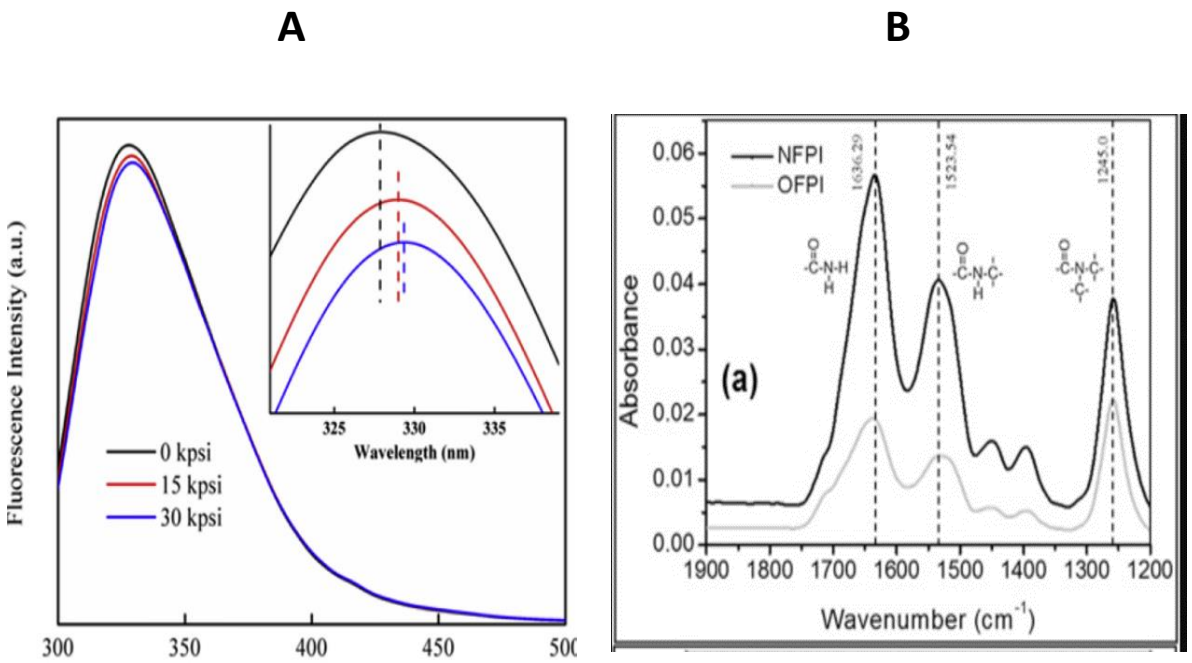

**Fig 4. (A)**Thermal curve of FBI and isolated storage proteins, legumin and vicilin in 0.05M Nacl (Arntfield et al., 1986); (**B**) Protein solubility profile of faba bean concentrate and isolate at different pH (Vogelsang-O'Dwyer et al., 2020).

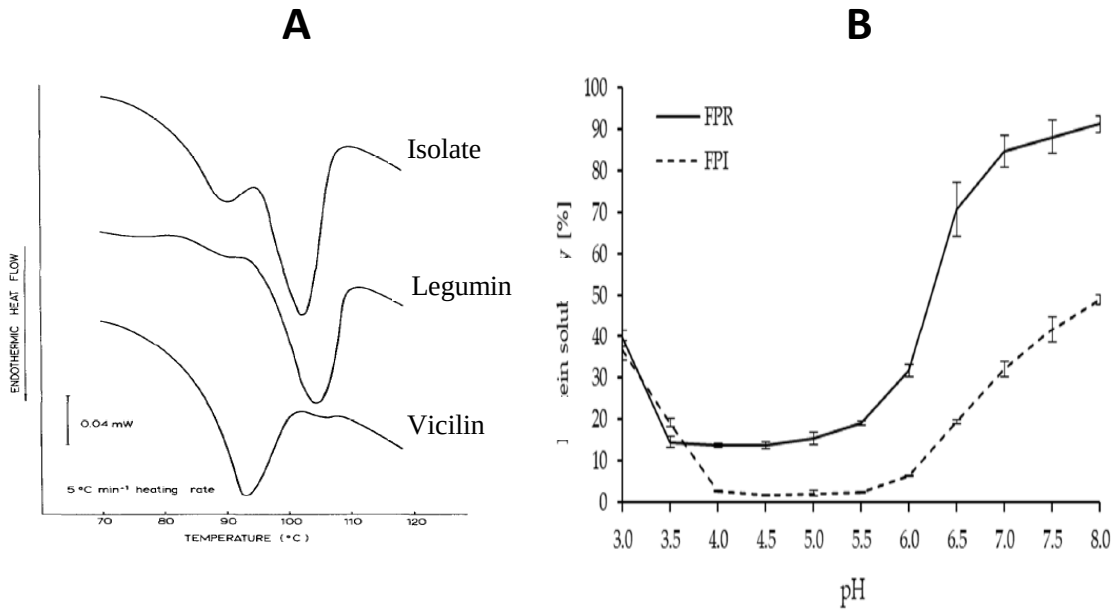


**Fig 5.** Confocal images of oil/water emulsion using FBC(FBP60), deflavoured concentrate (DefFBP60) and faba bean protein isolate (FBPI) at pH 2 and 7 (Keivaninahr et al., 2021).

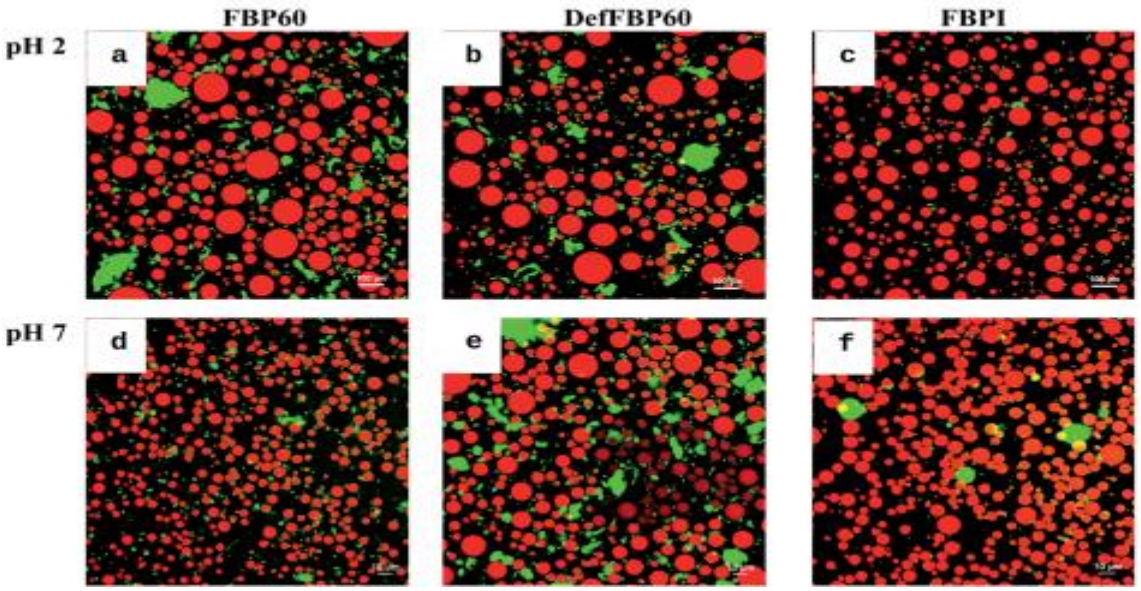

**Figure 6.** Principal component Analysis of faba bean ingredients evaluated at two conditions (pH 4 and 7). The impact of pH during modification on physicochemical and functional properties (foam and emulsion) is shown by different symbols (Sharan et al., 2021).

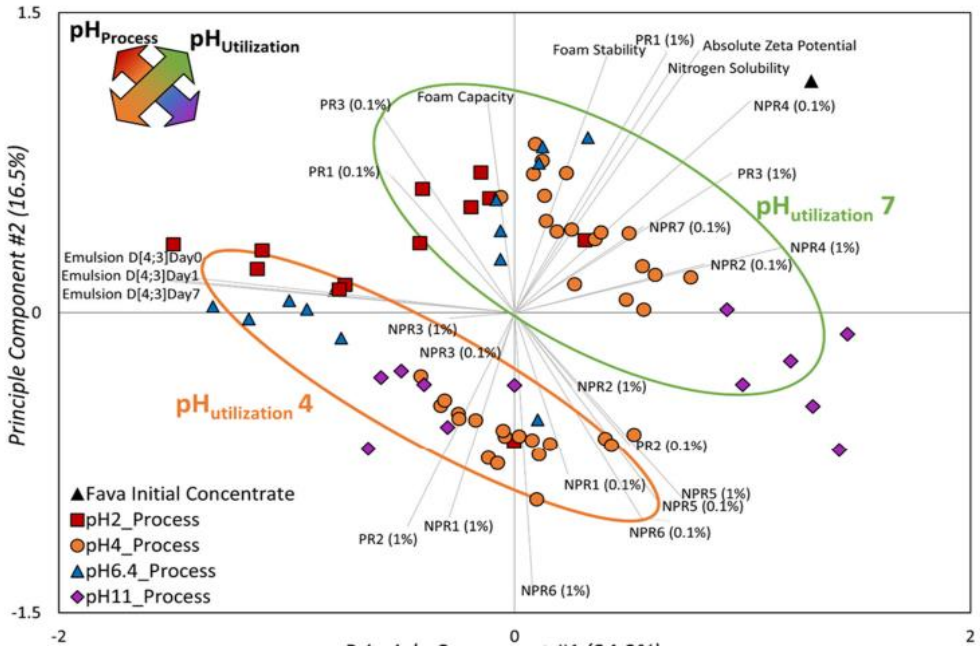


**Fig. 7.** Skeletal structure of vicine and convicine

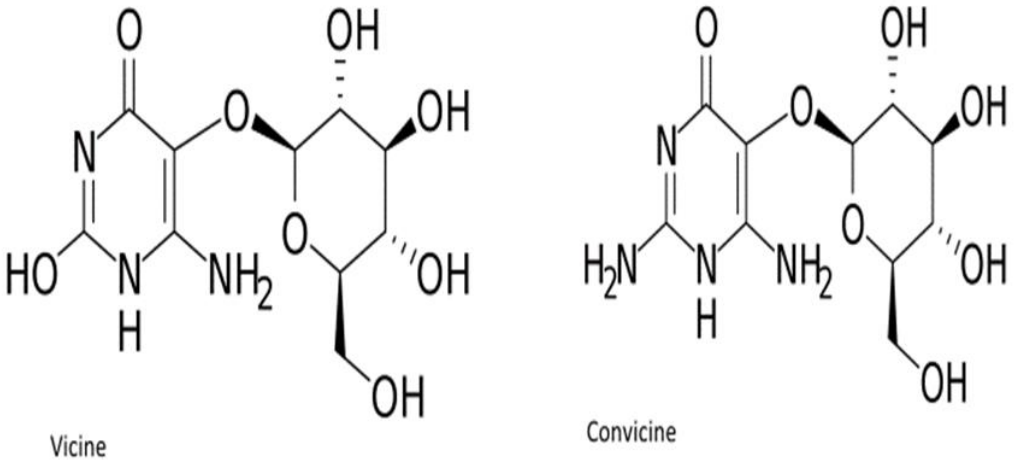


**Table 1**. Chemical composition (on % DM basis) of faba bean ingredients with other plant-based proteins. (n.d not determined; ISP Isoelectric precipitation)

| Sample | Protein | Fat | Ash | Carbohydrate | Fibre | Reference |
|---|---|---|---|---|---|---|
| **Faba bean flour** | 25.70 | 1.69 | 2.56 | 58.79 | n.d | (Kumar et al., 2021) |
| **Faba bean flour** | 30 | 1.7 | n. d | 63.3 | 26.7 | (Raikos et al., 2014) |
| **Wheat flour** | 12.6 | 1.4 | n. d | 68.5 | 3.1 | (Raikos et al., 2014) |
| **Green pea flour** | 26.7 | 0 | n. d | 60 | 26.7 | (Raikos et al., 2014) |
| **Faba bean Concentrate** | 64.1 | 2.43 | 4.8 | 28.7 | n.d | (Vogelsang-O'Dwyer et al., 2020) |
| **Faba bean Concentrate (Densification)** | 56.4 | 4.6 | 4.7 | 29.9 | n. d | (Felix et al., 2018) |
| **Faba bean Isolate (ISP)** | 90.1 | 4.36 | 5.2 | 0.34 | n.d | (Vogelsang-O'Dwyer et al., 2020) |
| | 92.4 | <0.1% | 3.2 | 4.4 | n. d | (Vioque et al., 2012) |
| **Whey protein isolate** | 86.8 | 0.03 | 0.6 | 5.8 | n. d | (Keivaninahr et al., 2021) |
| **Chickpea isolates** | 85.76 | 0.83 | 4.41 | 6.89 | n.d | |
| **Soy protein isolate** | 90.86 | 0.00 | 2.19 | 0.54 | n. d | (Johnston et al., 2014b) |

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1286 **Table 2.** Amino acid composition (% w/w) of faba bean ingredients and other protein sources

| **Amino acids** | **Concentrate** | **Protein Isolate** | | **Protein Fraction** | | **Other Protein** | | **FAO/WHO suggested requirement** | |
|---|---|---|---|---|---|---|---|---|---|
| | FBC[b] | FBI[b] Modified IEP | FBI[e] IEP | Legumin[g] | Vicilin[g] | SPI[h] | Casein[i] | 2–5-year old[J] | Adult[J] |
| Histidine | 2.39 | 3.49 | 2.80 | 2.44 | 1.95 | 2.81 | 2.70 | 1.90 | 1.60 |
| Isoleucine | 3.73 | 4.25 | 3.80 | 3.98 | 5.12 | 4.35 | 4.90 | 2.80 | 1.3 |
| Leucine | 7.10 | 8.09 | 8.0 | 7.84 | 9.21 | 6.79 | 8.40 | 6.60 | 1.90 |
| Lysine | 6.34 | 6.51 | 7.0 | 4.57 | 7.13 | 5.23 | 7.10 | 5.80 | 1.60 |
| Methionine | 0.60 | 0.54 | 0.100 | 0.59 | 0.31 | 0.92 | 2.60 | - | - |
| Phenylalanine | 4.13 | 4.68 | 4.90 | 3.56 | 5.20 | 5.14 | 4.50 | - | - |
| Threonine | 3.54 | 3.30 | 3.70 | 4.28 | 3.27 | 3.98 | 3.70 | 3.40 | 0.90 |
| Valine | 4.14 | 4.59 | 4.10 | 4.91 | 4.90 | 4.28 | 6.0 | 3.50 | 1.30 |
| Alanine | 3.85 | 3.94 | 4.40 | 6.10 | 4.87 | 3.72 | 2.7 | - | - |
| Arginine | 10.48 | 10.09 | 10.00 | 7.95 | 5.59 | 7.35 | 3.3 | - | - |
| Aspartic acid | 10.30 | 11.18 | 13.30 | 10.60 | 11.60 | 11.47 | 6.3 | - | - |
| Cysteine | - | 0.62 | 5.00 | 0.80 | 0.31 | 0.05 | 0.04 | - | |
| Glutamic acid | 16.25 | 17.96 | 19.90 | 16.40 | 15.30 | 20.67 | 19.0 | - | - |
| Glycine | 3.81 | 4.02 | 4.90 | 7.40 | 5.00 | 3.74 | 1.60 | - | - |
| Serine | 4.87 | 5.36 | 6.30 | 6.50 | 6.59 | 5.32 | 4.60 | - | - |
| Tyrosine | 3.05 | 3.74 | 2.63 | 2.61 | 2.59 | 3.61 | 5.50 | | - |
| Proline | 4.24 | 4.45 | 3.40 | - | - | 5.13 | - | - | - |

1287 Note. tryptophan was not quantified due to analytical challenges and low quantities. data obtained from
1288 b. (Vogelsang-O'dwyer et al., 2020), e (Vioque et al., 2012), g. (JACKSON et al., 1969), h. (Wang,
1289 X. et al., 2008), i (Tang et al., 2006), j. (Friedman & Brandon, 2001).

**Table 3.** Comparison of faba bean seed proteins functionality with other plant-based proteins

| Samples | Protein solubility (%) | Foaming capacity (%) | Foaming stability (%) | EAI($M^2$/g) or EC (%) | ESI(MIN) or ES (%) | Water holding capacity | Oil holding capacity | Gelling property | References |
|---|---|---|---|---|---|---|---|---|---|
| **Flour** | 1.70% at pH=4<br>11% at pH=7<br>12.5% at pH =10 | 40 at pH= 4<br>50 at pH= 7<br>70 at pH=10 | 2.7% at pH=4<br>5% at pH=7<br>7% at pH=10 | 12.5 $m^2$/g at pH=4<br>23.5 $m^2$/g at pH=7<br>38.2 $m^2$/g at pH= 10 | 33.6min at pH= 4<br>80min at pH= 7<br>135.4min at pH= 10 | 1.6 g/g at pH= 4<br>1.5 g/g at pH= 7<br>1.3 g/g at pH= 10 | - | LGC at pH 4,7 and 10 was 10% w/v | (Raikos et al., 2014) |
| **Concentrate** | 5% at pH =4<br>45% at pH = 7<br>55% at pH =9 | 85% at pH= 7 | 97% at pH=7 | 14 $m^2$/g at pH= 7 | 13min at pH= 7 | 2.5g/g at pH= 7 | 2.88g/g at pH= 7 | LGC at pH= 7 was 10% w/v | (de Paiva Gouvêa et al., 2023) |
| **Isolate** | 25 at pH = 7<br>2 at pH = 5 | 30% at pH= 5<br>65% at pH= 7 | 85% at pH=5<br>75% at pH=7 | 35 $m^2$/g at pH= 7 | 45min at pH= 7 | - | 5g/g at pH= 7 | - | (Eckert et al., 2019) |
| **Adzuki bean protein isolate** | 26.73 at Ph= 3<br>46.93 at PH= 7<br>69.66 at pH =8 | 350% at pH=8 | 66.6% at pH=8 | 60.7 $m^2$/g at pH=7 | 101.41min | - | - | - | (Barac, Pesic, Stanojevic, Kostic, & Bivolarevic, 2015) |
| **Soy protein isolate** | 50% at pH =3<br>60% at pH =7<br>80% at pH =10 | 25% at pH=7 | 90.54% at pH=7 | 48.2% | 47.5% | 60% | 311% | - | (Nunes et al., 2017) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Moringa seed protein** | 80% at pH =3<br>10% at pH =7<br>2% at pH =10 | 185% | 165% | 90% | - | - | 1.9g/g | - | (Jain et al., 2019) |

EAI, emulsion activity index; EC, emulsion capacity; ESI, emulsion stability index; FS, foaming stability; FC, foaming capacity; ES, emulsion stability

**Table 4**. Reported bioactivity of faba bean seeds and proteins

| Bioactivity | Study details | Reference |
|---|---|---|
| Antioxidation, in vitro and In vivo | FBH obtained from three enzymes (trypsin, chymosin and pancreatin) exhibited antioxidant activity (DPPH radical scavenging ability, $ABTS^{+}$) in mice. | (León-Espinosa et al., 2016) |
| | Peptides produced from fermented faba bean demonstrated varying antiradical activity indicated by $ABTS^{+}$<br>The fraction recovered from the sample fermented for three days at 30 °C showed the strongest antiradical activity (IC50 = 0.99 mg/mL). | (Jakubczyk et al., 2019b) |
| | Peptides produced from pepsin and trypsin exhibited a high scavenging activity. | |
| | FBH showed higher radical scavenging activity than that of the original substrate in ABTS and DPPH assay. Alcalase hydrolysates (4.19 mg/L) and combined pepsin and trypsin hydrolysates had the lowest IC50 values (indicating stronger chelating activity). Different enzyme hydrolysates contained a variety of antioxidant peptides. | (Ashraf et al., 2020) |
| | By using the TEAC assay, hydrolysates by pepsin at pH 3 produced antioxidant activity that was marginally better than that of hydrolysates of pepsin at pH 1.5. | (Samaei et al., 2020b) |
| | Following trypsin hydrolysis, the Faba bean peptides P5, P6, and P7, identified as LSPGDVLVIPAGYPVAIK, VESEAGLTETWNPNHPELR, and EEYDEEKEQGEEEIR, respectively, showed the strongest DPPH radical scavenging activity. | (Ali, 2019) |
| | After Alcalase hydrolysis, FBH at pH 8.0 displayed the highest antioxidant activity as evaluated by FRAP and ORAC assays. | |
| | FBH subjected to simulated gastrointestinal digestion demonstrated antioxidant properties using Hydroxyl Radical Assay, intestinal | (Karkouch et al., 2017b) |

| | | |
|---|---|---|
| | digestions, and most of them were able to inhibit $H_2O_2$ production too after SGID. | (Felix et al., 2021) |
| | The hydrolysates produced from alcalase exhibited high antioxidant activity and metal chelating activity while trypsin treatment showed lower DPPH radical scavenging activity. | (Dugardin et al., 2020) |
| | | (Seyedeh Parya Samaei, Mohammad Ghorbani, Alireza Sadeghi Mahoonak, & Mehran Aalami, 2020) |
| Hypocholesterolemic effects | FBH treated with trypsin showed a reduction in various atherogenic markers in male mice (10 mg/kg) | (León-Espinosa et al., 2016) |
| | Native faba bean peptides exhibit increased 3- hydroxy-3-methylglutaryl coenzyme A reductase (HMG Co-AR) inhibition (84.1 ± 2.7%) to thermally processed peptides (73.4 ± 1.7%). Heat treatment of the faba protein, which results in peptides that inhibit HMG Co-AR, had an impact on the enzymatic digestion of the protein. | (Ashraf et al., 2020) |
| Angiotensin I-converting enzyme (ACE) inhibition | Peptides fraction < 3kDa showed a higher potency against ACE than faba bean hydrolysates produced from a-amylase, pepsin and pancreatin hydrolysis. The peptide fraction obtained after fermentation | (Jakubczyk et al., 2019b) |

| | | |
|---|---|---|
| | for three days at 30 °C was reported to have the strongest ACE inhibitory activity (IC50 = 1.01 mg/mL).<br><br>Following in vitro simulated gastrointestinal, the FBH emulsions showed ACE inhibitory efficacy with 45% and 65% inhibition.<br><br>Peptides of FBH demonstrated a high good ACE inhibitor activity following simulated gastrointestinal digestion | (Felix et al., 2021)<br><br>(Dugardin et al., 2020) |
| Metal-binding | Among all the faba bean peptides synthesised only P5 peptide exhibited iron-chelating activity | (Karkouch et al., 2017b) |
| Serum glucose regulation | FBH generated a high dipeptidyl peptidase IV inhibitory potency when subjected to simulated gastrointestinal digestion. | (Dugardin et al., 2020) |
| Tyrosinase inhibitory Activity | Hydrolysate peptides P4 and P6 were found to be potent tyrosinase inhibitors.<br><br>The tyrosinase inhibitor potency of the hydrolysate made from immobilised protease was 1.6 times more than faba bean protein. By using RP-HPLC and HPSEXC, fraction F2, which had a high monophenolase inhibitor efficacy, was purified. | (Karkouch et al., 2017b)<br><br>(El-Sayed, Al-Azzouny, & Ali, 2019) |
| Antimicrobial Activity | With MBIC50 values ranging from 12 to 35 M, peptides P1, P5, P6, and P7 demonstrated remarkable antibiofilm efficacy against *P.aeruginosa*. | (Karkouch et al., 2017b) |

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