

ADVANCES IN PROTEIN EXTRACTION METHODS FROM FABA BEAN: A COMPREHENSIVE REVIEW

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Abstract

The extraction of proteins from faba beans is a key process in the food industry, driven by their high protein content and potential as a sustainable alternative to conventional sources. However, this process faces challenges, including the presence of anti-nutritional factors and the need to enhance protein recovery, purity, and quality. This review evaluates dry, wet, and hybrid extraction methods alongside pretreatment strategies, emphasizing their advantages, limitations, and industrial applications. Pretreatments, such as dehulling, soaking, and germination, are discussed for their ability to improve protein yield and reduce anti-nutritional factors. Dry extraction methods prioritize protein integrity, cost-efficiency, and sustainability, whereas wet methods excel in achieving high protein purity but are resource-intensive. Hybrid techniques, combining the strengths of dry and wet methods, are emerging as promising solutions to address existing limitations. This review also explores recent innovations in protein fractionation and processing technologies, with a focus on their potential impact on the food industry. Improving the sustainability and efficiency of protein extraction methods is vital to advancing their industrial adoption. By synthesizing current advancements, identifying research gaps, and proposing actionable insights, this work provides a roadmap for future studies and applications of faba bean protein extraction, contributing to sustainable food production.

KEYWORDS

Faba bean; plant-based protein; protein extraction; pretreatment; *Vicia faba* L

Introduction

In recent years, there has been a paradigm shift in global dietary patterns and agricultural landscape. This shift is driven by the growing recognition of the need for sustainable and nutritious food sources to meet the requirements of the world's growing population (which is expected to reach 10 billion by 2050, according to FAO), while mitigating environmental impacts (Boland et al. 2013; Henchion et al. 2017). To address these challenges, there has been a growing focus on investigating alternative protein sources. Pulses have emerged as strong candidates due to their nutritional value, environmental advantages, and versatility (Karkanis et al. 2018).

Among the diverse range of pulse crops, faba beans (*Vicia faba* L.) are particularly noteworthy due to their remarkable environmental and nutritional characteristics (Karkanis et al. 2018). Faba beans are known for their ability to fix atmospheric nitrogen through symbiosis with Rhizobia bacteria. This process enhances soil fertility,

reduces the need for synthetic nitrogen fertilizers, and mitigates greenhouse gas emissions (Guinet, Nicolardot, and Voisin 2020). The nitrogen fixation capacity of faba beans enhances the soil, benefiting not just the current plants themselves but also plants grown afterwards in rotation systems. This contributes to sustainable agricultural practices as highlighted by Multari, Stewart, and Russell (2015). Furthermore, compared to soybeans and peas, faba beans present a distinct environmental advantage. Faba beans have a deep root system that improves soil structure, reduces erosion, and enhances water infiltration, making them valuable in conservation agriculture. This legume has a smaller environmental footprint, positioning it as a more sustainable alternative for both human and livestock nutrition (Bangar and Kajla 2022).

From a nutritional perspective, faba beans are notable for their high protein content (20%–43%), substantial carbohydrates (~55%), dietary fibers, minerals (e.g. potassium, phosphorus, iron, and zinc), and vitamins (e.g. thiamin, riboflavin, and pyridoxine), while maintaining a low fat content (Boukid and Castellari 2022). It also offers a diverse array of health-promoting compounds, including peptides, phenolic compounds, and L-DOPA (Martineau-Côté et al. 2022). These bioactive constituents have been linked to various health benefits, including cholesterol-lowering, immune-modulating, and anticarcinogenic effects, underscoring the potential of faba beans as functional foods (Multari, Stewart, and Russell 2015). It is also gluten-free, which makes it an appealing option for consumers with dietary restrictions.

In addition to their environmental and nutritional benefits, faba beans exhibit valuable functional properties that make them versatile candidates for food formulations. Faba bean proteins possess foaming, emulsifying, water-binding, and gelling capabilities, contributing to the texture, stability, and sensory attributes of food products (Voisin et al. 2014). These functional characteristics give faba bean proteins great potential for a wide range of applications, including baked goods, meat analogues, dairy alternatives, and plant-based beverages. While their use in the food industry is still growing, their versatility makes them promising ingredients for the development of novel plant-based products (Boye, Zare, and Pletch 2010).

Due to this rising interest in utilizing faba bean protein, there is a need to investigate sustainable and efficient extraction methods that can isolate and concentrate proteins from faba bean seeds. The extraction of proteins from faba beans presents a challenge due to the complex composition of the bean matrix. Thus, extracting proteins from this complex matrix requires strategies to disrupt cell structures, solubilize proteins, and minimize damage to protein structures and functionality.

Conventional extraction methods, such as aqueous extraction and alkaline extraction, have been the primary approaches used for protein isolation from faba beans due to their simplicity, scalability, cost-effectiveness, and ability to achieve high purity while reducing anti-nutritional factors (ANFs). Aqueous extraction involves the use of water as a solvent to solubilize proteins from faba bean flour or protein concentrates, while alkaline extraction utilizes alkaline solutions (e.g. sodium hydroxide and potassium hydroxide) to enhance protein solubility by disrupting protein-ligand interactions. These methods raise concerns about resource consumption, environmental impact, and protein quality (Felix et al. 2018). In response to these limitations, the scientific community has increasingly turned its attention to dry methods, presenting a promising alternative to traditional methods. Mechanical processes such as milling, and air-classification are employed to isolate/concentrate protein fractions while minimizing water and chemical usage (Boukid and Castellari 2022). However, despite their potential advantages, dry extraction methods produce lower yields and less pure protein fractions, requiring further optimization for high protein yields and purity (Dangi et al. 2022), while the combination of wet and dry extraction is expected to result in milder process conditions that may help preserve the quality and functionality of native proteins.

Previous review works, such as those by Augustin and Cole (2022), Boukid and Castellari (2022), and Stone et al. (2024), have investigated various techniques for faba bean protein extraction, including alkaline extraction,

isoelectric precipitation, and dry extraction, demonstrating good yields. However, there remains a lack of comprehensive reviews that detail, compare, and evaluate these methods in terms of optimizing protein functionality and sustainability.

To address this gap in literature, this review aims to provide a comprehensive overview of recent advancements in faba bean protein extraction methods. The review will commence with an examination of the environmental and nutritional potential of faba beans, emphasizing their contribution to sustainable agriculture and human health. Subsequently, the focus will shift to pretreatments and protein extraction methods, evaluating both traditional wet techniques and emerging dry and hybrid approaches. This analysis will highlight the underlying principles, advantages, and limitations of each method, incorporating recent advancements in protein extraction technology. Finally, the paper will conclude by outlining future research directions for faba bean protein extraction and utilization and reflects on the potential impact of faba beans on sustainable agriculture and food production.

Faba beans: a promising protein source for human diets

AGRICULTURE AND BOTANICAL CHARACTERISTICS

Faba beans (*V. faba* L.) are a resilient cool-season legume, adaptable to diverse climatic and soil conditions, allowing for cultivation across various regions (Keneni and Ahmed 2016). However, their productivity is significantly impacted by abiotic stress factors, including drought, soil acidity, waterlogging, and inadequate cultural practices (Migdadi et al. 2016). Climate change has shifted cultivation patterns, decreasing the faba bean area in Egypt while expanding it in Sudan due to rising temperatures (FAOSTAT, 2016). Additionally, frost susceptibility during the reproductive phase remains a challenge, necessitating strategies like seedling hardening for improved cold tolerance (Arbaoui and Link 2008; Maqbool, Shafiq, and Lake 2010; Sallam, Martsch, and Moursi 2015).

In addition to its agronomic challenges, faba bean also faces environmental and economic limitations. European farmers were discouraged from cultivating faba beans due to their susceptibility to weather extremes, high disease and pest vulnerability, and volatility compared to other crops (Salmerón, Ávila, and Torres 2011). Although faba beans are resilient to cold, late frosts can damage blossoms and pods, highlighting the importance of suitable growing conditions (Flores et al. 2013; Karkanis et al. 2018). Faba beans are globally recognized for their nutritional significance, serving as a rich source of protein, carbohydrates, B-complex vitamins, and minerals (Dhull et al. 2022). Furthermore, the efficient nitrogen fixation capability of faba beans, which surpasses that of other pulses, underscores their ecological value (Mekkei 2014). The crop's ability to fix atmospheric nitrogen enhances soil fertility and reduces dependence on nitrogen fertilizers (Etemadi et al. 2018). This, coupled with their adaptability to various soil types and pH levels, positions faba beans as a sustainable option in cropping systems (Singh et al. 2013).

In conclusion, the faba bean holds immense potential as a sustainable and nutritious crop, despite facing various abiotic and biotic challenges. To unlock its full potential and contribute to a more sustainable and food-secure future, it is crucial to address these challenges through breeding programs for stress tolerance, integrated pest management strategies, and optimized agronomic practices.

NUTRITIONAL COMPOSITION

Faba bean seeds are a nutritious source of essential nutrients for human health. They contain high levels of protein, ranging from 20% to 43% of dry weight, with a favorable amino acid profile that includes lysine, an amino acid often lacking in cereal-based staple foods (Ayala-Rodríguez et al. 2022; Labba, Frøkiær, and Sandberg 2021; Millar et al. 2019; Multari et al. 2016). The reported protein content in faba beans varies depending on the nitrogen-to-protein

(N-to-P) conversion factor used across different studies. The standard factor of 6.25, based on the assumption that proteins contain 16% nitrogen, is commonly applied. However, some researchers (Mariotti, Tomé, and Mirand 2008) have suggested that a factor of 5.7 may be more appropriate for legumes, as it better accounts for their specific amino acid composition and the presence of non-protein nitrogen compounds. The choice of conversion factor can lead to significant variation in reported protein values, underscoring the need for consistency and transparency in methodology when comparing results across studies. While more precise estimations can be obtained through amino acid profiling, such approaches are less commonly used due to their complexity and resource requirements.

Faba beans contain a substantial amount of carbohydrates, ranging from 51% to 68%. Starch is the primary carbohydrate constituent, accounting for 22% to 45% of the total composition (Hoover and Sosulski 1991). Faba bean starch is classified as a high-amylose starch, with amylose content typically ranging from 30% to 40%, which is substantially higher than that of common cereal starches such as corn (~25%) or wheat (~20%). This high amylose level contributes to a higher gelatinization temperature, firmer gel structure, and greater resistance to enzymatic hydrolysis, making it particularly suitable for applications targeting slow digesting carbohydrates or resistant starch functionalities. Structurally, faba bean starch granules exhibit a mixed B- and C-type crystalline pattern, a hallmark of legume starches, in contrast to the A-type crystallinity predominant in cereal starches. The granule size of faba bean starch typically ranges from 20 to 40 μm (Ambigaipalan et al. 2011), which is moderate compared to pea starch (10–35 μm) and potato starch (20–100 μm), and this size distribution influences its hydration capacity, swelling power, and pasting behavior. These compositional and structural attributes position faba bean starch as a promising functional ingredient for food applications that require thermal stability, controlled digestibility, and enhanced textural properties.

Additionally, faba beans are rich in dietary fiber, including both soluble and insoluble fibers (Multari et al. 2016). They can contain dietary fiber ranging from 15% to 30%, which contributes to their role in promoting digestive health and aiding in weight management (Vidal-Valverde et al. 1998). While their lipid content is relatively low, ranging from 1.40% to 3.20%, faba beans contain triacylglycerols, phospholipids, and fatty acids such as oleic, linoleic, and linolenic acids, which contribute to their nutritional value (Moussou et al. 2019; Yoshida et al. 2009).

Furthermore, faba bean proteins demonstrate high digestibility and a balanced amino acid composition. Arginine is the most abundant amino acid across various genotypes (Nosworthy et al. 2018). Although methionine, cysteine, and tryptophan levels may be relatively lower in faba bean proteins, their amino acid profile complements cereals, which are deficient in lysine, thereby enhancing overall protein quality in mixed diets (Acevedo-Pacheco and Serna-Saldívar 2016; Martineau-Côté et al. 2022). However, enzyme inhibitors such as trypsin and chymotrypsin inhibitors can impair protein digestibility by inhibiting proteolytic enzymes in the gastrointestinal tract (Gueguen 1983). Various processing methods, including cooking, heating, fermentation, and germination, can reduce these inhibitors, improving protein digestibility and nutritional bioavailability (Multari, Stewart, and Russell 2015). Faba beans are rich in essential minerals, including sodium, potassium, calcium, copper, zinc, iron, manganese, magnesium, phosphorus, and sulfur. These minerals are crucial for various physiological functions in the human body (Khalil and Mansour 1995; Nosworthy et al. 2018). Additionally, they contain bioactive compounds such as polyphenolic antioxidants, including L-3,4-dihydroxyphenylalanine (L-DOPA), which has been studied for its potential therapeutic effects in treating Parkinson's disease (Barakat et al. 2017; Turco, Ferretti, and Bacchetti 2016; Singh et al. 2013).

Additionally, research indicates that faba beans have anticarcinogenic properties, making them a valuable addition to cancer-preventive diets (Mudryj, Yu, and Aukema 2014). Moreover, the amino acid profile of faba beans complements the dietary requirements of essential amino acids in adults when consumed alongside cereals, highlighting their potential as a staple food (Polak, Phillips, and Campbell 2015).

ANTI-NUTRITIONAL FACTORS (ANFS)

Despite their nutritional benefits, faba beans contain several anti-nutritional factors (ANFs) or antinutrients that can compromise their nutritional quality and pose health risks to consumers.

Vicine and convicine are unique ANFs found specifically in faba beans and belong to a class of pyrimidine glycosides. They are responsible for favism, a hemolytic anemia affecting individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Bressani and Elías 1988). G6PD is an enzyme essential for maintaining the integrity of red blood cells. When individuals with G6PD deficiency consume faba beans containing high levels of vicine and convicine, it can trigger hemolysis, leading to the destruction of red blood cells and associated symptoms like fatigue, jaundice, and anemia. Fortunately, breeding programs have successfully developed faba bean cultivars with significantly lower vicine and convicine content, mitigating the risk of favism for susceptible individuals (Khazaei et al. 2019).

Faba beans also contain other ANFs that can further reduce their nutritional value. Tannins are polyphenolic compounds that are mainly found in the seed coat. Studies have shown that they can reduce protein digestibility and hinder the absorption of minerals, especially iron and zinc (Ayala-Rodríguez et al. 2022). Moreover, faba beans contain phytic acid, protease inhibitors, lectins, and oligosaccharides that can cause flatulence, such as raffinose and stachyose. These compounds can interfere with nutrient absorption and digestion, leading to reduced bioavailability of essential nutrients and gastrointestinal discomfort (Kumar et al. 2021).

Various processing methods can be used to mitigate the effects of ANFs and improve the nutritional quality of faba beans. Traditional techniques, such as soaking (Hussein et al. 1986), germination, fermentation (Pulkkinen et al. 2019), and cooking (Khazaei et al. 2019), have been shown to reduce the levels of antinutrients, including vicine and convicine, in faba beans. By combining traditional processing techniques with modern breeding strategies, it is possible to produce faba beans with improved nutritional quality and reduced antinutrient content. This enhances their suitability for human consumption and promotes their inclusion in diverse diets.

SEED STORAGE PROTEINS

The protein content of pulse seeds, including faba beans, is influenced by numerous factors such as genotype, environmental conditions, and agronomic practices, leading to significant variability among different varieties and even within seeds from the same plant (Multari, Stewart, and Russell 2015). Faba beans accumulate large quantities of proteins during seed development, primarily stored in membrane-bound organelles called protein bodies (Duranti 2006). Faba beans rely on proteins to support seed germination and early seedling growth by providing essential amino acids and carbon skeletons (Duranti and Gius 1997). The major seed storage proteins in faba beans are globulins, which constitute 70%–79% of the total seed protein content. These proteins are soluble in diluted salt solutions but insoluble in water (Osborne 1924). They are rich in amino acids such as aspartic acid, glutamic acid, leucine, and arginine (El Fiel, El Tinay, and Elsheikh 2002). Other protein fractions include albumins (2%–20%), glutelins (1%–11.5%), and prolamins (2.7%–3.5%) (El Fiel, El Tinay, and Elsheikh 2002). Analytical centrifugation has identified two main types of globulins in faba bean seeds: the 11S legumin and the 7S vicilin (Wright and Boulter 1974). Legumin, which makes up approximately 40–45% of the total protein, has a molecular weight ranging from 300 to 400 kDa and comprises hexameric structures (composed of two trimeric subunits (legumin α and β) linked by disulfide bonds), while vicilin, which constitutes 20%–25% of the total protein, has a molecular weight of approximately 158–180 kDa and forms trimeric complexes (Stone et al. 2015; Warsame et al. 2020). The ratio of legumin to vicilin in faba bean seeds can vary depending on several factors, including genotype, environmental conditions during growth, and the extraction method used. During seed development, legumin is synthesized before vicilin, making it the dominant storage protein in mature faba bean seeds (Gueguen and Cerletti 1994). Minor

globulin components, such as convicilin, have been identified, although their precise role and relationship to vicilin remain areas of ongoing investigation (Warsame et al. 2020).

In conclusion, the seed storage proteins in faba beans, mainly globulins, are essential for seed development and contribute significantly to their nutritional value. It is crucial to understand the composition, structure, and variations of these proteins, particularly legumin and vicilin, to optimize their functionality and nutritional profile.

Faba bean processing: pretreatments and protein extraction methods

In this section, we discuss the processing of faba beans, focusing on the various pretreatments applied to enhance protein extraction, the milling techniques used to prepare the powder for extraction, and the different methods employed to efficiently extract proteins from the milled faba bean material. The overall process is illustrated in **Figure 1**, which provides a comprehensive overview of the steps and techniques involved.

PRETREATMENTS

Pretreatments play a crucial role in enhancing the quality and utilization of faba beans for further processing efficiency. Before processing, faba beans often undergo various pretreatments to improve their texture, reduce anti-nutritional factors, and increase digestibility (Agrawal, Panigrahi, and Eri 2024). Common pretreatments include physical pretreatments such as thermal (drying, roasting, microwaving, cooking, pressure cooking and autoclaving, and extrusion) and non-thermal (dehulling, soaking, gamma irradiation, and ultrasound), and biological pretreatments like germination, fermentation, and enzyme treatment (Rahate, Madhumita, and Prabhakar 2021). These pretreatments contribute to enhancing the protein separation efficiency (PSE) and are therefore important steps in the processing of faba beans for diverse food applications.

PHYSICAL TREATMENTS

Thermal treatments.

Drying. Drying is a crucial post-harvest treatment for seeds that significantly influences product quality, shelf life, and subsequent processing efficiency. In milling processes, achieving appropriate seed moisture levels is essential for maximizing efficiency. Common drying methods, such as sun-drying and hot-air drying, are widely used, with hot-air drying offering better control over final product characteristics. However, temperatures exceeding 60 °C during drying can negatively impact splitting efficiency, degrade sensory quality (Amiri Chayjan and Shadidi 2014) and cause protein denaturation, potentially affecting its functionality and digestibility. Despite these benefits, scaling up drying presents challenges related to energy consumption and specialized equipment, which can become prohibitively expensive for small producers. On an industrial scale, drying energy consumption ranges between 0.8 and 1.5 kWh/kg, depending on the drying technology used, significantly impacting operational costs and environmental footprints (Boukid and Castellari 2022).

Among artificial drying methods, fluidized bed drying has shown advantages, including uniform moisture content, enhanced energy efficiency, and cost optimization, making it a favorable choice for industrial-scale applications. Research conducted by Amiri Chayjan and Shadidi (2014) demonstrated that faba bean drying in a fluidized bed

follows a defined kinetic pattern, with optimal conditions at airflow velocities of 1.5–2.5 m/s and temperatures ranging from 50 °C to 70 °C for effective moisture removal.

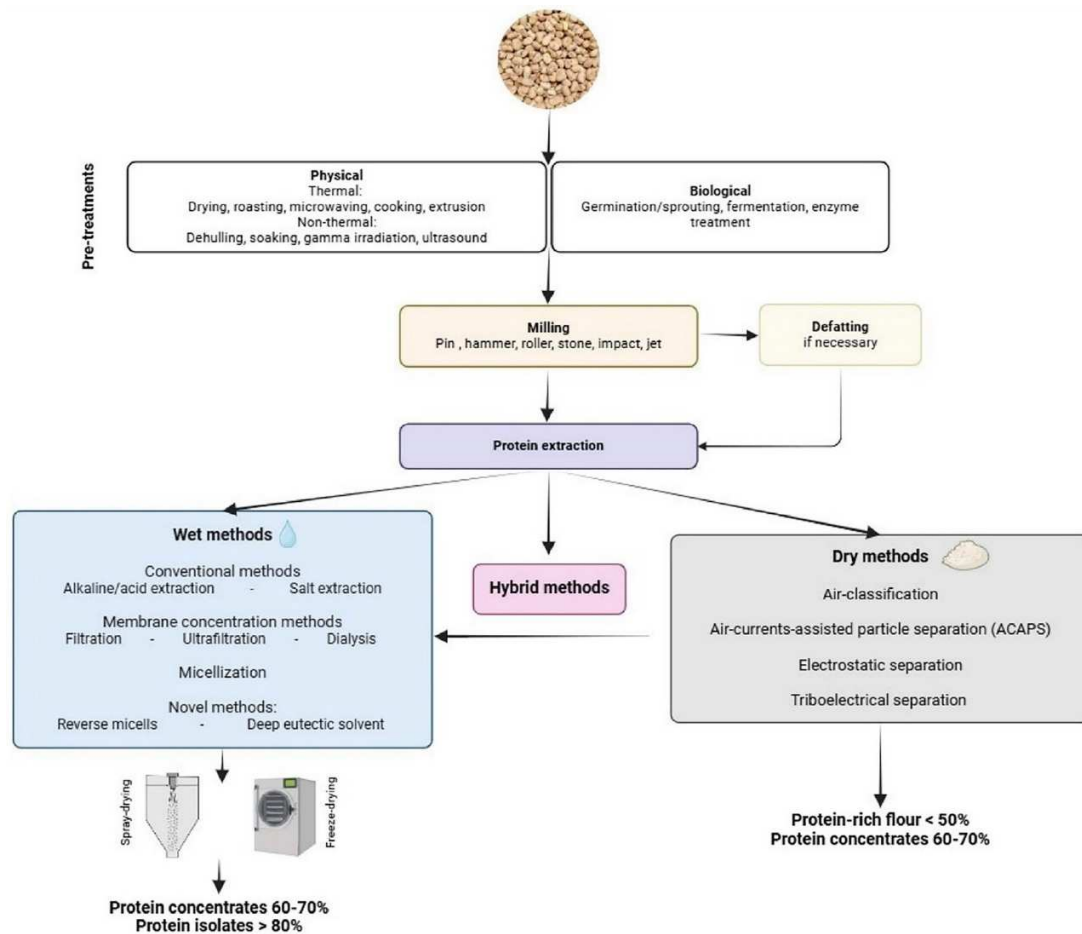


Figure 1. Overview of processing of faba beans: pretreatments, milling, and fractionation techniques.

Drying also facilitates effective separation of the hull from the endosperm, which is critical for hulling processes. Fernando (2021) found that a combination of tempering and drying at 50–55 °C effectively removed the seed coat while maintaining flour quality. However, lowering moisture content too much can reduce protein separation efficiency during subsequent fractionation steps, as reported by Pelgrom et al. (2013, 2015). This suggests that while drying enhances hull removal and milling yields, it requires careful optimization, especially in industrial settings, to balance protein integrity with cost-effectiveness and energy usage. **Roasting.** Roasting faba beans at 120 °C for 15 min has been shown to reduce trypsin inhibitor activity (TIA), thereby improving protein digestibility (Anderson et al. 1994). However, such high temperatures can also cause protein denaturation, particularly 7S vicilin and 11S legumin, typically denature at temperatures between 75 and 95 °C (Shevkani et al. 2019). Exposure to temperatures exceeding 100 °C may lead to irreversible structural changes, reducing protein solubility and altering functional properties. This process also results in a decrease in calcium and phytic acid content, possibly due to the formation of insoluble phytins that include phytic acid and certain minerals in the beans (Vidal-Valverde et al. 1998). Furthermore, roasting generates new phenolic compounds (Turco, Ferretti, and Bacchetti 2016), but its effect on vicine levels varies among cultivars, with some showing no change or an increase in vicine levels, while others experience significant reductions in both vicine levels (Oyeyinka, Adebó, and Kesa 2022). In addition, roasting is used as a heat pretreatment prior to dry milling, which reduces the levels of phenolic compounds and alters their specific profile (Siah et al. 2014), while also reducing vicine and convicine levels (Cardador-Martínez et al. 2012), although

less effectively than traditional cooking. However, both heat treatments eliminate L-DOPA, a heat-labile molecule, from the seeds (Cardador-Martínez et al. 2012; Siah et al. 2014).

Microwave.

Microwave heating has emerged as a promising pre-milling treatment for faba beans, offering numerous benefits that contribute to improving overall product quality. Studies by Jiang et al. (2016), Collado et al. (2020), and Pysz et al. (2012) have demonstrated the effectiveness of microwave treatment in reducing the levels of anti-nutritional factors in faba beans. These ANFs, including phytic acid, tannins, saponins, and trypsin inhibitors, are heat-sensitive compounds that are degraded during microwave heating, resulting in improved nutritional quality and reduced adverse effects upon consumption (Jiang et al. 2016). In addition, microwave treatment has been shown to improve the milling properties of faba beans by reducing seed hardness, thereby facilitating the milling process and resulting in the production of higher quality flour (Jiang et al. 2016). Furthermore, the inactivation of enzymes such as peroxidases and lipoxygenases by microwave heating plays a crucial role in minimizing off-flavors in faba beans, thus improving the sensory attributes of the final product. However, while microwave heating offers significant benefits, careful optimization of processing parameters is required to ensure that desirable effects such as improved protein digestibility are balanced against potential drawbacks such as excessive protein denaturation. In addition, achieving cooking uniformity remains a challenge associated with microwave processing. Understanding the underlying mechanisms of microwave heating, which primarily affects polar molecules such as water and ionic compounds, is essential for optimizing its application in faba bean processing and maximizing its potential benefits (Jiang et al. 2016; Collado et al. 2020; Pysz et al. 2012). Further research in this area is warranted to explore the full spectrum of effects and refine microwave processing techniques for optimal results in faba bean processing and utilization.

Cooking, pressure cooking, and autoclaving.

Cooking, using various methods such as pressure cooking and autoclaving, is a critical step in improving the nutritional profile and functional attributes of faba beans. Extensive research highlights the efficacy of high-pressure cooking in reducing antinutrient compounds such as convicine, phytic acid, tannin, trypsin inhibitor, and vicine, thereby promoting improved protein digestibility (Luo and Xie 2013). Furthermore, prolonged high-pressure cooking times enhance these reductions, resulting in improved protein digestibility (Sharma and Sehgal 1992; Luo and Xie 2013). In addition, cooking induces starch gelatinization, which increases starch digestibility while decreasing the proportion of resistant starch, ultimately promoting better nutrient absorption (Setia et al. 2019). Besides, pressure cooking has been found to preserve B vitamins better compared to traditional cooking techniques (Khalil and Mansour 1995; Oyeyinka, Adebo, and Kesa 2022). Autoclaving is another important cooking method that effectively reduces anti-nutritional factors such as phytic acid, tannins, trypsin inhibitors, vicine, and lectins, thereby enriching the overall nutritional value of faba beans (Osman et al. 2014). In addition, autoclaving, when combined with soaking and dehulling, has a synergistic effect that significantly increases *in vitro* protein digestibility (Luo and Xie 2013). The process also eliminates hemagglutinin activity and preserves more B group vitamins compared to conventional cooking methods, ensuring the preservation of nutritional value (Luo and Xie 2013; Sharma and Sehgal 1992). Functionally, both pressure-cooked and autoclaved faba beans exhibit superior properties, such as increased water solubility index and water absorption capacity, due to structural changes induced by processing, including pressure cooking (Kumar, Sadiq, and Anal 2022).

Extrusion.

Extrusion processing has emerged as a pivotal technique in the food industry, especially in enhancing the nutritional quality of plant-based proteins, with a focus on faba beans (Alonso, Aguirre, and Marzo 2000; Masoero, Pulimeno, and Rossi 2005; Hejdysz, Kaczmarek, and Rutkowski 2016). By subjecting raw materials to high temperatures and pressures within a high-moisture environment, extrusion effectively dismantles ANFs such as phytic acid, condensed

tannins, and polyphenols, thereby facilitating improved digestibility of proteins and starch (Hejdysz, Kaczmarek, and Rutkowski 2016). Research indicates that extrusion not only increases the bioavailability of amino acids but also mitigates *in vitro* protein degradability while markedly enhancing starch digestibility (Alonso, Aguirre, and Marzo 2000). Moreover, this process induces a rise in soluble dietary fibers and a reduction in various anti-nutritional factors, including trypsin inhibitors, chymotrypsin inhibitors, and α -amylase inhibitors. The utilization of preconditioning techniques before extrusion further reinforces nutritional enrichment by significantly reducing trypsin inhibitor levels (Adamidou et al. 2011). Widely adopted across the food sector, extrusion offers multifaceted benefits, including enhanced nutrient retention and textural refinement. Noteworthy is its superior performance compared to alternative thermal treatments like soaking and sprouting, particularly in terms of increasing protein and starch digestibility. As such, extrusion emerges as a pivotal process in food manufacturing, underscoring its pivotal role in optimizing nutritional outcomes while catering to consumer preferences for quality and palatability.

Non-thermal treatments.

Dehulling. The process of dehulling, or decortication, improves the economic value, sensory quality, cooking time, and digestibility of seeds such as faba beans by separating the seed coat from the cotyledons (Saldanha do Carmo et al. 2020; Singh et al. 2013). This process reduces anti-nutritional factors such as phytic acid, condensed tannins, and polyphenols, which slow nutrient absorption (Alonso, Aguirre, and Marzo 2000). Dehulling methods include abrasive dehullers for tightly attached hulls and attribution-type dehullers for lightly attached hulls (Fernando 2021). Pretreatments, including soaking, conditioning with water or oil, enzyme or chemical treatments, and hydrothermal treatments, facilitate decortication by loosening the adhesion between the seed coat and cotyledon, minimizing breakage and enhancing flour quality (Fernando 2021). Conditioning involves the addition of water to increase the moisture content of the seed, which facilitates the removal of the hull. Dehulling generally improves the nutritional profile by enhancing protein quality and digestibility. However, the effect of dehulling on phytic acid levels remains controversial, with conflicting results reported in the literature. While Alonso, Aguirre, and Marzo (2000) observed an increase in phytic acid levels following dehulling, Sharma and Sehgal (1992) reported a decrease, suggesting that variations in processing parameters such as soaking conditions may influence the outcome. Nevertheless, the agreement among researchers (Saldanha do Carmo et al. 2020; Fernando 2021) is that dehulling remains a valuable processing technique for improving the nutritional profile of pulses such as faba beans, offering numerous benefits in terms of improved protein quality, increased digestibility, and reduced antinutrient content.

Soaking.

Soaking faba beans prior to cooking serves as an important pretreatment method that has been documented to increase their nutritional value and digestibility (Revilla 2015). This technique triggers the activation of endogenous enzymes in faba beans, resulting in the degradation of ANFs such as phytic acid and lectins (Abdel-Aal et al. 2019). This degradation process consequently improves the bioavailability of minerals and reduces potential digestive problems associated with these antinutrients (Dhull et al. 2022). Furthermore, soaking has been shown to reduce the cooking time of faba beans and promote softening, resulting in a more desirable texture in the final product (Shi, Arntfield, and Nickerson 2018). However, it is important to recognize the potential drawbacks associated with soaking. This process can leach out water-soluble vitamins and minerals, such as folate and thiamin, as well as dissolve some proteins present in the faba beans (Hefni, Shalaby, and Witthöft 2015). The effectiveness of soaking in mitigating antinutrient levels has been shown to be influenced by several factors, including soaking duration, temperature, and the specific variety of faba bean being processed (Luo and Xie 2013). By carefully optimizing soaking conditions, processors can maximize the benefits of this pretreatment method while minimizing potential nutrient losses, ultimately achieving a balance between improved digestibility, reduced cooking time, and optimal nutritional content.

Gamma irradiation.

Gamma irradiation represents a promising technique for enhancing the nutritional value of faba beans (*V. faba* L.). The research demonstrates that irradiation can significantly diminish anti-nutritional factors, particularly tannins, thereby enhancing protein digestibility and bioavailability (Osman et al. 2014). The impact of irradiation on phytic acid is dependent on the dosage administered. Lower doses have been observed to reduce phytic acid levels, whereas higher doses may elicit an inverse effect (Osman et al. 2014). The application of gamma irradiation at safe doses has been observed to exert minimal effects on the overall mineral profile and proximate composition of faba beans (Osman et al. 2014; Dhull et al. 2022). Furthermore, studies by Rady et al. (2020) and Dangi et al. (2022) indicate that gamma irradiation enhances the antioxidant properties and phenolic content of faba beans, while maintaining the integrity of their constituents.

Nevertheless, several obstacles impede the industrial implementation of gamma irradiation. The necessity for specialized equipment and infrastructure contributes to a high capital investment and operational expenses, estimated at \$2–\$5 per kg (FAO 2020). As a result, it is less feasible in comparison to other pretreatments, particularly for small-scale producers. Additionally, consumer acceptance represents a significant challenge, as concerns about radiation safety and nutritional degradation persist despite assurances from regulatory bodies (Boukid and Castellari 2022).

Regarding the legislation, the use of gamma irradiation is subject to strict regulation, with permissible doses and labeling requirements varying between countries. The European Union permits the use of gamma irradiation for certain foods but requires clear labeling. In contrast, the United States allows the use of gamma irradiation for a broader range of products, with dose limits between 1 and 10 kGy for pulses (EU Regulation 1999/2/EC; US FDA 2020).

In summary, while gamma irradiation offers benefits such as reducing anti-nutritional factors and enhancing antioxidant properties, its high cost, limited consumer acceptance, and strict regulations limit its industrial scalability.

Ultrasound.

Ultrasound pretreatment has been widely studied for enhancing the extraction and functional properties of faba bean proteins. Scientifically, it has shown promising results, such as increasing protein yield, enhancing foaming capacity, and reducing particle size, which improves water solubility (Boukid and Castellari 2022; Kaya, Tuncel, and Yılmaz Tuncel 2017). Ultrasound also promotes the formation of protein-polyphenol conjugates, enhancing solubility, foaming capacity, and stability (Alavi, Chen, and Emam-Djomeh 2021). Martínez-Velasco et al. (2018) and Iscimen et al. (2023) further confirm that ultrasound causes structural modifications in proteins, thereby improving functional properties.

Even if ultrasound-assisted extraction (UAE) has been widely explored as a method to enhance protein extraction, its application can vary significantly depending on the stage of the extraction process and the physical state of the material being treated. For instance, ultrasound applied directly to dry flour helps disrupt cellular structures and improve protein release during subsequent steps (Martínez-Velasco et al. 2018). When used as a pretreatment before wet extraction, it facilitates cell wall breakdown and enhances solubility. UAE can also be applied in liquid suspensions, often in combination with alkaline or enzymatic treatments, to improve mass transfer and protein dispersion (Alavi, Chen, and Emam-Djomeh 2021). The technique's efficiency depends on ultrasound parameters such as frequency, power, duration, and treatment medium. If applied excessively, high-power ultrasound may cause protein denaturation, while optimized conditions can enhance extraction yields and functional properties (Iscimen et al. 2023). These studies demonstrate UAE's potential for improving extraction outcomes while reducing solvent use and processing time. However, the method's scalability and energy efficiency are significant challenges for real-world application, as large-scale systems require high energy input and uniform ultrasonic treatment, making the

process cost-intensive (Boukid and Castellari 2022). Moreover, ultrasound may reduce *in vitro* protein digestibility (Ouraji et al. 2020) and must meet strict regulatory standards for safety. Although promising in controlled laboratory settings, ultrasound pretreatment's industrial application faces obstacles including high operational costs and scalability issues, requiring further optimization to balance enhanced functionality with practical feasibility.

BIOLOGICAL TREATMENTS

Germination/sprouting.

Germination is a key process for enhancing the nutritional quality and sensory properties of food, particularly in pulses such as faba beans. This natural phenomenon activates endogenous enzymes, which catalyze biochemical transformations within the seeds. Studies show that germination reduces anti-nutritional factors such as phytic acid, polyphenols, and lectins, thereby improving the overall nutritional profile of pulses (Vidal-Valverde et al. 1998). In addition, germination increases the digestibility of major constituents such as proteins and starch. The enhancement of the starch granules' enzymatic breakdown and subsequent digestion is primarily attributed to the disruption of the protein and fiber matrix surrounding them (Setia et al. 2019). Moreover, the activation of endogenous phytase during germination contributes to the increased bioavailability of essential minerals, including iron, calcium, and copper. Phytase-mediated reduction in phytic acid levels can enhance the absorption of bound minerals in the digestive tract (Alonso, Aguirre, and Marzo 2000). This process also reduces flatulence-causing oligosaccharides and increases folate levels (Hefni, Shalaby, and Witthöft 2015; Dhull et al. 2022). However, germination of faba beans can produce toxins due to the enzymatic conversion of vicine and convicine into harmful metabolites (divicine and isouramil) (Wei, Wanasundara, and Shand 2022). These metabolites may cause hemolytic anemia (favism) in individuals with G6PD deficiency. It is important to note that while germination can improve certain functional properties of faba beans, such as *in vitro* protein digestibility, it may also lead to a reduction in the levels of specific essential amino acids, such as threonine (Setia et al. 2019). Nonetheless, the general agreement highlights germination as a valuable pretreatment method to enhance the nutritional quality and functional properties of pulses, especially faba beans.

Fermentation.

Fermentation is a safe and cost-effective method for improving the nutritional properties of faba bean flour and its fractions. This method, especially lactic acid bacteria like *Lactobacillus plantarum*, has become a powerful method for addressing these challenges and improving the overall nutritional quality of faba beans (Coda et al. 2015; Verni et al. 2019). Studies by Coda et al. (2015) and Verni et al. (2019) have shown that fermentation significantly reduces undesirable compounds (i.e. trypsin inhibitory compounds, phytic acid, tannins, vicine, and convicine), leading to improvements in nutrient digestibility and bioavailability. Furthermore, fermentation promotes the synthesis of essential amino acids, such as lysine and methionine, and the production of bioactive compounds, such as γ -aminobutyric acid (GABA), which is known for its health benefits, including blood pressure regulation. In addition, fermentation lowers the glycemic index of faba beans, making them suitable for individuals managing diabetes (Coda et al. 2015; Verni et al. 2019). Incorporating fermented faba bean flour into food products, such as bread and beverages, can enhance protein content and digestibility. This contributes to the development of functional foods with improved nutritional qualities (Coda et al. 2015; Verni et al. 2019). Fermentation is a powerful tool to unlock the nutritional potential of faba beans. It offers a safe, effective, and sustainable means to improve their utilization and contribute to global efforts toward enhanced food security and nutrition. However, fermentation may lead to microbial variability affecting consistency, and the development of undesirable acidic flavors if not well-controlled (Coda et al. 2015; Verni et al. 2019).

Enzyme treatment.

Enzymatic treatments have emerged as a promising approach for modifying the composition of faba beans, with the dual objective of enhancing flavor and reducing anti-nutritional factors. To enhance flavor, dextran produced via dextransucrase from *Weissella confusa* A16 was employed to effectively mask undesirable off-notes, including bitterness, pea odor, and astringency, while simultaneously enhancing sweetness and fruity characteristics. This was achieved by reducing lipid oxidation products and anti-nutritional compounds (Wang et al. 2024). Furthermore, enzymatic hydrolysis enhanced functional properties, which indirectly influenced flavor perception (Sharan et al. 2021). To mitigate the antinutritional effects of phytic acid and flatulence, the use of phytase, an enzyme known for catalyzing the hydrolysis of phytic acid, has been the subject of extensive study. A reduction in phytic acid levels has been demonstrated in studies using exogenous phytase, with variations influenced by enzyme dosage, incubation time, and pH conditions (Rizzello et al. 2019; Luo, Xie, and Cui 2010). Similarly, α -galactosidases were employed to reduce galactooligosaccharides, thereby addressing concerns related to flatulence (Nyyssölä et al. 2021). Phytase, derived primarily from microbial sources such as *Aspergillus* species, exhibits optimal activity at slightly acidic pH levels and enhances mineral bioavailability, thereby improving zinc and iron absorption (Rahate, Madhumita, and Prabhakar 2021; Luo et al. 2012). Moreover, the activation of endogenous phytase during the early growth stages of faba beans has also been demonstrated to reduce phytic acid levels (Oatway, Vasanthan, and Helm 2001). Despite its effectiveness, enzyme treatment in faba beans presents challenges such as high enzyme costs, the need for precise processing conditions, and regulatory constraints for large-scale applications (Rizzello et al. 2019; Wang et al. 2024). While these enzymatic approaches offer controlled reactions and minimal undesirable by-products, further research is needed to optimize processes for commercial-scale application and ensure regulatory compliance.

MILLING

Milling faba beans is of critical importance for the efficient separation of protein bodies from starch granules while preserving their integrity. The cotyledon cells contain starch granules (25–40 μm) within a protein matrix (1–3 μm), necessitating precise milling to avoid excessive starch damage. Various milling techniques, including pin milling, hammer milling, and jet milling, are employed, each with a distinct mechanism and impact on the material.

Pin milling, which employs impact forces against a rotating pin disk, is preferred for its capacity to produce fine flour with minimal starch damage (Schutyser et al. 2015). Hammer milling, which utilizes high-speed rotors with fixed or swinging hammers, generates considerable heat and consumes more power, potentially affecting material properties (Vitelli et al. 2020). Jet milling reduces particle size through high-velocity air streams, leading to minimal energy consumption and preserving protein functionality (Létang et al. 2002). Achieving an optimal particle size distribution is crucial for effective separation. Efficient separation occurs when the particle sizes of protein bodies and starch granules are distinct. Increasing the classifier wheel speed during ultrafine grinding can reduce particle size, but this may result in a loss of product yield due to the adherence of fine particles to the grinding chamber (Pelgrom et al. 2015). Material properties, including chemical composition, moisture content, and the adhesion between cellular components, significantly influence milling behavior. In faba beans, high crude fiber content, particularly in the seed coat, increases energy demand and reduces protein separation efficiency, making dehulling an essential preprocessing step. However, faba beans have relatively low fat content, which has minimal impact on milling efficiency compared to oil-rich legumes such as soybeans (Schutyser et al. 2015; Pelgrom et al. 2015; Tyler 1984).

In conclusion, dry milling of faba beans necessitates a meticulous equilibrium between the selection of optimal techniques and the optimization of parameters to achieve an efficient separation of protein and starch. An understanding of the interrelationship between milling methods and material properties is of importance to produce high-quality, protein-rich fractions for a multitude of food applications.

WET EXTRACTION METHODS

Wet pretreatments, such as soaking, fermentation, and germination, enhance wet extraction by improving protein solubility and reducing anti-nutritional factors, which is crucial for efficient wet fractionation.

Protein extraction from faba beans employs wet fractionation, which involves the dissolution of faba bean flour in alkaline solutions, followed by acidification for protein precipitation (Ouraji et al. 2020). Alternative methods, such as the use of dilute salt solutions and ultrafiltration, are employed for protein isolation (Boye, Zare, and Pletch 2010). Optimization studies focus on pH, temperature, and extraction time to enhance efficiency and minimize denaturation (Eckert et al. 2019). Research aims to improve protein functionality by studying properties like solubility and foaming capacity (Langton et al. 2020). Overall, the goal is to develop sustainable methods for producing high-quality faba bean protein isolates suitable for diverse food applications (Sharan et al. 2021).

ALKALINE EXTRACTION FOLLOWED BY ISOELECTRIC PRECIPITATION

The alkaline extraction followed by isoelectric precipitation method (AE-IP) represents a highly effective approach for isolating proteins from pulses, offering a significant degree of protein purity (~90%) (Langton et al. 2020; McCurdy and Knipfel 1990). Initially, proteins are solubilized in an alkaline solution, typically at a pH between 8 and 10, where the pH adjustment enhances protein solubility by inducing negative charges on the protein molecules (Boye, Zare, and Pletch 2010). Studies have demonstrated that protein solubilization rates can reach up to 85% at pH 10 compared to 76% at pH 7 (McCurdy and Knipfel 1990). This alkaline environment enhances protein dispersion stability, facilitating the removal of insoluble materials through centrifugation, typically at a solvent/feed (S/F) ratio of 5 to 10 (Boye, Zare, and Pletch 2010). Subsequently, the pH of the protein-rich supernatant is carefully adjusted to the isoelectric point of the desired proteins, typically between pH 4 and 5.5, leading to their precipitation (Boye, Zare, and Pletch 2010; Vioque, Alaiz, and Girón-Calle 2012). Protein recovery is reported to be higher at lower pH (approximately 4.0), but protein purity may decrease due to the co-precipitation of non-protein compounds (McCurdy and Knipfel 1990). The pH at which protein precipitation occurs plays a crucial role in determining both recovery yield and purity. Lower pH values (e.g. pH 4.0) are often associated with higher protein recovery, as more proteins reach their isoelectric point and precipitate efficiently. However, at such acidic conditions, there is also a greater tendency of co-precipitation of non-protein components, such as polysaccharides, fibers, and phenolic compounds, potentially lowering the final purity of the protein isolate. On the other hand, precipitation at slightly higher pH values (e.g. pH 4.5–5.0) may enhance purity by reducing unwanted co-precipitation, but this can come at the cost of lower protein recovery. Studies have shown that optimizing the pH balance between 4.2 and 4.8 can improve purity while maintaining an acceptable recovery rate. In addition, factors such as the ionic strength of the extraction medium, solvent-to-feed ratio, and centrifugation conditions also influence the selectivity of protein precipitation. Moreover, the presence of phytochemicals and other anti-nutritional factors can further impact purity. Some studies suggest that an additional washing step after precipitation can help remove residual contaminants, improving the protein purity without significantly reducing recovery. Alternative fractionation techniques, such as ultrafiltration or membrane filtration, are often used in combination with isoelectric precipitation to enhance purity while retaining a high yield. Overall, while lower pH favors higher recovery, a balance must be achieved to optimize both purity and functionality for industrial applications. Centrifugation is then employed to separate the precipitated proteins from the solution, followed by washing, neutralization, and drying steps to obtain the final protein isolate (Flink and Christiansen 1973). This method offers several advantages, including high protein purity and efficient protein recovery. It is therefore a valuable technique for industrial protein isolation processes. However, this method also has disadvantages, including high water and chemical consumption, potential protein modifications due to extreme pH conditions, and environmental concerns related to wastewater disposal (Boye, Zare, and Pletch 2010).

A multitude of studies have been conducted to investigate the details of this method, employing a variety of pretreatment parameters with the objective of optimizing the extraction process. For example, various pretreatment steps, including dehulling, defatting, and specific pH adjustments, have been employed to enhance protein extraction efficiency in faba beans (Ayala-Rodríguez et al. 2022; Cepeda, Villarán, and Aranguiz 1998; Fernández-Quintela et al. 1997; Hewage et al. 2024; Karaca, Low, and Nickerson 2011).

In these studies, the protein content of the resulting isolates exhibited a wide range, spanning from 81.2% to 97.41%, with corresponding yields varying between 37% and 74.35%. Such variability underscores the importance of optimizing extraction conditions to achieve desirable outcomes (Ayala-Rodríguez et al. 2022; Cepeda, Villarán, and Aranguiz 1998; Fernández-Quintela et al. 1997; Hewage et al. 2024; Karaca, Low, and Nickerson 2011). Furthermore, the solubility of the extracted proteins has emerged as a critical factor influenced by pH and solvent selection. Studies have reported maximal solubility at low acidic pH and high alkaline pH, further emphasizing the need for precise control over extraction conditions to attain desired protein characteristics (Ayala-Rodríguez et al. 2022; Cepeda, Villarán, and Aranguiz 1998; Fernández-Quintela et al. 1997; Hewage et al. 2024; Karaca, Low, and Nickerson 2011). Moreover, the physicochemical properties of the protein isolates have been a subject of considerable investigation, with some studies indicating enhanced surface charge and emulsifying properties compared to others. Such variations underscore the intricate effects of extraction conditions on the final protein product, underscoring the necessity for thorough characterization for specific applications (Ayala-Rodríguez et al. 2022; Cepeda, Villarán, and Aranguiz 1998; Fernández-Quintela et al. 1997; Hewage et al. 2024; Karaca, Low, and Nickerson 2011).

It is important to note that the protein isolates derived from the alkaline extraction and isoelectric precipitation method have demonstrated a decrease in the levels of anti-nutritional factors such as trypsin inhibitors, phytic acid, and tannins. This reduction not only enhances the digestibility of the protein isolates but also improves their overall nutritional quality, rendering them suitable for a wide range of food and nutritional applications (Ayala-Rodríguez et al. 2022; Cepeda, Villarán, and Aranguiz 1998; Fernández-Quintela et al. 1997; Hewage et al. 2024; Karaca, Low, and Nickerson 2011).

In conclusion, the investigation of the alkaline extraction and isoelectric precipitation method for faba bean protein extraction demonstrates its efficacy in producing protein isolates with high purity, solubility, and enhanced functional properties.

SALT EXTRACTION

In contrast to conventional pH-shift methods, gentle fractionation approaches offer a more sustainable means of extracting proteins from pulses. These approaches require less water and energy and avoid the use of chemical additives (Geerts et al. 2018). Among these techniques, aqueous salt extraction stands out as a common and effective method in wet fractionation, particularly for isolating faba bean proteins based on their solubility (Wang et al. 2010). This method capitalizes on the prevalence of salt-soluble globulins in faba beans, which constitute approximately 69%–78% of their total storage proteins (Multari, Stewart, and Russell 2015).

Low salt concentrations induce a “salting-in” effect, increasing protein solubility by promoting protein-water interactions (Jarpa-Parra et al. 2014; Singhal et al. 2016). However, high salt concentrations lead to “salting-out,” causing protein precipitation due to disrupted hydration layers and promoted hydrophobic interactions (Singh et al. 2013). Polyvalent salts, such as ammonium sulfate, are more effective in salting out than univalent salts (Hofmeister 1888).

The subsequent removal of salt, often by dialysis, allows the retrieval of proteins in a solubilized form (Boye, Zare, and Pletch 2010). The process of salting out involves the dehydration of proteins with high-neutral salt solutions (>1 M), which results in protein aggregation and precipitation via hydrophobic interactions (Boye, Zare, and Pletch 2010).

The yield and purity of the protein isolates obtained from salt extraction depend on specific conditions. For instance, in water and salt extraction at 23 °C followed by dialysis at 23 °C, the soluble protein content reaches 92% (yield: 23.98%), while the insoluble protein content is 87.85% (yield: 1.36%). Conversely, water and salt extraction at 23 °C followed by ultrafiltration at 23 °C yields a protein content of 50% (yield: 15.76%) and 58.87% (yield: 12.85%) for two faba bean varieties Snowbird low-tannin (LT) and Athena high-tannin (HT) extracts, respectively. Furthermore, salt extraction at 23 °C with subsequent dialysis at 4 °C results in a protein content of 79.14% (yield: 20.27%) and 81.58% (yield: 22.10%) for LT and HT extracts, respectively (Jeganathan, Vasanthan, and Temelli 2023).

The protein isolates obtained from salt extraction demonstrate varying effects on proteins and anti-nutritional factors. While some studies indicate reduced levels of trypsin inhibitors, lectins, and condensed tannins, others highlight increased protein content and solubility. These protein isolates have diverse applications in food and nutrition due to their high protein content and functional properties.

DEEP EUTECTIC SOLVENT (DES) EXTRACTION

Deep eutectic solvents (DES) have emerged as eco-friendly, biodegradable, and cost-effective alternatives for extracting plant proteins. They have been demonstrated to be effective in sources such as rapeseed, evening primrose cake, oats, and Brewer's spent grain, with yields ranging from 7.98% to 79% and protein content from 36% to 55.28% (Patra, Prasath, and Pandiselvam 2023). Typically composed of a hydrogen bond donor (HBD) and acceptor (HBA), such as choline chloride (ChCl) and glycerol (Gly), DES are known for their low toxicity and high biodegradability. The addition of water can enhance extraction by adjusting solvent polarity. DES work by solubilizing proteins and disrupting cell walls, aided by hydrogen bonding.

Regarding the extraction of proteins from faba beans using deep eutectic solvent, Hewage et al. (2024) used a system comprising choline chloride and glycerol, which had been optimized through response surface methodology (RSM). The optimal conditions were a 28:1 w/w liquid to solid ratio, a 1:2 choline chloride to glycerol molar ratio, a temperature of 50 °C, a duration of one hour, and a water content of 40%. In these conditions, DES extraction achieved a protein content of 92.33%, a yield of 65.42%, and a recovery rate of 23.15%, which outperformed conventional alkaline extraction, which had a similar protein content (92.50%) but a lower yield (60.76%) and recovery rate (21.74%). DES-extracted proteins exhibited a higher α -helix content (21.37%) than alkaline-extracted proteins (10.68%), which displayed a greater abundance of β -turns (19.71%) and intermolecular β -sheets (7.61%) (Hewage et al. 2024).

Unlike traditional alkaline extraction, which requires harsh chemicals such as NaOH or KOH, DES are composed of hydrogen bond donors (HBDs) and acceptors (HBAs), forming a stable eutectic mixture that facilitates protein solubilization under milder conditions. This helps preserve protein structure and functionality, reducing the risk of excessive denaturation or modification.

Despite these advantages, one of the main criticisms of DES extraction is that reported protein yields are often similar to those obtained using conventional alkaline extraction. Given this, the key motivation to adopt this method lies in their lower environmental impact, reduced water and chemical consumption, and potential for reusability. Unlike alkaline extraction, which generates large volumes of wastewater requiring treatment, DES systems can be partially recovered and recycled through water evaporation or phase separation techniques, thereby reducing solvent waste. The feasibility of recovering and reusing DES depends on their composition. Certain DES, such as those based on choline chloride and glycerol, remain stable after extraction and can be separated from the protein fraction using membrane filtration or selective precipitation. However, the efficiency of this recovery process still requires further optimization for industrial-scale applications. Cost-effectiveness is another factor to consider although DES may incur higher initial costs, their reusability could offset these expenses over time.

From a food-grade perspective, not all DES are suitable for food processing. Nevertheless, formulations composed of food-safe components (e.g. choline chloride, citric acid, and glycerol) have shown potential for GRAS (Generally Recognized as Safe) status, making them viable for food protein extraction. However, regulatory approval for industrial food applications remains limited, and additional toxicological and safety assessments are needed to support their adoption.

In conclusion, while DES extraction may not drastically surpass alkaline extraction in terms of protein yield, its benefits in terms of sustainability, functional protein preservation, solvent recyclability, and potential food-grade use make it a promising area for future research. Optimization of recovery methods, cost analysis, and regulatory validation will be crucial to unlocking its full potential in the food industry.

DIALYSIS

Dialysis plays a pivotal role in the extraction and purification of proteins from faba beans. This technique serves as a process whereby soluble components are separated from insoluble ones by means of a semi-permeable membrane (Karaca, Low, and Nickerson 2011). This allows small molecules, such as salts and proteins, to pass through the membrane, while larger molecules, such as starches and fibers, are retained.

By employing dialysis, researchers can effectively enhance the purity and quality of the faba bean protein fractions, rendering them more suitable for various downstream applications in the food and biotechnological sectors. Furthermore, dialysis offers a gentle and efficient means of protein purification, minimizing the risk of denaturation or degradation commonly associated with harsher techniques.

In the study by Jeganathan, Vasanthan, and Temelli (2023), two cultivars of faba beans, Snowbird (LT) and Athena (HT), were subjected to dialysis extraction at 23 °C using a water and 1% salt solution. The soluble fractions obtained from this process exhibited varying yields and compositions. The soluble fraction of Snowbird (LT) exhibited a yield of approximately 23.98%, whereas Athena (HT) yielded about 20.37%. These soluble fractions contained protein contents of $92 \pm 0.54\%$ for Snowbird (LT) and $91.29 \pm 2.53\%$ for Athena (HT). It is noteworthy that the insoluble fractions from both cultivars exhibited starch content, with Snowbird (LT) containing $6.72 \pm 0.04\%$ and Athena (HT) containing $4.08 \pm 0.01\%$. This study indicates that the dialysis extraction method effectively separates protein-rich soluble fractions from starch-rich insoluble fractions in faba beans, by producing protein isolates with ~91% purity, while starch contamination in the protein fraction was minimal (<0.5%).

MICELLIZATION

Micellization precipitation is an effective method for extracting high-purity protein isolates from plants such as peas and faba beans, yielding isolates with over 98% protein content. The process involves the dissolution of plant material in a diluted salt solution (e.g. 0.1 M NaCl), followed by centrifugation to remove insoluble components. The addition of cold water to the supernatant significantly reduces ionic strength, promoting protein micelle formation through hydrophobic interactions. Proteins dissociate and form micelles, which grow and precipitate out, then are recovered by centrifugation or microfiltration (Murray, Myers, and Barker 1979). Despite its high purity, micellization often yields less protein than alkaline extraction-isoelectric precipitation (AE-IP). Stone et al. (2015) reported yields of 30.7%–31.1% for peas using micellization versus 62.6%–76.7% with AE-IP, with similar protein contents. Jeganathan, Vasanthan, and Temelli (2023) also noted low yields with micellization but high purity. Conversely, Abdel-Aal et al. (1986) observed higher yields and protein content for faba beans when using micellization (86.3% protein; 74% yield) compared to AE-IP (75.2% protein; 66.2% yield), although with a higher ash content.

This method was demonstrated as an effective means of removing sucrose and raffinose family oligosaccharides, including raffinose, stachyose, and verbascose, from non-precipitated fractions. While micellization preserves

protein functionality, it faces challenges in yield efficiency and industrial scalability. Further optimization is necessary for broader commercial use (Jeganathan, Vasanthan, and Temelli 2023; Jeganathan et al. 2022; Sun and Arntfield 2010).

ULTRAFILTRATION

Ultrafiltration (UF) is a prominent method for protein concentration in food processing, offering advantages over traditional techniques like isoelectric precipitation (IP) following alkaline extraction (AE). By leveraging pressure-driven membrane filtration, UF effectively separates proteins based on molecular size, without the need for heat, as demonstrated in studies by Boye, Zare, and Pletch (2010) and Klupšaitė and Juodeikienė (2015). This process, which employs molecular weight cutoff membranes with a range of 1000–100,000 kDa, allows the retention of proteins of interest while removing unwanted components. Furthermore, the combination of ultrafiltration (UF) with diafiltration (DF) enhances product recovery and purity, as highlighted by Singhal et al. (2016). In comparison to AE-IP, UF/DF processes result in protein concentrates with lower levels of anti-nutritional factors, thereby enhancing the overall nutritional value of protein ingredients (Fuhrmeister and Meuser 2003; Mondor et al. 2009). Despite the challenges of higher operating costs and membrane fouling, ultrafiltration (UF) remains an appealing option for large-scale industrial applications due to its ability to produce high-purity protein ingredients (Eckert et al. 2019). The industrial application of ultrafiltration in faba bean protein production is exemplified by US4208323A (1980), which describes a process for extracting and purifying legume proteins including from faba beans via aqueous salt extraction followed by ultrafiltration or diafiltration. This step in the process serves to concentrate the proteins while preserving their functionality. While the patent encompasses various legumes, faba bean is specifically mentioned as a suitable raw material. Furthermore, alternative protein recovery techniques, including microfiltration, cryoprecipitation, electrodialysis, and the use of organic solvents, offer additional ways for protein extraction. Each of these techniques has unique benefits and considerations (Boye and Barbana 2012; Bazinet, Lamarche, and Ippersiel 1998). In conclusion, ultrafiltration (UF) is a versatile and efficient method for protein concentration in food processing, with the potential for significant advancements in enhancing protein quality and nutritional value.

In the study by Jeganathan, Vasanthan, and Temelli (2023), the ultrafiltration method is examined alongside mild fractionation methods and traditional technologies for isolating faba bean proteins. The ultrafiltration process involves water and salt extraction at 23 °C, followed by ultrafiltration at the same temperature. The results reveal that this method yields protein isolates with varying protein contents and yields. For Snowbird (LT) extract, the protein content is around 50% with a corresponding yield of 15.76%. In contrast, Athena (HT) extract shows a higher protein content of 58.87% but with a slightly lower yield of 12.85%.

DRY EXTRACTION METHODS

Dry pretreatments, such as dehulling and drying, are essential for effective dry fractionation, particularly air-classification.

Dry fractionation is emerging as a promising method for protein extraction from plant sources, offering advantages over aqueous fractionation approaches. This method minimizes water and energy consumption, avoids the need for drying processes or chemical additives post-extraction, and preserves the native structure and functionality of proteins (Fernando 2021; Pelgrom et al. 2013; Vogelsang-O'Dwyer, Zannini, and Arendt 2021). Furthermore, it facilitates the removal of bitter/astringent components, and the improvement of protein color (Saldanha do Carmo et al. 2020; Pelgrom et al. 2013; Vogelsang-O'Dwyer, Zannini, and Arendt 2021). Nevertheless, challenges persist, including lower purity and yield of extracted proteins, particularly without pretreatment, due to carbohydrate and fiber fragmentation during milling (Fernando 2021). Despite these limitations, dry fractionation methods hold

promise as sustainable alternatives for protein enrichment, with ongoing research focusing on optimization to enhance protein content and purity (Schutyser et al. 2015).

AIR-CLASSIFICATION

Air-classification has emerged as a prominent method for separating protein-rich fractions from starch-rich legume flours, thereby enriching their protein content. This process relies on differences in particle size and density, with centrifugal and gravitational forces separating flour into fine protein and coarse starch fractions (Schutyser et al. 2015). This method is controlled by setting a cut size, which determines the size of particles that are separated into fine and coarse fractions. The optimization of milling parameters, including speed, significantly influences the efficiency of protein separation. The application of higher speeds has been demonstrated to result in an enhancement of the protein content within the fine fractions (Pelgrom et al. 2013). However, excessive milling can induce starch damage and reduce separation efficiency (Pelgrom et al. 2014). Despite its advantages, challenges such as random particle collisions and inefficient separation of similar-sized particles persist (Assatory et al. 2019).

Protein-rich fractions have a protein content of approximately 48.6%–66%, with a maximum protein recovery of 26%–49% (Coda et al. 2015; Martinez et al. 2016; Saldanha do Carmo et al. 2020; Vogelsang-O'Dwyer et al. 2020). The dry process does not necessitate the employment of water or chemicals, consequently resulting in a minimal generation of waste (Felix et al. 2019). The main drawback of this method is that the purity of the fractions is inferior to that achieved by the wet extraction. Furthermore, the anti-nutritional compounds (vicine and convicine) were not completely removed (Felix et al. 2018; Vogelsang-O'Dwyer et al. 2020).

In conclusion, air-classification is a versatile and effective technique for fractionating legume flours into protein-rich and starch-rich fractions, with a wide range of applications in food technology and nutrition.

AIR-CURRENTS-ASSISTED PARTICLE SEPARATION (ACAPS)

In the context of dry processing methods, sieving is a technique that has gained considerable traction. However, it is not without its challenges, including the potential for clogging with fine sieves, a reduction in throughput, and limitations in separation efficacy (Pelgrom et al. 2013; Schutyser et al. 2015). In response to these limitations, air-currents-assisted particle separation (ACAPS) has emerged as a promising alternative. The ACAPS method employs vacuum and high-pressure air pulsing to induce dynamic air currents within a sieving device (Vasanthan 2018). This innovative technology has demonstrated the ability to concentrate specific components, such as barley β -glucan in coarse fractions and starch in fine fractions. Recent studies by Jeganathan, Gao, et al. (2023) have explored the application of ACAPS to bean flour, yielding coarse fractions enriched in protein and dietary fiber. Variations in the composition of these fractions were observed based on sieve sizes (75 μm and 250 μm). Subsequent processing of these fractions has yielded valuable co-products, including protein isolates, starch isolates, and dietary fiber concentrates (Lu et al., 2020). Nevertheless, further research is necessary to elucidate the role of coarse and fine fractions resulting from ACAPS in subsequent wet fractionation processes, as well as the influence of upstream processing steps, such as defatting, on particle size-based separation (Lu et al., 2020). This highlights the necessity for continued research to optimize the application of ACAPS and its integration into existing processing frameworks.

TRIBOELECTRIC SEPARATION

Triboelectric separation has been identified as a promising technique for the fractionation of starches and proteins in various food processing applications. This method is grounded in the principle of inducing electric charges on particles upon contact with charging plates, which facilitates the selective separation of proteins while maintaining starches neutral within an electric field (Zhu et al. 2021). Although primarily employed in industries such as mining,

plastics, and pharmaceuticals, its potential within the food sector is significant (Tabtabaei et al. 2017). It is noteworthy that triboelectric separation has been successfully applied in protein enrichment across diverse legumes, including soybean, navy bean, pea, lentil, and chickpea (Xing et al. 2018; Tabtabaei et al. 2017). However, its application to faba beans remains unexplored, presenting an avenue for future investigation (Zhu et al. 2021). The mechanism of this method involves particle electrification through electron or ion transfer, with parameters such as particle size and environmental factors influencing its efficacy (Tabtabaei et al. 2017). Further research is necessary to optimize this technique for potential applications in faba bean protein concentration and other food processing endeavors.

HYBRID EXTRACTION METHODS

Hybrid fractionation processes, which integrate both dry and wet separation methods, are highly promising techniques for extracting high-purity protein fractions from faba beans. These approaches are economically viable and sustainable, as highlighted in studies by Schutyser et al. (2015) and Dumoulin et al. (2021). By using a combination of techniques, such as suspending fine fractions in water without chemicals and fractionating coarse starch-rich fractions, these hybrid processes significantly reduce water and energy consumption in comparison to conventional extraction methods (Avila Ruiz et al. 2016). The major advantage of this approach lies in its ability to pre-concentrate proteins during dry fractionation, thereby decreasing the volume of material requiring wet extraction. This directly impacts water usage, which is reduced from 85 kg of water/ kg of extracted protein in traditional wet methods to 15.5 kg/kg in hybrid processes (Schutyser and van der Goot 2011), representing a 5.5-fold reduction. In addition to water savings, energy consumption is significantly reduced in hybrid systems. Wet fractionation alone is highly energy-intensive due to drying and solvent evaporation. By incorporating air-classification as a preliminary step, a large portion of proteins are concentrated before undergoing wet extraction, which lowers the overall drying energy requirements. Air-classification requires only 11 MJ/kg of protein-rich flour (Pelgrom et al. 2013), making it a significantly lower-energy alternative to direct wet extraction.

Beyond resource efficiency, hybrid methods retain protein functionality better than conventional wet extraction, as the proteins undergo milder processing conditions. However, challenges remain in terms of scalability and process optimization, as hybrid systems require precise control over multiple processing steps. While initial capital investment costs may be higher, the long-term benefits in terms of reduced operational costs and environmental sustainability make hybrid processes a promising alternative for large-scale protein extraction.

Although wet fractionation can recover a significant amount of protein, it may not always result in a substantial reduction of anti-nutritional factors, as noted by Dumoulin et al. (2021). To address this issue, hybrid fractionation strategies have been proposed, which combine elements from both dry and wet methods. Hybrid processes reduce process volumes in energy-intensive steps and improve protein purity. However, their scalability and industrial feasibility remain moderate due to multi-step processing and the need for specialized equipment. These benefits have been discussed in studies by Shanthakumar et al. (2022) and Ferreira et al. (2022).

Three studies have provided comprehensive insights into varied approaches and outcomes. Dumoulin et al. (2021) developed a hybrid dry-wet extraction process characterized by meticulous steps such as dehulling, micronization, air-classification, and subsequent wet extraction. The method used yielded protein recovery rates ranging from 53.6% to 60.6%, which is praiseworthy. However, there was an associated increase in anti-nutritional factors. The study emphasized the preservation of native protein structures, which suggests potential applications in functional food formulations. In contrast, Jeganathan et al. (2022) used a sequential pearling approach followed by wet fractionation to enhance protein isolation. This method involves pretreatments such as dehulling and sieving, resulting in protein isolate yields of 20%–22%. The process induces minimal conformational changes in proteins and achieves a substantial reduction in tannin content, thereby improving protein purity and mitigating potential

nutritional concerns. Complementing these findings, Jeganathan, Gao, et al. (2023) investigated air-currents assisted particle separation (ACAPS) technology in conjunction with wet extraction. The study showed protein recovery rates ranging from 39% to 59%, while preserving secondary protein structures and reducing tannin levels in the protein isolates. ACAPS technology also demonstrated potential in refining particle separation and enhancing protein purity, making it a viable option for sustainable and efficient protein extraction processes.

Collectively, these studies highlight the potential of hybrid extraction in optimizing protein recovery, purity, and techno- functional properties. However, scalability and industrial implementation remain moderate due to multi-step processing and specialized equipment requirements. These insights establish a foundation for further research into refining hybrid extraction protocols and improving industrial feasibility.

Discussion

Faba beans (*V. faba* L.), with their high protein content of (20–43 g/100 g), are increasingly recognized as a promising alternative to traditional animal protein sources, particularly in the context of addressing global protein demands sustainably. However, the complexity of efficiently extracting high-quality proteins from faba beans, while retaining their nutritional and functional properties, remains a significant technical challenge. A systematic evaluation of the interplay between pretreatments, extraction techniques, and operational conditions is essential to achieve a balanced outcome. To provide a key comparison, this discussion examines pretreatments and extraction methods, considering their protein yield, purity, reduction of anti-nutritional factors (ANFs), and overall cost-effectiveness and scalability. The discussion integrates **Tables 1, Table 2, Figure 2, and Figure 3** for a more comprehensive analysis.

PRETREATMENT METHODS AND THEIR IMPACT

The pretreatment stage is crucial in determining the efficiency of subsequent protein extraction. Effective pretreatment aims to modify the physical and chemical properties of faba beans to enhance protein yield and purity while reducing ANFs and preserving functional properties. Different pretreatment methods, including physical (thermal, non-thermal), and biological approaches, are evaluated in **Figure 2**, which provides an overview of their effects on protein content, ANFs reduction, and process feasibility.

Thermal pretreatments, such as roasting, microwaving, autoclaving, and extrusion, serve a dual role by enhancing protein extractability and reducing ANFs like vicine, convicine, and phytic acid. Roasting and microwaving are known to promote protein denaturation, which exposes hydrophobic groups and reactive sites, facilitating improved solubility and extractability (Langton et al. 2020). These processes also reduce bitterness and off-flavors, making the extracted protein more suitable for incorporation into food systems.

However, thermal treatments introduce significant risks, particularly related to protein over-denaturation and the formation of Maillard reaction products, which reduce the nutritional value of proteins by reacting with amino groups

Table 1. Reported reductions of anti-nutritional factors (ANFs) in faba beans as affected by pretreatment methods.

Pretreatment method	Tannins reduction	Phytic acid reduction	RFOs reduction	Vicine and convicine reduction	Other ANFs (Saponins, Lectins, Oxalates)	References
Drying	70%–95%	50%–80%	60%–90%	80%–98%	–	Amiri Chayjan and Shadidi (2014) and Boukid and Castellari (2022)
Roasting	Limited effect	20%–40%	10%–30%	30%–60%	Saponins reduction	Anderson et al. (1994)
Microwaving	30%	30%	–	–	Saponins, lectins and reduction	Collado et al. (2020) Pysz et al. (2012)
Cooking	Moderate	Limited effect	Moderate	High	Saponins reduction in some studies	Osman et al. (2014)
Autoclaving	Moderate	Limited effect	Moderate	High	Lectins eliminated, oxalates reduced	Luo and Xie (2013) and Osman et al. (2014)
Extrusion	12%–25%	26.7%	Effective	Effective	–	Alonso, Aguirre, and Marzo (2000) and Masoero, Pulimeno, and Rossi (2005)
Dehulling	High (seed coat removal)	May increase (cotyledon concentration)	–	–	–	Alonso, Aguirre, and Marzo (2000) and Sharma and Sehgal (1992)
Soaking	Moderate	Limited effect	Moderate	High (up to 99%)	Saponins reduction	Luo and Xie (2013) and Shi, Arntfield, and Nickerson (2018)
Gamma irradiation	Reduced (dose-dependent)	60%–85%	–	70%–95%	–	Osman et al. (2014)
Ultrasound	40%–80%	30%–60%	40%–70%	60%–85%	–	Iscimen et al. (2023) and Martínez-Velasco et al. (2018)
Germination	–	Effective	60%–100%	Effective	–	Dhull et al. (2022) and Hefni, Shalaby, and Witthöft (2015)
Fermentation	High (complete in some cultivars)	90%	Effective	Effective	–	Coda et al. (2015) and Verni et al. (2019)
Enzyme treatment	–	Up to 89% with phytase	Reduced by α -galactosidase	–	Lectins reduction	Rizzello et al. (2019) and Luo, Xie, and Cui (2010)

Table 2. Comparative analysis of faba bean protein extraction methods: pretreatment methods, protein yields, impact on ANFs, advantages, and drawbacks.

Wet methods	Method	Pretreatment	Protein content	Yield	ANFs	Advantages	Drawbacks	References							
Alkaline extraction followed by isoelectric precipitation	Dehulling, soaking, autoclaving, defatting	81.2% to 97.41% (Nitrogen-to-protein conversion factor 6.25)	37% to 74.35%	Vicine and Convicine: absence or low contents in protein fractions. Trypsin Inhibitor Activity (TIA): low levels. Tannins and Phytates: lower concentrations compared to seeds. FODMAPs: lower content in protein isolates	High protein purity and yield Improved functional properties such as emulsifying and foaming properties Enhanced nutritional quality and reduced ANFs Easily scaled up for industrial production Cost-effective on a large scale	Protein structural alteration through the disruption of non-covalent interactions Loss of gelation capacity Generation of wastewater with different pH levels, which requires proper treatment before disposal Resource intensiveness in terms of energy, chemicals, equipment...	Ayala-Rodriguez et al. (2022) Cepeda, Villarán, and Aranguiz (1998) Fernández-Quintela et al. (1997) Hewage et al. (2024) Karaca, Low, and Nickerson (2011) Langton et al. (2020) McCurdy and Knipfcl (1990) Ouraji et al. (2020) Otegui et al. (1997) Ruckmangathan et al. (2022)								
								Salt extraction	Dehulling	79.14%–87.11% (Nitrogen-to-protein conversion factor 6.25) 43.1–92% (Nitrogen-to-protein conversion factor 5.7)	12.85%–28.43%	Reduced trypsin inhibitors. Lower tannin content	Mild extraction method preserving protein functionality. Higher amino acid content: with specific methods (e.g. dialysis)	Lower protein yield compared to other wet methods. Requires extensive dialysis for purification	Shi and Nickerson (2022) Suchintita Das et al. (2023) Vioque, Alaiz, and Giron-Calle (2012) Vogelsang-O'Dwyer et al. (2020) Żmudzinski, Goik, and Praszek (2021) Jeganathan, Vasanthan, and Temelli (2023) Karaca, Low, and Nickerson (2011) Suchintita Das et al. (2023) Sussmann et al. (2013) Hewage et al. (2024)
Dialysis	Dehulling	93.2%–96.4% (Nitrogen-to-protein conversion factor 6.25) 98.7%–99.1% (Nitrogen-to-protein conversion factor 6.25)	27.51%–28.48%	Reduced trypsin inhibitory activity and tannins. Reduced raffinose family oligosaccharides	High protein purity and functional properties. Effective in reducing ANFs High protein quality and purity. High IVPDCAAS (<i>in vitro</i> protein digestibility corrected amino acid score) values. Suitable for clean-label applications	Labor-intensive process. Resource-intensive (water, time) Requires precise control of conditions and not easily scalable	Jeganathan, Vasanthan, and Temelli (2023) Jeganathan, Vasanthan, and Temelli (2023)								
								Micellization	Dehulling	50%–58.87% (Nitrogen-to-protein conversion factor 6.25)	11.67%–12.11%	–	High protein quality and purity. High IVPDCAAS (<i>in vitro</i> protein digestibility corrected amino acid score) values. Suitable for clean-label applications	Requires specialized equipment and extensive processing	Jeganathan, Vasanthan, and Temelli (2023)
Ultrafiltration	Dehulling	12.85%–15.76%	–	Produces protein fractions with high solubility and functionality	Requires specialized equipment and extensive processing	Jeganathan, Vasanthan, and Temelli (2023)									

(Continued)

Table 2. Continued.

Method	Pretreatment	Protein content	Yield	ANFs	Advantages	Drawbacks	References
Dry methods	Dehulling	- Classifier wheel speed 6200–8000 rpm: 48.6%–62.7% - Classifier wheel speed 15000 rpm: 51.49% - Classifier wheel speed 11500 rpm: 75.1% - Classifier wheel speed 12000 rpm: 64%–66.6% - Classifier wheel speed 15000 rpm: 60%–60.9% - Classifier wheel speed 9600 rpm: 61.4% (Nitrogen-to-protein conversion factor 6.25)	21.1%–64.7%	Concentration of vicine, convicine, trypsin inhibitor activity, and hemagglutinating activity in protein-rich fractions, while starch-rich fractions have lower levels. Phytic acid levels remain comparatively low in some protein-rich fractions	Effective separation of protein and starch with high recovery rates. Enhanced foaming, and emulsifying properties, suitable for food applications. High lysine and essential amino acid content, with added nutritional value. Environmentally sustainable method, using less water, chemicals, and energy compared to wet extraction	Concentration of ANFs (Vicine, convicine, phytic acid) in protein fractions, limiting use without further processing. Different faba bean varieties respond variably, impacting protein yield and quality. Air-classified protein fractions have lower solubility, affecting certain applications. Increased rancidity during storage, likely due to fat concentration. Requires optimization of processing parameters to achieve the desired ingredient properties	Cloutt, Walker, and Pike (1987) Coda et al. (2015) Martinez et al. (2016) Saldanha do Carmo et al. (2020) Saldanha do Carmo et al. (2022) Skylas et al. (2023) Sosulski and McCurdy (1987) Tyler (1984) Tyler, Youngs, and Sosulski (1981) Tyler, Youngs, and Sosulski (1984) Vogelsang-O'Dwyer et al. (2020)
Hybrid methods	Air-currents-assisted particle separation (ACAPS) Dehulling, pearling Micronization Alkaline extraction followed by isoelectric precipitation Dehulling Air-currents assisted particle separation (ACAPS) followed by wet extraction by micellization	34.59%–38.96% (Nitrogen-to-protein conversion factor 6.25) 53.6% in protein-rich fraction (air-classification) and 60.6% in protein-rich fraction (wet extraction) (Nitrogen-to-protein conversion factor 6.25) 87.26%–97.74% (Nitrogen-to-protein conversion factor 6.25)	18.25%–59.67% 69% for air-classification 87% for the whole process 48%–54%	– Increase in ANFs rates (between 40% and 330%) Major reduction of tannins	Environmentally friendly method High protein recovery rate, reduced energy and water consumption (5.5 times less) Chemical-free process with effluent water recycling High purity and yield Maintains protein structure which makes them suitable as functional food ingredients Appropriate for underutilized high-tannin cultivars	Requires optimization of processing parameters to achieve the desired ingredient properties Higher ANFs levels compared to some unprocessed legumes Requires precise control of conditions and not easily scalable	Jeganathan, Gao, et al. (2023) Dumoulin et al. (2021) Jeganathan, Gao, et al. (2023)



Pretreatment	Protein purity	Nutritional quality	Techno functional quality	Sensory quality	Cost	Scalability	Industrialization	Environmental impact
Physical 								
Thermal								
Drying	●	●	●	●	●	●	●	●
Roasting	●	●	●	●	●	●	●	●
Microwaving	●	●	●	●	●	●	●	●
Cooking	●	●	●	●	●	●	●	●
Autoclaving	●	●	●	●	●	●	●	●
Extrusion	●	●	●	●	●	●	●	●
Non-thermal								
Dehulling	●	●	●	●	●	●	●	●
Soaking	●	●	●	●	●	●	●	●
Gamma irradiation	●	●	●	●	●	●	●	●
Ultrasound	●	●	●	●	●	●	●	●
Biological 								
Germination	●	●	●	●	●	●	●	●
Fermentation	●	●	●	●	●	●	●	●
Enzyme treatment	●	●	●	●	●	●	●	●

Figure 2. Overview of pretreatment methods for faba bean protein extraction and their impact on key para. Color coding: ● Red (Low), ● Yellow (Moderate), ● Green (High).




Extraction method	Protein purity	Nutritional quality	Techno functional quality	Sensory quality	Cost	Scalability	Industrialization	Environmental impact
Wet methods 								
AE-IP	●	●	●	●	●	●	●	●
Salt extraction	●	●	●	●	●	●	●	●
DES extraction	●	●	●	●	●	●	●	●
Dialysis	●	●	●	●	●	●	●	●
Micellization	●	●	●	●	●	●	●	●
Ultrafiltration	●	●	●	●	●	●	●	●
Dry methods 								
Air-classification	●	●	●	●	●	●	●	●
ACAPS	●	●	●	●	●	●	●	●
Triboelectric separation	●	●	●	●	●	●	●	●
Hybrid methods 								
Air-classification AE-IP	●	●	●	●	●	●	●	●
ACAPS Micellization	●	●	●	●	●	●	●	●

Figure 3. Comparative analysis of wet, dry, and hybrid extraction methods for faba bean protein and their impact on key parameters. Color coding: ● Red (Low), ● Yellow (Moderate), ● Green (High).

and depleting essential amino acids like lysine. The extent of Maillard reactions is influenced by temperature and time, and over-processing can lead to browning, reduced solubility, and deterioration in techno-functional properties like gelation and emulsification (Karaca, Low, and Nickerson 2011). These negative impacts must be carefully controlled, as excessive protein denaturation can limit the application of thermally treated proteins in products where functionality, such as solubility or gelling, is crucial.

Additionally, extrusion represents a specific form of thermal pretreatment involving both heat and mechanical forces, which is used to alter the structural characteristics of faba bean flour. Extrusion effectively reduces ANFs but is associated with increased shear forces that may cause aggregation and a decrease in protein digestibility (Singh et al., 2007). This highlights the critical need to balance the benefits of ANFs reduction with the preservation of protein quality during thermal pretreatment.

Non-thermal pretreatments, such as dehulling, soaking, gamma irradiation, and ultrasound, aim to minimize the thermal damage associated with protein extraction while maintaining ANFs reduction. Dehulling involves mechanically removing the seed coat, which contains fibers, polyphenols, and phytic acid that reduce protein digestibility and interfere with mineral absorption. By removing the hull, dehulling increases protein concentration and improves nutritional bioavailability (Martineau-Côté et al. 2022). Furthermore, dehulling generates valuable by-products that can be used in animal feed, thus adding economic value.

Soaking is another non-thermal method that initiates enzymatic activity leading to the breakdown of lectins-proteins that bind carbohydrates and interfere with nutrient absorption (Multari, Stewart, and Russell 2015). Soaking also rehydrates the beans, softening cell walls and facilitating the subsequent mechanical milling process. Gamma irradiation and ultrasound offer additional non-thermal methods to modify protein structure and enhance extractability. Gamma irradiation has proven effective in reducing microbial load and ANFs, but it also leads to oxidative stress, which results in the fragmentation of protein molecules and the loss of sensitive amino acids, thereby impairing nutritional quality (Rady et al. 2020).

Ultrasound, which relies on acoustic cavitation, helps disrupt cellular structures and promotes mass transfer during soaking or other chemical treatments, improving protein yield without employing harsh solvents. However, ultrasound treatment is energy-intensive and requires specialized equipment, which poses challenges in terms of scalability.

Biological pretreatments, including fermentation, germination, and enzyme treatments, are increasingly explored for their capacity to reduce ANFs while improving the nutritional and functional properties of proteins. Fermentation with lactic acid bacteria helps reduce phytic acid and tannins, which enhances the bioavailability of nutrients like calcium, iron, and zinc, thus improving overall protein digestibility and nutritional quality (Saldanha do Carmo et al. 2022). Moreover, fermentation contributes to the breakdown of carbohydrates, such as raffinose-family oligosaccharides, reducing flatulence-causing components and improving consumer acceptability.

Enzyme treatments involve using proteases to break down complex protein structures, thereby improving protein solubility and emulsification properties (Martineau-Côté et al. 2022). Enzyme-assisted extraction also allows for targeted hydrolysis, enabling the extraction of specific protein fractions with desirable functional characteristics for different applications, such as foaming or gelling. Nevertheless, biological pretreatments have inherent challenges, such as the need for precise process control, which affects their scalability, as well as time intensity, making them less feasible for continuous industrial operations without appropriate process automation.

A synthesis of the current findings on the impact of pretreatment methods on anti-nutritional factors (ANFs) in faba beans is presented in **Table 1**. This table offers a comparative overview of the relative efficacy of different pretreatment methods on tannins, phytic acid, raffinose-family oligosaccharides (RFOs), vicine and convicine, and other ANFs, including saponins, lectins, and oxalates.

EXTRACTION METHODS: WET, DRY, AND HYBRID APPROACHES

After pretreatment, extraction methods, whether wet, dry, or hybrid, play a crucial role in determining the protein content, yield, and quality of the final product. **Figure 3** provides a comparative analysis of these methods, showing their respective performances in terms of protein content, ANFs reduction, process feasibility, and environmental impact.

Wet extraction methods, such as alkaline extraction followed by isoelectric precipitation (AE-IP), are effective in achieving high protein purity (81.2%–97.41%, **Table 1**). During alkaline extraction, proteins are solubilized in an alkaline solution, which disrupts ionic and hydrogen bonds, thereby enhancing protein separation from starch and fiber. Subsequently, isoelectric precipitation exploits the isoelectric point (pI) of proteins, allowing them to precipitate selectively while impurities remain in solution (Rivera, Siliveru, and Li 2024). Ultrafiltration is another crucial step in some wet extraction processes, used to concentrate proteins based on their molecular size, thereby increasing protein purity without thermal degradation (Karaca, Low, and Nickerson 2011).

Despite their advantages in terms of yield and purity, wet extraction methods have notable drawbacks, including their high resource intensity. The requirement for large volumes of water, energy, and alkaline chemicals not only elevates operational costs but also raises significant environmental concerns (Ouraji et al. 2020). The disposal of

alkaline waste adds to the environmental burden, necessitating appropriate wastewater treatment to mitigate negative impacts on ecosystems. Furthermore, the use of harsh alkaline chemicals can result in protein modifications, which may alter functional properties such as solubility, emulsification, and sensory characteristics, thereby affecting the quality of the final product (Langton et al. 2020).

Dry extraction methods, such as air-classification, involve mechanical separation of proteins from starch and fiber, based on differences in particle size and density. Air-classification is particularly appealing due to its low environmental impact, as it does not require water or chemical solvents. Protein purities obtained through air-classification range from 48.6% to 75.1%, depending on the classifier's wheel speed (**Table 1**). The preservation of protein structure during dry extraction is one of its key advantages, as it maintains important techno-functional properties like emulsification and foaming, which are essential for certain food products (Schutyser and van der Goot 2011).

However, dry extraction has inherent limitations, particularly in its ability to remove ANFs. Since ANFs like tannins and phytic acid are concentrated within the protein fraction, dry extraction methods often require subsequent processing to improve protein purity and reduce these undesirable components.

Moreover, mechanical milling, a necessary step in dry fractionation, can lead to protein denaturation and aggregation, resulting in diminished solubility and functionality (Vogelsang-O'Dwyer et al. 2020). This reduction in protein quality is a significant drawback that limits the application of dry-extracted proteins, especially in formulations requiring high solubility.

Hybrid extraction methods, which integrate dry and wet techniques, have emerged as promising approaches for overcoming the limitations of individual extraction methods. These methods aim to maximize protein yield and purity while minimizing the environmental and economic costs associated with wet processing. For example, combining air-classification with wet micellization allows to produce high-purity protein isolates with significantly reduced water and chemical consumption compared to conventional wet extraction alone. Hybrid methods can produce protein purities of 87.26%–97.74%, while also reducing tannin content and enhancing functional properties (Jeganathan, Gao, et al. 2023).

Figure 3 illustrates that hybrid extraction methods achieve a moderate environmental impact and an improved scalability, making them a viable solution for industrial applications. One notable advantage of hybrid approaches is their ability to pre-concentrate proteins during dry processing, thereby reducing the volume of material requiring wet extraction. This not only enhances efficiency but also helps in addressing the environmental footprint associated with water and energy usage (Saldanha do Carmo et al. 2022).

However, the implementation of hybrid methods requires sophisticated equipment and precise control of multiple processing steps, which increases capital costs and operational complexity, potentially limiting their adoption in resource-constrained settings. Additionally, achieving consistent protein quality across different batches requires strict monitoring of both dry and wet processes, adding to the challenges of scaling up these methods for continuous production.

The comprehensive evaluation presented in **Table 1**, **Table 2**, along with the insights provided by **Figures 2** and **3**, reveals the inherent tradeoffs associated with different pretreatment and extraction methods for faba bean proteins. Thermal pretreatments offer substantial benefits in terms of ANFs reduction and enhanced digestibility, but they also carry significant risks of over-processing, leading to protein denaturation and compromised functional properties. Non-thermal and biological pretreatments provide milder alternatives that retain nutritional integrity, but they face challenges related to scalability, time intensity, and process complexity.

In summary, each protein extraction method presents a distinct set of tradeoffs in terms of yield, purity, functional properties, and environmental impact. Wet extraction techniques, particularly alkaline extraction followed by

isoelectric precipitation, are the most effective in achieving high protein purity (~90%), making them suitable for high-quality applications. However, they are resource-intensive, requiring large volumes of water and chemicals, which raises concerns regarding environmental sustainability, wastewater treatment, and potential protein denaturation under extreme pH conditions.

In contrast, dry fractionation offers a more sustainable and energy-efficient alternative, with significantly lower water and energy requirements. However, it typically yields lower protein purity (~50–60%) due to incomplete separation from starch and fiber fractions and offers limited effectiveness in removing anti-nutritional factors (ANFs).

Hybrid extraction methods aim to combine the strengths of both wet and dry techniques. By pre-concentrating proteins through dry fractionation before applying a milder wet process, these approaches can improve overall protein recovery while significantly reducing water and energy consumption. Nevertheless, hybrid systems require careful process integration and optimization to manage operational complexity and capital costs, and to ensure consistent product quality at scale.

Future research should prioritize optimizing hybrid extraction processes by integrating advanced pretreatments, such as enzyme-assisted extractions, which can synergistically improve protein yield and functionality while reducing ANFs content. Innovations such as ultrasound-assisted hybrid extractions or the development of green solvents for wet processing could further enhance sustainability and reduce reliance on harsh chemicals. Moreover, there is a need for more cost-effective process designs that can make these methods accessible across different industrial settings, particularly in regions with limited resources.

Achieving a balance between protein quality, environmental sustainability, operational feasibility, and economic viability is crucial for the successful commercialization of faba bean proteins. This will require a multidisciplinary approach, combining advancements in food chemistry, process engineering, and environmental technology to develop efficient, scalable, and sustainable protein extraction systems. The ongoing research in this field has the potential to transform faba beans into a major plant-based protein source, contributing to the global shift toward a more sustainable and resilient food system, addressing the rising consumer demand for high-quality alternative proteins. While significant progress has been made in developing sustainable and efficient protein extraction techniques, several technical and economic barriers remain for large-scale adoption. Membrane technologies, electrodialysis, and low-temperature drying present promising avenues to enhance protein purity and functionality while reducing energy demand. Moreover, the valorization of process by-products (e.g. starch, fiber fractions, and residual biomass) could improve economic viability and contribute to circular food production systems. Future research should prioritize integrated processing approaches, ensuring that protein quality, sustainability, and industrial feasibility are optimized simultaneously.

Conclusion

The extraction of proteins from faba beans is a complex process that demands careful consideration of numerous factors to optimize yield, purity, and functionality. In this review, we have examined a range of extraction methodologies, including wet, dry, and hybrid approaches. Each of these methodologies offers distinct advantages and challenges in the quest for efficient protein extraction.

Wet extraction methods are effective for producing high protein yield and purity, suitable for refined protein isolates with excellent functionality. Techniques like alkaline extraction and isoelectric precipitation achieve high purity and recovery, making them ideal for certain food applications. However, the resource intensity of wet methods, including high water, energy, and chemical use, set environmental and economic challenges. Innovations in waste recycling, process optimization, and greener reagents are necessary to enhance their feasibility at an industrial scale.

Dry extraction methods provide a more sustainable alternative by reducing resource consumption and minimizing environmental impact. Techniques such as air-classification preserve the native protein structure, but face challenges related to lower protein purity and the concentration of anti-nutritional factors (ANFs) in protein fractions. Protein denaturation during milling can also affect functional properties like solubility and emulsification. Ongoing research is focused on optimizing milling parameters and integrating pretreatments to enhance efficiency and sustainability.

Hybrid extraction methods integrate elements from both wet and dry techniques, leveraging their respective strengths while minimizing limitations. By combining dry fractionation with wet purification, hybrid methods can achieve high protein yield and purity with reduced resource consumption. For example, air-classification followed by wet extraction can lower water and chemical use. Hybrid methods offer greater flexibility in achieving specific protein functionality goals, but require sophisticated equipment and precise process control, which can increase capital investment and operational complexity.

Advances in sustainability, efficiency, and scalability are driving research in faba bean protein extraction. Future innovations will involve emerging pretreatment technologies and novel extraction approaches. Enzyme-assisted extraction shows promise in improving protein solubility and functional attributes without harsh chemicals. Green solvents like deep eutectic solvents (DES) and ultrasound-assisted or microwave-assisted techniques can further enhance yield and purity while reducing environmental impact.

Efforts to improve process integration and automation will be essential for scaling up extraction methods economically. Developing continuous extraction systems that integrate pretreatment, extraction, and purification can reduce costs and improve efficiency. Advances in membrane filtration offer opportunities to improve separation without compromising protein quality. Adopting circular economy principles, including the process of water recycling and using by-products, can enhance the sustainability of these processes.

In conclusion, achieving an optimal balance between protein quality, environmental sustainability, operational feasibility, and economic viability is critical for the successful commercialization of faba bean proteins. This requires a holistic and interdisciplinary approach that integrates advances in food science, process engineering, and environmental technology. Collaboration among researchers, industry stakeholders, and policymakers is crucial to develop integrated extraction systems that optimize yield, purity, and functionality while minimizing resource consumption and environmental impact. With continuous innovation in pretreatment, extraction, and purification technologies, faba beans can become a major sustainable source of plant-based protein, contributing to a resilient global food system and supporting a sustainable, nutrition-secure future.

Author contributions

Mariem Boukraa conducted the literature review, structured the manuscript, and drafted the initial version of the paper. Nicolas Jacquet and Christophe Blecker contributed to revising the manuscript for intellectual content and approved the final version to be published.

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