Journal of
Composites Science

MDPI

Review

Advancing Faba Bean Protein Purification Using Membrane Technology: Current State and Future Perspectives

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Abstract: Plant-based proteins are gaining popularity because of their appeal to vegetarians and vegans, alignment with scientific and regulatory recommendations, and the environmental impact associated with livestock production. Several techniques are employed for the separation, isolation, and purification of plant-based proteins including membrane-based separation, diafiltration, centrifugation, chromatography, electrophoresis, micellar precipitation, and isoelectric precipitation. Despite decades of application, these techniques still have some limitations such as scale-up challenges, high solvent consumption, chemical/biological disposal, and the possibility of protein loss during precipitation or elution. Membrane separation processes are the most effective purification/concentration technology in the production of plant-based protein isolates and concentrates due to their selective separation, simple operational conditions, and easy automation. Membrane separation processes yielded products with higher protein content compared to isoelectric precipitation, and all concentrates presented good functional properties with expected variability among different legumes. This review critically focuses on the membrane technology advances and challenges for the purification of plant-based protein isolates. This study also highlights the plant-based diet trend, the market, composition, and the protein isolate of the faba bean, in addition to the emerging technologies for the elimination of antinutritional compounds.

Keywords: plant-based protein; purification; membrane technology; faba bean; isolate

Citation: Ehsani, M.; Westphalen, H.; Doan, H.; Lohi, A.; Abdelrasoul, A. Advancing Faba Bean Protein Purification Using Membrane Technology: Current State and Future Perspectives. *J. Compos. Sci.* **2024**, *8*, 15. https://doi.org/10.3390/jcs8010015

Received: 6 November 2023
Revised: 7 December 2023
Accepted: 26 December 2023
Published: 2 January 2024

1. Introduction

It has been proven that proteins play a vital role in the growth and development of the body and are essential for a healthy lifestyle. Increasing awareness about the importance of high-quality proteins in the diet has led researchers and nutritionists to seek environmentally friendly and sustainable protein sources. Proteins are found in both animals and plants. However, there are a number of issues associated with animal protein, including cost, supply, direct environmental impact, biodiversity loss, and even human health issues. In addition, there has been a growth in the population of vegetarians, vegans, and people who have difficulty relying on animal proteins [1–5]. In terms of environmental impact, a significant amount of greenhouse gas emissions (GHG) is attributable to the modern food system, which constitutes 21 to 37% of total greenhouse gas emissions. According to GHG life cycle assessments, livestock production accounts for 18% of anthropogenic greenhouse gas emissions [6]. In this regard, a wide range of plant-based proteins are increasingly being utilized in human diets as economical and versatile substitutes for animal proteins. Alternative dairy and meat products made from plant-derived proteins can meet the same

nutritional needs at considerably good prices while preserving forests and reducing greenhouse gas emissions [7]. In addition to their anti-diabetic properties, plant proteins are low in calories and fat, and have a high level of antioxidant activity, essential amino acids, minerals, and vitamins [8].

Industrial-scale production and commercialization of plant-derived proteins have become commonplace, such that they are widely used in edible products including food supplements, edible coatings, food stabilizers, bioactive peptides (BAPs), and hydrogels [1,7]. Legumes, soy proteins, lentils, and cereals are the most common plant-based protein sources. Proteins can be isolated and purified using a wide range of approaches, determined by their physicochemical properties and the biological characteristics of their sources.

A growing market for plant-based proteins has led to many studies on legumes, including faba beans. Faba beans are the third most popular legume after soy and peas and like other legumes, they provide a high amount of lysine-rich protein. In addition to its essential nutrients, the faba bean is one of the most affordable protein sources in developing countries [9–11]. Although faba beans are becoming increasingly popular as a source of protein, some of their low functional properties and antinutritional compounds such as pyrimidine glycosides (vicine and convicine), condensed tannins, and protease inhibitors limit their use. Thus, various processing methods would be required to remove or degrade antinutritional compounds from faba beans in order to improve their features and ensure their safety for consumption [12–15].

Protein purification takes place to achieve high purity standards, concentration enrichment, inhibition of undesired catalysis, meet product specifications, improve protein stability, and minimize protein denaturation. There are a number of methods that can be utilized to purify proteins, including membrane-based separation, diafiltration, centrifugation, chromatography, electrophoresis, micellar precipitation, and isoelectric precipitation [1,16–19]. Although these techniques have been used for protein purification for years, they still have some limitations, such as: scale-up challenges, high solvent consumption, chemical/biological disposal, low purity, and potential loss of proteins due to precipitation or elution [20–22].

Membrane technology has been proven to be one of the most sustainable and cost-effective approaches for protein purification/recovery [23]. The use of membrane-based processes has gained growing attention in recent years owing to their ability to separate and purify proteins based on their size and charge. It has been found that pressure-driven processes such as microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) are the most promising techniques among the membrane-based separation processes [24–29]. Membrane filtration possesses beneficial features such as its ability to work at mild operational conditions (low temperature and pressure) without phase changes, bioactivity preservation, molecular separation, high separation efficiency, low footprint and chemical consumption, high protein recovery yield, and easy scale-up, which make it suitable for a broad range of applications [30–32].

This review paper focuses primarily on membrane technology for the purification of plant-derived proteins, particularly faba beans. Afterward, an overview of recent developments on membrane technology as a purification technique, in addition to its challenges and future outlook, will be provided.

2. Plant-Based Diet Trend

With rapid global population growth, food production needs to significantly increase in order to meet the large population's nutritional demand. It is estimated that agrarian production would have to duplicate between 1999 and 2050 when the world population reaches about 9 billion [33]. However, due to the environmental impacts caused by food production, the consumption of animal-based food needs to meet climate goals and future global food demands [34]. With that, it is necessary to invest in and increase food production through sustainable agriculture and environmentally responsible manufacturing processes.

Over the past few years, the general public has become more aware of the environmental impact caused by meat and highly processed food production. Over many decades, plant-based diets have been associated with a healthier lifestyle and lower risk of many diseases such as type 2 diabetes (T2D), obesity, cancer, and coronary heart disease, which is the leading cause of death globally [35,36].

Many authors have defined plant-based diets differently, and for that reason may have misled studies to certain conclusions. Recent studies have focused on the quality of the plant-based products included in certain diets and created two dietary groups: overall plant-based diet index (PDI), in which the main focus is to reduce animal food intake; and healthy plant-based diet index (hPDI), which aims at the consumption of plant foods associated with improved health outcomes, such as: whole grains, fruits, and vegetables [36,37]. One of the strategies that can break this paradigm is to change the image of plant-based protein products such as beans and tofu. According to Jallinoja et al., plant-based products should be depicted and associated with pleasurable, fulfilling, and energizing foods [38].

3. Faba Bean Market

Also known as broad beans, horse beans, and field beans, faba beans are a vetch and not a true bean. Along with other grain legumes, faba beans are cultivated worldwide and its origin has been tracked back to 10,000 years ago in Eurasia [39]. In 2013, faba beans were reported as the third most important feed grain legume, being produced in 58 countries on large scale [40]. Recent market reports show that the global production of faba beans reached 4.8 million tons in 2016 with an annual growth rate around 1% between 2014 and 2018. It is predicted that the faba bean production should reach 5 million tons by 2022 [41,42]. China is currently the largest producer of faba beans, representing around 30% of the global production. In 2018, the exporting market was led by Australia—which accounts for 40% of the global export volumes—followed by France, United Kingdom, Ethiopia, United States, Egypt, China, Canada, Lithuania, and Latvia. For the same year, the primary importing country was Egypt, followed by Saudi Arabia, Sudan, Norway, Canada, Indonesia, Spain, United Arab Emirates, France, and Italy [42]. Singh et al. reported that in the United States and northern Europe faba beans are not cultivated in large quantities and are mostly used for livestock pasturage, hay, and silage [40]. In developing countries, faba beans are used as human food whereas in industrialized countries it is used as livestock feed, mainly for pigs, horses, poultry, and pigeons [40].

3.1. Faba Bean in Food

Faba beans, whether consumed in their green, immature state or as dried and stored seeds [39], offer a wealth of plant proteins, nutrients, dietary fiber, and bioactive compounds. They serve diverse purposes, functioning as food, feed, forage, and even medicine for both humans and animals [9]. Faba beans offer medicinal value as a nutrient-rich food with potential benefits for heart health, blood sugar regulation, weight management, and anti-inflammatory effects. They are particularly noteworthy for their contribution to bone health, digestive well-being, and as a source of folate for prenatal care [9]. Notably, faba beans constitute a substantial share of the pulses market, alongside other legumes like chickpeas, lentils, and beans [43]. Pulses have grown in popularity specially in developing countries, representing an alternative towards a healthier diet. Globally, the production volume of pulses reached 84.7 million tons in 2017, with an annual growth rate of 3.6% between 2010 and 2017 [44]. The high contents of digestible proteins and starch in the faba bean seeds contribute towards its wide food use [42].

For sensitive human subjects, the intake of raw or cooked faba beans can induce a disease called favism, which is characterized by hemolytic anemia. This condition is frequently observed in the Middle East and Mediterranean basin, and is associated with the presence of vicine and convicine [45]. This is one consequence of the antinutritional effect. Despite this specific adverse effect, non-sensitive subjects can benefit from faba bean

consumption. Studies indicate that this protein source can help to combat chronic disorders such as diabetes, cardiovascular disease, obesity, and cancer [45].

In the food industry, proteins are frequently used as emulsifiers. Motivated by the growing trend towards the substitution of animal-based proteins with plant-based proteins, Karaca et al. reported an investigation in the emulsifying properties of different legume proteins, including faba bean proteins, produced by isoelectric precipitation and salt extraction [46]. Another use of faba beans in the food industry is the complete or partial substitution of wheat flour that has been associated with many chronic diseases [47]. Gimenez et al. successfully introduced faba bean flour into the composition of pasta, improving its nutritional composition without sacrificing sensorial properties valued by the consumer [48].

3.2. Faba Bean in Livestock Feed

In addition to being a great alternative to meat-based products for humans, faba beans can potentially replace soy proteins in livestock feed. However, some components can also have antinutritional effects in monogastric animals, limiting the use of faba beans as feed. A high-protein diet is extremely important for animals, especially poultry, and although faba beans can be a great source of protein, antinutritional compounds can cause a reduction in digestibility and other adverse effects [49,50].

According to the reported literature, pigs can be fed up to 350 g/kg of faba beans with or without tannins. The tannins present in the faba bean reduce its nutritional value for pigs, both for energy and protein. However, growth performance can be hindered at feed rates more than 100 g/kg. Lactation has not been affected by the use of faba beans [49].

For poultry, the inclusion of 250 g/kg of faba beans in broiler diets can almost completely replace soybean meal. However, tannins, vicine, and convicine can present a limitation to the use of faba beans as feed. Tannins impact protein digestion negatively and reduce energy and starch digestibility. Vicine has been reported to negatively affect the egg size of certain species and has been attributed to the rupture of red blood cells. A successful strategy to increase starch and protein digestibility is pelleting, due to the destruction of antinutritional components by the heat generated during the process [45]. However, there is still no agreement among authors regarding the effect of faba bean feed on the feed conversion ratio and growth performance [49].

Multari et al. reported that rabbits showed no sensitivity to antinutritional factors, unlike pigs and poultry. Furthermore, the partial introduction of faba beans into certain fish diets is also possible [45].

The introduction of faba beans into ruminant diets as a replacement of soybean meal has shown no significant improvement in feed consumption, milk production, or milk consumption. However, in general, cows, lambs, and bulls have adapted well to the faba bean [49].

4. Faba Bean Composition

4.1. Nutrient Composition

Faba beans are primarily used for improving the nutritional quality and health benefits of food and feed products, and they contain 31–34% protein, 44–47% carbohydrate, 8% dietary fiber, and 3.5–4% ash [9]. Faba beans have high contents of digestible proteins and starch in their seeds [49], as well as dietary fiber, choline, lecithin, folate, and polyphenols [9]. The protein content in faba beans varies for different genotypes and environmental conditions, ranging from 27 to 34% of seed dry matter [41,51]. Moreover, faba beans are a good source of iron, potassium, magnesium, selenium, zinc, and copper [41]. When compared to most legumes, faba beans have a relatively low fat content with a good amount of dietary fibre and B-complex vitamins [41]. Crépon et al. [49] summarized the chemical composition of faba bean seeds, as shown in Table 1. In addition to their high protein and fiber content, faba beans are beneficial for lowering the plasma LDL cholesterol level, preventing chronic diseases such as diabetes and cardiovascular disease, and possibly

managing Parkinson's disease [9,52]. Despite the numerous health benefits linked to faba bean consumption, certain seed components can induce toxicity or allergies in humans [42].

Table 1. Chemical composition of faba bean seeds (dry basis).

	Duc et al. [53] Mean [a]	Sauvant et al. [54] Mean (*SD*)
High-tannin faba beans		
Crude protein (g/kg)	310	294 (25)
Starch (g/kg)	412	443 (31)
Crude fiber (g/kg)	99	91 (13)
Sugars (g/kg)	38	35 (9)
Fat (g/kg)	19	15 (4)
TIA (UTI/mg) [b]	2.9	
Condensed tannins (g/kg)	6.6	
Vicine + Convicine (g/kg)	8.3	
Lysine (g/kg)	20.3	19.2
Methionine (g/kg)	2.7	2.1
Cysteine (g/kg)	3.9	3.7
Tryptophane (g/kg)	2.7	2.4
Low-tannin faba beans		
Crude protein (g/kg)	319	311 (26)
Starch (g/kg)	427	433 (27)
Crude fiber (g/kg)	88	87 (10)
Sugars (g/kg)	44	43 (8)
Fat (g/kg)	20	13 (2)
TIA (UTI/mg) [b]	2.9	
Condensed tannins (g/kg)	0.1	
Vicine + Convicine (g/kg)	7.6	
Lysine (g/kg)	19.5	20
Methionine (g/kg)	2.6	2.2
Cysteine (g/kg)	3.6	3.9
Tryptophane (g/kg)	2.7	2.6

[a] Means of four low-tannin lines carrying gene zt1, compared to the mean of their high-tannin isogenics. [b] UTI: Units of trypsin inhibitor activity (Valdebouze et al. [55]).

4.2. Antinutriet Composition

According to Multari et al., particular molecules present in the raw faba bean seeds can have antinutritional effects, that is, the potential to cause adverse effect on nutrition, reducing digestibility and leading to some pathogenic conditions [45,50]. Studies have shown that saponins, tannins, vicine, and convicine have antinutritional effect in the diet of monogastric animals [2,8]. In human nutrition, divicine and isouramil—active derivates of vicine and convicine—are seed components that are toxic to individuals affected by a genetic disorder known as glucose-6-phosphate dehydrogenase (G6PD) deficiency.

The molecular structure of the favism-inducing components is shown in Figure 1 [50]. Figure 1 demonstrates two possible tautomeric forms of the aglycones. The concentrations of vicine and convicine have been detected at levels up to 5 mg and 2 mg/g of dry weight, respectively.

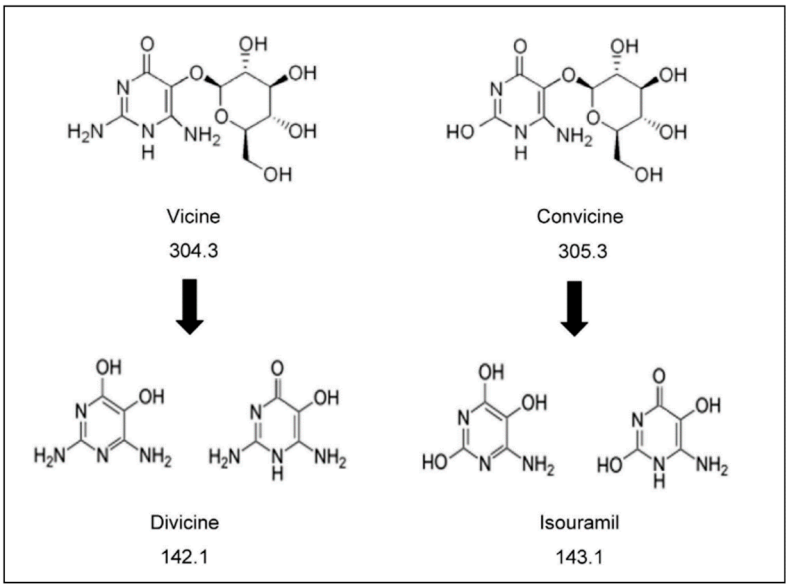

Figure 1. Molecular structure of vicine, convicine, and their respective aglycones, divicine, and isouramil [50].

5. Faba Bean Protein Isolate

Faba bean isolate refers to a protein extracted from faba beans, which is used as an ingredient in various food products. The goal of using plant-based protein isolates in food products is to create more sustainable and environmentally friendly food choices by reducing reliance on animal protein sources [7,56]. Faba bean isolate consists of approximately 80–95% crude protein [57] and has two main protein fractions: globulin and albumin. The globulin fraction is composed of vicilin and legumin, and the faba bean protein isolate consists of this fraction [58]. Multari et al. reports that the production of protein isolates and concentrates can significantly improve the nutritive value of legumes [45] and the protein structure plays a very important role in its functional properties. Various chemical, enzymatic, and physical treatments can be used to modify protein structures and tailor protein isolates to specific applications [58].

Because the composition of faba beans includes antinutritional components, the production of the protein isolate is a promising approach to produce high-quality functional nutritional foods and supplements free of favism-inducing components [45,59]. However, the manufacturing process, that is, the method and conditions of isolation, is a determinant factor on the composition and functional properties of the protein isolate, such as solubility, foam expansion, gelation capacity, emulsifying capacity, and others [45].

According to GEA, protein isolate manufacturing consists of three main processes: extraction, purification, and drying, as shown in Figure 2. For the first step, the alkaline condition promotes the dissolution of protein fractions in the aqueous extract, yielding a vegetable isolate with a protein content of 80% [60]. However, various techniques can be used besides alkaline extraction, and different extraction techniques can yield different properties. Afterwards, by using an appropriate technique such as decantation or isoelectric precipitation, the aqueous extract can be separated from other solids. The proteins that are dissolved in water can be precipitated and separated. Further dilution, pH adjustments, thermal treatment, and finally drying are necessary to obtain the protein isolate powder [60].

Figure 3 shows the protein isolate process based on alkaline extraction used by Vioque et al. [59]. According to the authors [59], the process yielded 92% protein isolate with a high oil absorption capacity; favism-inducing components were almost completely eliminated. Furthermore, by-products presented a great potential use in the food industry.

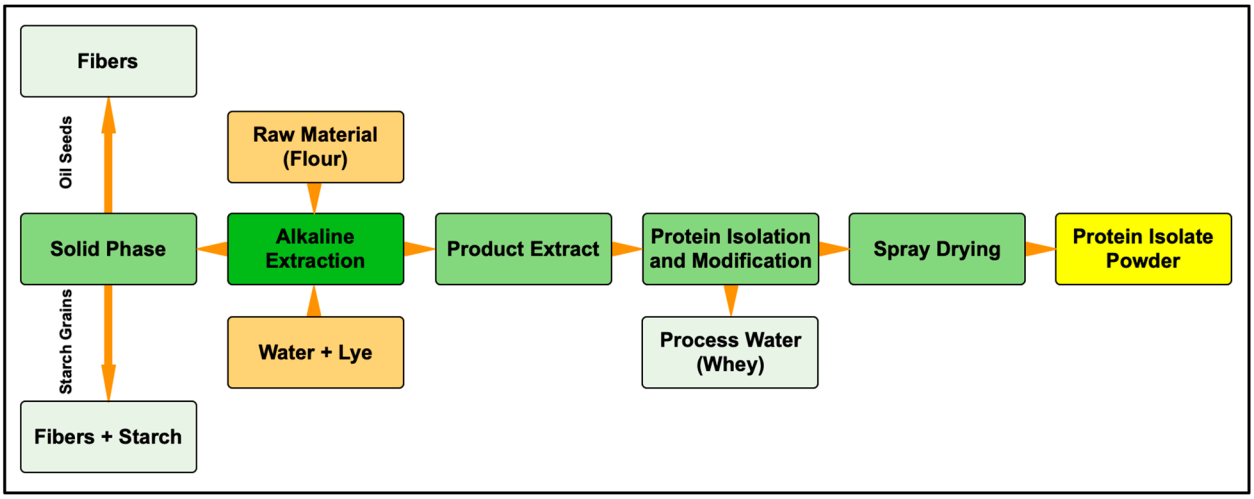

Figure 2. Example of protein isolate process [60].

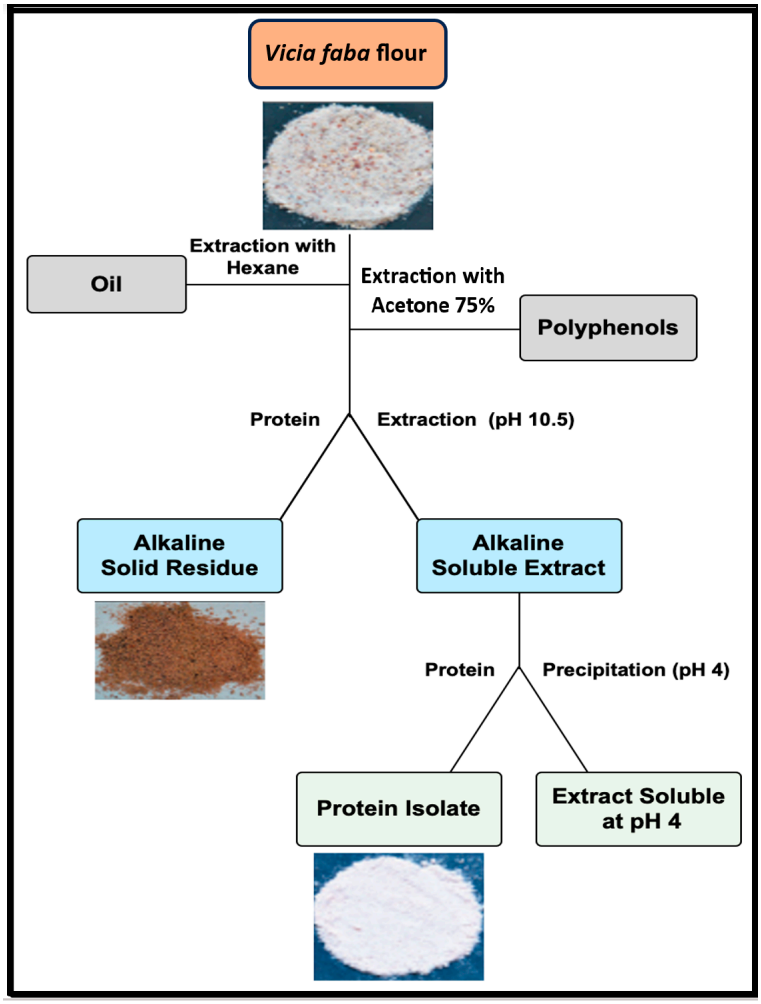

Figure 3. Production process for *Vicia faba* protein isolates [59].

Martínez-Velasco et al. investigated the effect of high-intensity ultrasound treatment of faba bean proteins. Physicochemical and surface properties were analysed, as well as foaming ability, stability, morphology, bubble size, and rheology foams. Lower interfacial tension, zeta potential and viscosity, and higher solubility were observed. Furthermore, the structure and relative digestibility of the faba bean protein isolate were studied. According

to the response surface methodology, an optimized faba bean protein isolate was obtained under amplitude of 72.7% for 17.3 min [61].

6. Faba Bean Processing Methods

While faba beans boast various nutritional properties, their application in the food and feed industry is limited by significant antinutritional factors. Processing techniques are essential to safely incorporate faba beans into diverse diets, particularly since these factors are more concentrated in their raw form [62]. Processing techniques include soaking, dehulling, cooking, roasting, autoclaving, germinating, fermenting, and recently extrusion cooking [14,63–65]. Cooking techniques such as boiling, roasting, and frying can reduce the content of the mentioned compounds from 20% up to 40% [45]. However, these methods can also affect the nutritional properties of faba beans [64,66].

Van der Poel et al. applied dehulling, reconstitution, extrusion, and reconstitution prior to extrusion and evaluated the effect of the processing methods on the tannin content [67]. The flow diagram for processing faba beans used by Van der Poel et al. is shown in Figure 4 [67]:

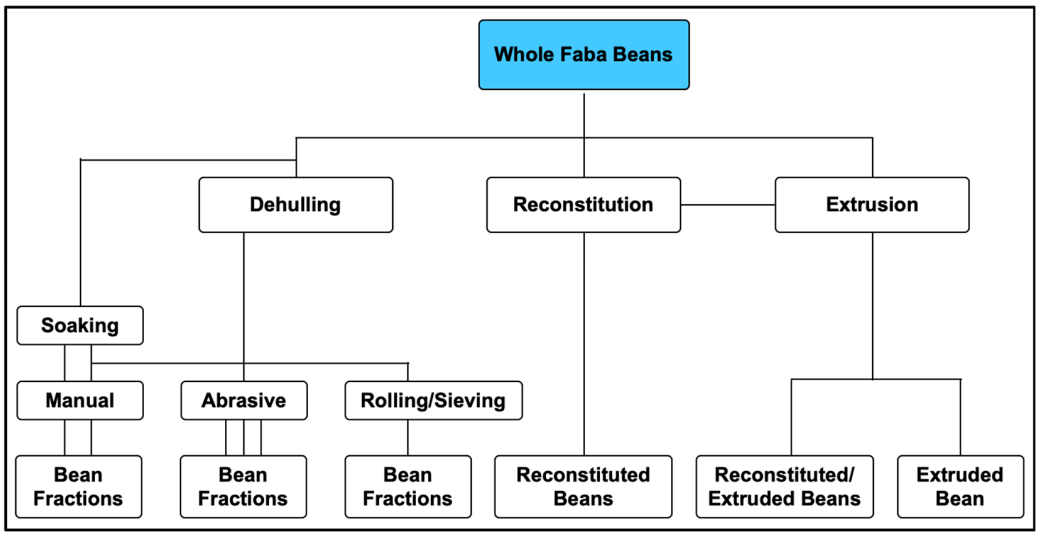

Figure 4. Flow diagram of processing faba beans [67].

The study showed that the level of tannins in faba beans can be reduced using fractionation procedures such as dehulling or thermal treatments. Dehulling can completely remove all tannin fractions; however, it also removes protein. Steaming, reconstruction, or extrusion can cause a change in the chemical structure of tannins leading to lower extractability, for example [67].

Luo et al. analysed the effect of various processing techniques on antinutritional factors. It was reported that different methods affected the components differently; for instance, dehulling and soaking increased levels of phytic acid and trypsin inhibitor activity, but it was ineffective for lectin activity [62]. Alonso et al. reported that dehulling significantly decreased condensed tannin and polyphenol levels; however, it also reduced the protein content [64].

In previous study, Jamalian et al. attempted removing vicine and convicine using four different techniques: stepwise soaking, autoclaving, soaking in a continuous flow of an acid solution, and flow soaking in tap water at varied temperatures and soaking times. Only flow soaking in tap water for 72 h at 50 °C, 60 h at 55 °C, or 48 h at 60 °C with flowrate of 0.5 mL/min could completely remove vicine and convicine from whole faba beans [68]. However, other properties were compromised during the proposed procedures, and a great amount of contaminated water, which is required to be treated before disposal, might compromise the economic feasibility of this technique.

Rizello et al. reported a study on the degradation of those two components. The authors believed that complete hydrolysis of vicine and convicine could avoid their adverse effects in sensitive subjects. The hydrolysis kinetics of vicine and convicine and their derivates during fermentation of faba bean flour was investigated using a specific liquid chromatography–mass spectrometry (LC–MS) method. The fermentation process enhanced the flavour and nutritional properties of faba bean flour [50].

7. Emerging Technology for Elimination of Antinutritional Compounds

While faba beans are recognized as a protein-rich source, their low functional properties restrict their applications. The demand for faba beans has been hindered by the presence of antinutritional compounds such as pyrimidine glycosides (vicine and convicine), condensed tannins, and protease inhibitors [12,13]. To enhance functional properties and ensure the safety of faba bean consumption, various methods can be employed to remove or degrade these antinutritional compounds [14,15]. Vicine and convicine (pyrimidine glycosides) are thermostable seed components and not easily removed. There is evidence that vicine and convicine are responsible for favism in susceptible individuals, and reduction in animal production systems like the size of chicken eggs [69].

Many treatments have been proposed, but most are difficult to be scaled up and often incompatible with food and feed industry [50]. Genetic modifications have been studied over decades in an attempt to select low vicine, convicine, and tannin content genotypes [49,70]. However, these genetic modifications lead to low yields due to the fact that vicine and convicine provide protection against insects and fungi in faba seeds [71]. The traditional methods discussed in the previous section cannot completely remove vicine and convicine. Therefore, it is extremely important to use suitable and accurate techniques to detect their occurrence. Pulkknien et al. successfully applied reversed-phase, high-performance liquid chromatography with UV detection to observe both components in the isolate fraction and extract made from faba beans and in faba bean suspension [72].

In 2014, Osman et al. presented an investigation on the effect of gamma irradiation and/or cooking on the composition and presence of antinutritional factors. The results obtained revealed that a low dose of gamma irradiation and/or cooking treatment could significantly reduce the contents of antinutritional factors and increase digestibility in faba bean seeds. Furthermore, no significant changes in chemical composition and mineral contents were observed [73].

8. Added-Value Products from Faba Bean and Future Application

As per previous studies, the incorporation of faba beans in food and feed products can enhance their nutritional composition, dependent on how the faba beans are used—as seed, protein isolate, pellets, etc. Chieab et al. evaluated the content of polyphenols and antioxidant capacity of thirteen faba bean genotypes. It was found that faba beans are a good source of natural antioxidants; hence, they could be used to increase the shelf life of food and feed products [74]. Different forms of faba beans have been incorporated into different products such as pasta, yielding a gluten-free product [75]. Gluten-free products have gained a lot of attention and shelf space due to increased customer awareness on health and new dietary trends. Faba bean protein isolate has also been used as an emulsifying agent in the food and feed industry. A recent study by Liu et al. evaluated the potential use of microbial transglutaminase (MTG)-treated faba bean protein isolate (FBPI) as an emulsifier in oil-in-water emulsion. The product was successful in maintaining physical stability and improving lipid oxidative stability in emulsion [75,76].

Pietrzak et al. explored the use of faba bean seeds in the production of bioethanol, feed components, and biomass as an integrated process. A summary of the experimental procedures is shown in Figure 5. By combining different treatments, a total of six different processes were tested [77].

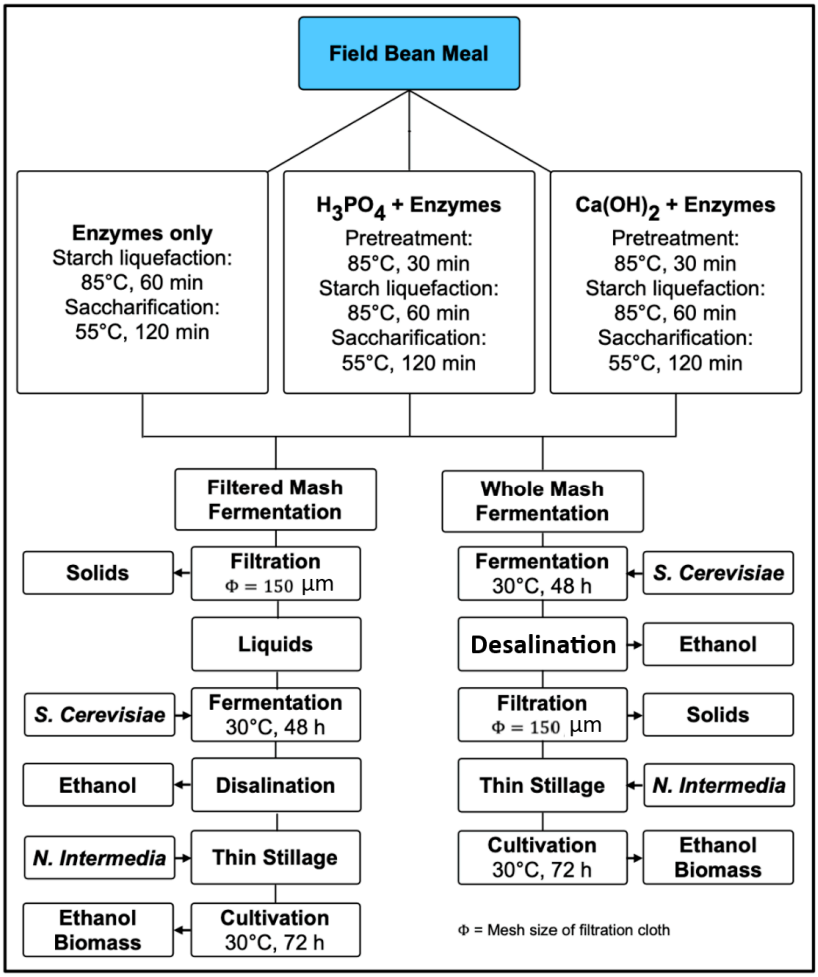

Figure 5. Flowchart of experimental procedures [77].

The average ethanol yield was considerably different between filtered mash and whole mash fermentation—75% and 37%, respectively. The crude protein content recovery was comparable between both cases, with an average of 79% and 63%. According to the study, the solid residues contained up to 32% protein, and the content of antinutritional factors was reduced. Overall, the authors considered faba beans a feasible feed-stock for an ethanol biorefinery [77].

9. Membrane Technology for Purification of Plant-Based Protein Isolate

Following the enrichment procedure, proteins need to be purified. There has been an increasing implementation of membrane technologies in the industrial processing of food products/by-products. Membrane separation processes have been adopted as purification/concentration procedures in the production of plant-based proteins and protein nanofibrils due to their selective separation, simple operational conditions, and easy automation [78–81]. The difference between protein isolates and concentrates is the protein content in each one. Protein isolates should have at least 70% of protein content and protein concentrates at least 90% of protein content, both on a dry basis [82]. Moreover, membrane separation can provide specific benefits for the purification of different plant-based proteins, including preserved protein properties, low recovery cost, and high recovery yields and purity [31,82–85]. According to the reported literature, traditional methods for plant-based protein production are unable to efficiently remove phytic acid (an antinutritional factor) and insoluble carbohydrates. Furthermore, most methods require acid precipitation that can affect the functional properties of the proteins [82].

9.1. Advantages of Membrane-Based Separation in Plant-Based Protein Purification

In the realm of plant-based protein purification, membrane-based separation offers a number of distinct advantages over other methods. Membrane-based separation has been proven to be a viable technique for large-scale production in industrial applications [86]. Membrane filtration can be used for the separation/purification of soluble proteins, based on their size, through a pressure-driven process (microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF)) without altering their structure (preserving their functional and nutritional properties) [32,87–89]. Ultrafiltration (mean pore size of 1–100 nm) is the most effective process to purify/isolate proteins and other macromolecules, while microfiltration is ideal for separating fine particles sized 0.1–10.0 µm [24]. Nanofiltration (mean pore size of 0.2–2 nm) can also be employed in the separation/purification of the smaller proteins and some types of peptides/amino acids. Wang et al. [90] employed UF and NF processes for the purification of glutathione after extraction. According to their findings, the combination of UF and NF processes showed promising results for the concentration/purification of glutathione from yeast extracts. UF was used first to concentrate glutathione in the permeate stream (larger particles were separated). Subsequently, the NF process was applied to the glutathione-rich solution obtained from the UF permeate to purify glutathione.

The efficacy of protein hydrolysates can be enhanced through the use of UF. The application of appropriate UF membranes would produce highly purified, food-grade proteins with a desired molecular size [91]. The functional properties of faba bean protein isolates were found to be inadequate for use in food applications owing to their low solubility. In this regard, Eckert et al. [92] aimed to enhance the solubility and functional properties of faba bean proteins through the application of the UF technique following enzymatic hydrolysis. UF membranes with two different molecular weight cut-offs of 10 and 5 kDa have been used for the fractionation of faba bean hydrolysates. According to their results, the use of ultrafiltration resulted in enhanced foaming and oil holding capacity, as well as significantly improved emulsifying capacity. Therefore, it was deduced that ultrafiltration following enzymatic hydrolysis is a viable approach for markedly enhancing the solubility and functional characteristics of faba bean proteins. The protein composition of faba beans reveals that a significant portion, ranging from 69 to 78% of the storage proteins, is comprised of salt-soluble globulins, which are primarily located in the membrane-bound protein bodies. In order to produce clean-label proteins that are low in salt or free of added salt, filtration techniques such as dialysis, ultrafiltration, or diafiltration could be employed over wet processing, which can denature proteins through heat and pH changes [57,93,94]. Membrane-based separation presents a promising alternative to the conventional acid-leaching process for protein separation and isolation. This approach involves the use of a variety of membranes that selectively separate and extract components based on their molecular sizes [94]. As reported by Vose [95], ultrafiltration was utilized to isolate protein from faba beans, and it resulted in a protein yield of 94% (w/w). The protein obtained from this process exhibited comparable foaming and emulsifying properties to those obtained from isoelectric precipitation, which yields 91% (w/w) of proteins. In another study by Jeganathan et al. [96], ultrafiltration and dialysis were employed to isolate faba bean proteins without using alkali/acid and thermal treatments (clean-label proteins). The results demonstrated that protein isolates obtained using a cellulose membrane with a molecular weight cut-off of 6–8 kDa, following water extraction, at 35 °C and a solvent/feed (S/F) ratio of two, had a higher protein yield, recovery rate, and protein content, as compared to the protein concentrates that have been produced through alkali extraction followed by acid precipitation. However, due to the impracticality of performing large-scale dialysis for the extraction, ultrafiltration (Nuetch filter, LJ Star W.T. Maye) was used as a substitute. High-tannin faba bean dehulled flour was subjected to water extraction at a S/F ratio of three, followed by ultrafiltration and spray drying, which yielded a protein fraction of 16.46 ± 0.12% with a purity of 82.80 ± 0.03% and recovery rate of 40.08 ± 0.28%.

9.2. Diverse Applications of Ultrafiltration in Protein Processing

One specific membrane technology that has gained significant prominence in protein processing is ultrafiltration. Its diverse applications extend across various facets of protein purification and processing, offering unique advantages for different protein sources and end products. Aside from in faba bean isolation, ultrafiltration was used in a study on protein enrichment from ryegrass and alfalfa, and it was compared to coagulation/centrifugation. Despite the fact that crude protein yields were almost identical between these methods, ultrafiltration resulted in a higher protein solubility and 14% higher crude protein recovery [97]. In another study, Vishwanathan et al. assessed the ability of MF and UF membranes of various pore sizes/MWCO to eliminate non-protein substances from okara—a by-product of the soymilk production—and soy protein extract [82]. Figure 6 shows the lab-scale procedure utilized in the study. The system consisted of a cross-flow flat sheet membrane operated in batch mode. The results obtained by Vishwanathan et al. indicated that both MF and UF are feasible processes for the purification/concentration of okara and soy protein concentrates. The protein content in okara extract increased by approximately 13% for both processes, reaching about 80%. Soy extract presented better protein content improvement, reaching 85%. All membranes tested had a similar performance, but the larger pore size offered reduced processing time as the result of a higher average flux. Overall, the study indicated that membrane technology can be successfully applied to produce protein concentrates without compromising protein properties and adding value to underutilized products such as okara [82]. In another study, a UF membrane was used in the purification of Lupin proteins following liquid/solid extraction at various pH levels by Albe-Slabi et al. [98]. The protein retention rate and permeate flux were measured using an Akta Flux®6 system coupled with a hollow fiber cartridge with different molecular weight cut-offs (MWCO) (10, 30, 100, and 300 kDa). The UF membrane with MWCO of 10 kDa retained proteins completely with a flux of 0.09 mL/min·cm^2. It has also been reported that even 300 kDa MWCO results in 97% protein retention (flux of 0.11 mL/min·cm^2). After washing with five diafiltration volumes (DV) using ultrapure water, the rejected proteins were collected and freeze-dried.

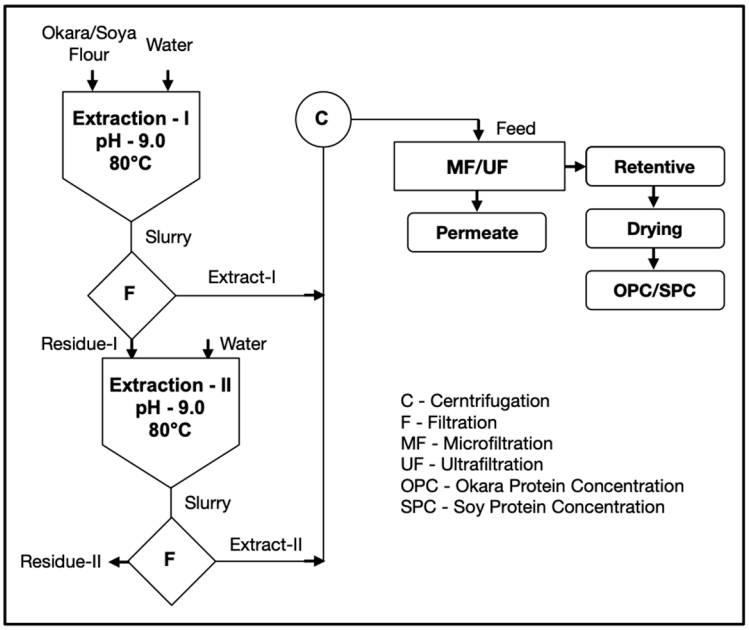

Figure 6. Scheme for processing of SPC/OPC using membrane technology [82].

Soybeans have played an important role in the human diet as a rich source of protein; and its functional forms such as flours, isolates, and concentrates became very popular. Traditionally, the proteins from the soybean were extracted using defatted flour with acid

or alcohol, followed by a separation process such as centrifugation or filtration [83]. Kumar et al. adopted a membrane separation process to produce soybean protein concentrates as shown in Figure 7. Using a polyvinylidene difluoride membrane (18 kDa), ultrafiltration (UF) was conducted in batch mode while diafiltration (DF) was operated continuously. According to the study, UF presented higher yields than conventional processes but still presented limitations. The retention of salts in the retentate, high viscosity, and higher solids losses in the permeate at high concentration factors limited the protein level to 60–70%. To overcome these limitations, UF was combined with DF, and optimum results were obtained with the following configuration: UF-DF-UF. A higher protein content was obtained (90%), while sugars were almost completely removed [83].

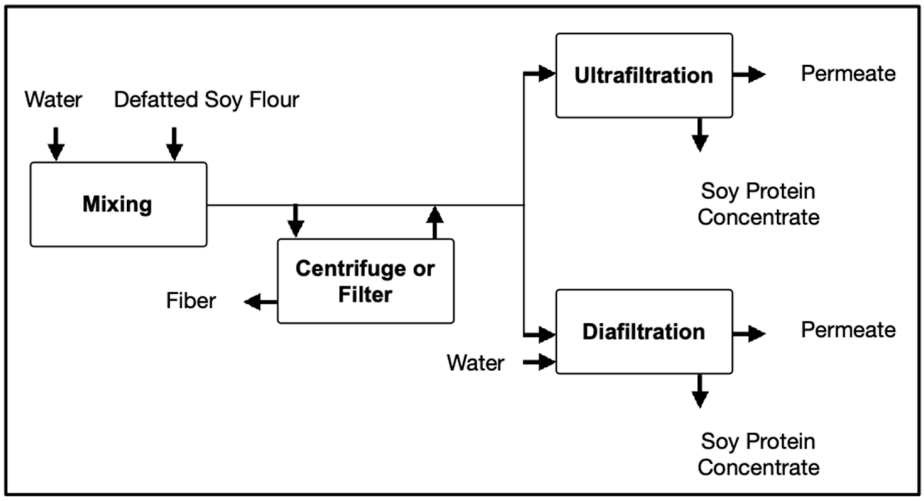

Figure 7. Schematic of ultrafiltration process for soy protein concentrates. UF, ultrafiltration concentration; DF, diafiltration [83].

Mondor et al. investigated the impact of four different sequences of UF and DF to purify soy protein extract with pH 6. The pH 6 extract was obtained via electro-acidification and the filtration was performed using a polysulfone hollow fibre membrane. The study concluded that the UF/DF sequence had a significant impact on membrane fouling, permeate flux, and protein concentrate properties such as ash and phytic acid content and solubility. Also, the most effective process which yielded a higher protein content was the one in which DF was performed continuously with a more concentrated solution. However, it was also the one more severely affected by membrane fouling [84]. Hernández-Marín et al. [99] used a combination of ultrafiltration and diafiltration (5 kDa membranes) for the purification of Huauzontle seed protein after alkaline extraction. The protein isolation/purification process was completed successfully with about 66% purity for precipitated protein isolate (SPI). To improve purity, they repeated the process with a 10 kDa membrane. A membrane with larger pore sizes was used to separate compounds with higher molecular weights and allow the transport of all proteins to the permeate, resulting in a 78% SPI purity. Taherian et al. compared properties of commercial and membrane-processed pea protein isolates from yellow peas. Four pea protein isolates were obtained using KCl extraction followed by UF and DF. The level of phytic acid was reduced in the range of 28–68% and functional properties were enhanced [100]. UF and DF following alkaline extraction have also been used in the production of protein isolates from Camelina sativa, as reported by Sarv et al., yielding a protein content of 67% in the protein isolate [101]. Boye et al. compared the functional properties of the protein concentrate from pea, chickpea, and lentil using UF/DF and isoelectric precipitation [102]. Figure 8 summarizes the process used in the study to obtain the protein extracts. The membrane separation process yielded products with a higher protein content (69.1–88.6%, w/w) compared to isoelectric precipitation (63.9–81.7%, w/w), and all concentrates presented good functional properties (in terms of solubility, wa-

ter holding capacity, emulsifying properties, and foam stability) with expected variability among different legumes [102].

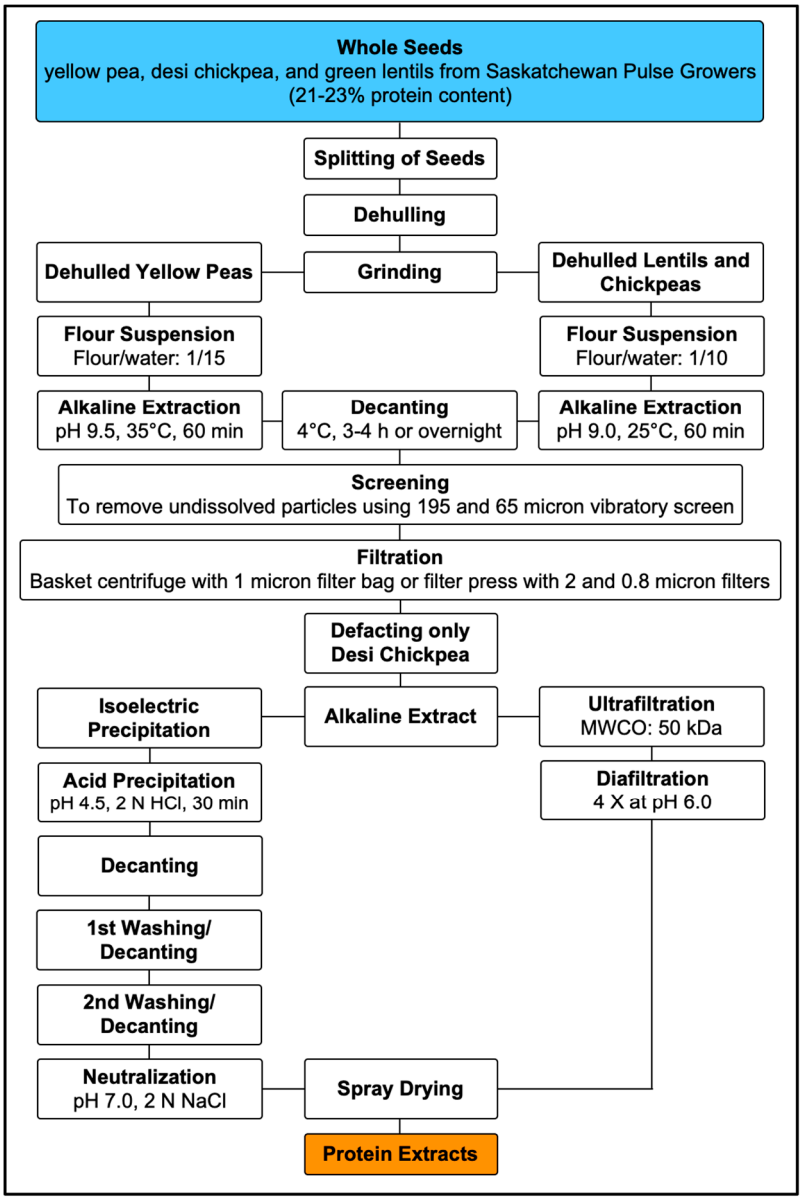

Figure 8. Schematic of the process used for the pilot scale production of the pulse protein concentrates [102].

Membrane dialysis has also been used in protein purification to improve separation efficiency and yield. In a study on protein extraction from soybean, Khan et al. [103] used membrane dialysis to remove the salt from the trypsin inhibitor protein extract. The supernatant from protein extraction was subjected to centrifugation followed by ammonium sulfate precipitation. Membrane dialysis was then used to successfully purify the centrifuged pellets. Hansen et al. [104] also used dialysis for pea protein purification following alkaline solubilization, isoelectric precipitation, and salt solubilization. The results (protein purity, yield, and ash content) were compared with those obtained from purification via ultrafiltration and the combination of dialysis and ultrafiltration (Vivaflow® membrane with 3 kDa). It was found that ultrafiltration did not completely remove salt from the proteinaceous supernatant, leaving a low protein purity and high ash content. In contrast, dialysis increased the protein purity and decreased the ash content (still noticeable).

The most favorable results were obtained from the combination of UF and dialysis, such that protein purity, yield, and ash content reached 92.8%, 72%, and 1.56%, respectively.

Plant-based peptides (short chains of amino acids [105]) have been widely used in the food, pharmaceutical, and cosmetic industries [106–108]. Membrane technology has made it possible to fractionate/isolate peptides from complex feedstocks based on their electric charge, size, or molecular weight [109,110]. According to Nuchprapha et al. [111] ultrafiltration membranes with 3, 5, and 10 kDa molecular weight cut-offs were employed sequentially to separate the peptides from protein hydrolysates (from longan seeds). Peptides with four different ranges of molecular weight cut-offs were separated (>10 kDa, 5–10 kDa, 3–5 kDa, and <3 kDa). According to their research, ultrafiltration improved the purification efficiency of small peptides which also had the most angiotensin-converting enzyme inhibition (ACEI) activity (ACEI helps in controlling hypertension and promoting cardio protection). The combination of electrodialysis with ultrafiltration membranes (EDUF) proved to be an effective method to fractionate peptide mixtures with charged solutes that have a similar molecular weight. This method has been employed in several studies for bioactive peptide separation [112–116]. In EDUF, electrodialysis and electrophoresis principles are combined using ultrafiltration membranes, which serve as a molecular barrier and separate the components by the electric potential difference [112]. Firdaous et al. [117] employed EDUF for the separation of bioactive peptides from a plant-based (alfalfa) protein hydrolysate. It has been reported that EDUF was able to overcome some of the fouling issues associated with conventional pressure-driven processes and also separate/concentrate charged peptides simultaneously at a transport rate of up to 7.3 $g/m^2 \cdot h$. More recently, González-Muñoz et al. [118] used EDUF for the separation of peptides from quinoa. The results showed no significant fouling development, and peptide fractionation from quinoa hydrolysate was proven successful as an antihypertensive and antidiabetic food alternative. In another study conducted by Doyen et al. [119] it was reported that using ultrafiltration facilitated the separation of peptides with lower molecular weights (300–500 Da).

10. Challenges of Membrane Technology in Purification of Plant-Based Proteins

Membrane-based processes have a wide range of applications and exhibit promising results. However, there are some limitations in their operation, for which optimization of the process is required.

Membrane fouling is one of the major drawbacks in membrane filtration processes, due to particle deposition and accumulation on the membrane surface or within the internal pores [6,110,120–124]. Fouling development during the filtration process negatively affects filtration performance in terms of permeate flux. The amount of particles retained on the membrane surface and inside the pores increases with time and causes continued flux decay [29,125]. As reported by Mondor et al. [126], permeate flux decreased by more than 45% once 1600 mL of solution was used in one sequence of ultrafiltration followed by continuous diafiltration for soy protein isolation (using a hollow fiber polysulfone membrane with the area of 650 cm^2). The membrane resistance increased from $96 \times 10^{12} \pm 7.75 \times 10^{10}$ m^{-1} for the clean membrane to $2.62 \times 10^{13} \pm 2.41 \times 10^{10}$ m^{-1} for the fouled membrane. It was also observed that the permeate flux was improved by about 20% when discontinuous diafiltration was employed in the system (resistance of $2.41 \times 10^{13} \pm 3.05 \times 10^{12}$ m^{-1}). However, ultrafiltration with discontinuous diafiltration resulted in a lower protein content and a higher ash content. Therefore, a complete economic analysis is required to determine the most feasible process for protein isolation/purification.

Membrane fouling and particle aggregation may occur as a result of the protein's low water solubility in the solution. In this regard, researchers have suggested combining enzymatic hydrolysis with membrane filtration to improve protein solubility [127]. However, this method is more effective for the dead-end process system with a short filtration time. In a continuous process, particle aggregation and fouling may increase over time as the amount of insoluble protein increases and enzyme activity diminishes.

Qu et al. [128] evaluated membrane fouling development by measuring the transmembrane pressure when separating ACE-inhibitory peptides from defatted wheat germ protein in an ultrafiltration process following an enzymatic hydrolysis. Their findings indicated that membrane fouling caused by insoluble substances increased with time, resulting in an increase in transmembrane pressure. During the first 90 min, the ultrafiltration pressure was relatively constant (6.5 psi), then slowly increased from 90 to 150 min (6.5 to 8.5 psi), and then rapidly increased until it reached 15 psi by 210 min.

Solute–membrane interactions, which could lead to fouling formation, are strongly influenced by membrane properties such as hydrophobicity/hydrophilicity, pore size, and surface charge [22,129]. There is a risk of fouling development both in hydrophilic and hydrophobic polymeric membranes. However, membranes with a highly hydrophilic nature are less susceptible to severe fouling development during the separation and purification of organic matter like proteins [22,32,129]. In this regard, Leberknight et al. [130] used polyethersulfone (PES) and regenerated cellulose membranes (5 kDa) in an ultrafiltration process for protein recovery from a corn ethanol process. Those two membranes were both hydrophilic; however, PES appears to be less hydrophilic than the regenerated cellulose membrane, according to their water contact angles data. The results of the protein separation indicated that permeate flux decay was significantly higher for the PES membrane, showing more severe fouling formation (flux decay of 20% for regenerated cellulose and more than 40% for PES). Similar results were reported by Zhang et al. [131] when they used UF polymeric membranes (commercial PES membranes) for protein recovery from alfalfa wastewater. According to their observation, hydrophilic modified membranes were found to be more resistant to protein fouling. Since hydrophilic membranes are less susceptible to protein fouling, membrane modification is an effective way to improve the fouling resistance of membranes by incorporating hydrophilic groups onto membrane surfaces or in polymeric solutions. This enhances the membrane's hydrophilicity and antifouling properties. There are several studies using hydrophilic additives like poly(ethylene oxide) or poly(ethylene glycol) (PEG) to improve the protein fouling resistance of UF membranes such as polyacrylonitrile (PAN) [132,133], poly(vinylidene fluoride) (PVDF) [132,134], polysulfone (PSf) [135,136], etc. There is a need for similar studies to enhance membrane fouling resistance against plant-based proteins.

Membrane selectivity is an important factor in measuring the quality of the separation process. Selectivity measures how well desired molecules are separated from unwanted molecules. In pressure-driven filtration, the pore size and pore size distribution of a membrane greatly influence the separation capacity and the permeation/rejection [137]. In spite of the low energy input, high separation efficiency, simplicity of operation, lack of use of costly solvents and effluents, and excellent scalability, no membranes are capable of separating compounds with nearly similar polarity and molecular weight (like proteins, peptides, and amino acids). In pressure-driven membrane processes, low selectivity becomes the major challenge for peptide separation and purification from complex protein hydrolysates. In this scenario, the permeate would be contaminated with undesirable compounds that are smaller than the membrane pores and have polarities that are similar to the target species [138,139]. Bioactive peptides contain a limited number of amino acid chains (2–6) and usually have low molecular weight (150–600 Da) [140]. In spite of the fact that pressure-driven UF and NF are the most commonly used membranes for peptide separation and are capable of continuous production/separation of low molecular weight molecules, they often fail to provide sufficient selectivity for peptides with a close molecular weight and different charges [112,137,141]. In order to improve the migration and selective separation of charged molecules, electrically driven membrane processes including electromembrane filtration (EMF) and electrodialysis with filtration membrane (EDFM) have been employed using ion-exchange and ultrafiltration membranes [141,142]. Langevin et al. [143] used both pressure-driven NF (MWCO of 300–500 Da) and EDUF for the separation of bioactive peptides from soy protein hydrolysate. Peptides with a high molecular weight were removed from the primary protein hydrolysate using the

UF process (10 kDa). According to their observations, even though NF led to a higher mass flux, EDUF recovered a greater quantity of polar amino acids and showed enhanced selectivity towards charged molecules. In another study by Firdaous et al. [117], it was reported that the combination of ultrafiltration (using a PES membrane) with EDUF showed promising results in improving the migration rate and overcoming some of the fouling issues associated with conventional pressure-driven processes in the separation of bioactive peptides from plant-based (alfalfa) protein hydrolysates. It is possible for the membrane surface charge density to influence membrane selectivity and protein separation [117]. Membrane surface modification can therefore be an effective method of improving protein charge selectivity by increasing attraction or repulsion between target molecules and the membrane surface [144,145].

Enzymatic protein degradation can also be a challenge in membrane filtration due to the elevated proteolytic activity caused by the operating temperature (normally room temperature) and filtration process duration (several hours to days) [87]. The time length of filtration and temperature are two factors playing an important role in protein degradation. Koschuh et al. [97] reported that after 24 h of storing Rubisco protein at 30 °C, 99% of the protein was degraded, while this value was about 20% at 0 °C. In terms of storage duration, it was observed that about 35% of the protein was degraded in the first 3 h and it reached 99% after 24 h.

11. Outlook

A growing market for plant-based proteins has led to many studies on legumes, including faba beans, which are rich in plant proteins, nutrients, dietary fiber, secondary metabolites, and bioactive compounds. Faba beans have been used for human nutrition in their green, immature, and/or dried states for future use. Although faba beans are becoming increasingly popular as a source of protein, some of their low functional properties and antinutritional compounds such as pyrimidine glycosides (vicine and convicine), condensed tannins, and protease inhibitors limit their use. Thus, various processing methods would be required to remove or degrade antinutritional compounds from faba beans in order to improve their features and ensure their safety for consumption.

Advances in biotechnology and analyses of various types of protein structures and functions have led to notable breakthroughs in protein purification and separation techniques. In recent years, membrane-based technologies have been increasingly used as a means of protein purification following the enrichment process in various industries.

Membrane filtration processes have some advantages, including their ability to operate at low temperatures and pressures without phase changes, preserve bioactivity, molecular separation, high efficiency, small footprint and chemical consumption, high protein recovery yield, and ease of scaling up which makes them ideal for many applications. It has been proven that pressure-driven processes, such as MF, UF, and NF, are the most effective methods for separating proteins from extracts. The combination of those processes with dialysis and electrodialysis would lead to a higher yield and protein purity.

Despite all its advantages, membrane filtration still has some limitations when it comes to protein purification, including membrane fouling, the inability to separate molecules with nearly similar polarities and molecular weights (MWs), and enzymatic protein degradation.

To achieve the desired purification efficiency, it is essential to select the correct membrane material in the separation process. For instance, water and alcohol (known as highly polar compounds) are transported more efficiently using hydrophilic materials, such as cellulose acetate (CA), polyvinyl alcohol (PVA), sodium alginate, chitosan, and polylactic acid (PLA) [32]. On the other hand, hydrophobic membranes (also referred as organophilic) such as poly(octylmethylsiloxane) (POMS), polydimethylsiloxane (PDMS), polyether block amide (PEBA), or poly(1-(trimethylsilyl)-1-propyne) (PTMSP), preferentially transport nonpolar molecules (or molecules with less polar properties) [32]. In spite of the factual material being selected according to the system requirement, fouling may still occur. Therefore, it is necessary to modify membranes in order to improve their separation efficiency and prevent

fouling development. Composite membranes demonstrated superior separation rates and antifouling properties/fouling resistance in protein separation [22,146,147].

The global plant-based protein market was worth USD 12.2 billion in 2022, and was anticipated to grow at a rate of 7.7% during 2022–2027 to hit USD 162 billion by 2030 in terms of value, according to a Bloomberg Intelligence report. Therefore, an increase in the plant-based protein economy is expected to drive the demand for membrane separation technologies. However, there is an urgent need to improve the fouling resistance and separation efficiency in purification of various types of plant-based proteins and bioactive peptides/charged solutes that have similar size/molecular weight. The development of mixed matrix membranes is expected to witness a higher rate as an alternative to conventional UF membranes for the purification of plant-based proteins. Figure 9 shows the impact of membrane modification on the improvement of solute migration, selectivity, and fouling resistance. To enhance selectivity and solute migration, it is recommended that research efforts be focused on synthesizing new membranes with impregnated nanomaterials that could create strong interactions with protein isolates. The interactions should include hydrogen bond interactions, electrostatic interactions, and π–π interactions, as demonstrated in Figure 9. On the other side, the innovative membranes should have a tuned chemistry that provides higher repulsion forces to peptides and amino acids that have similar polarities and molecular weights.

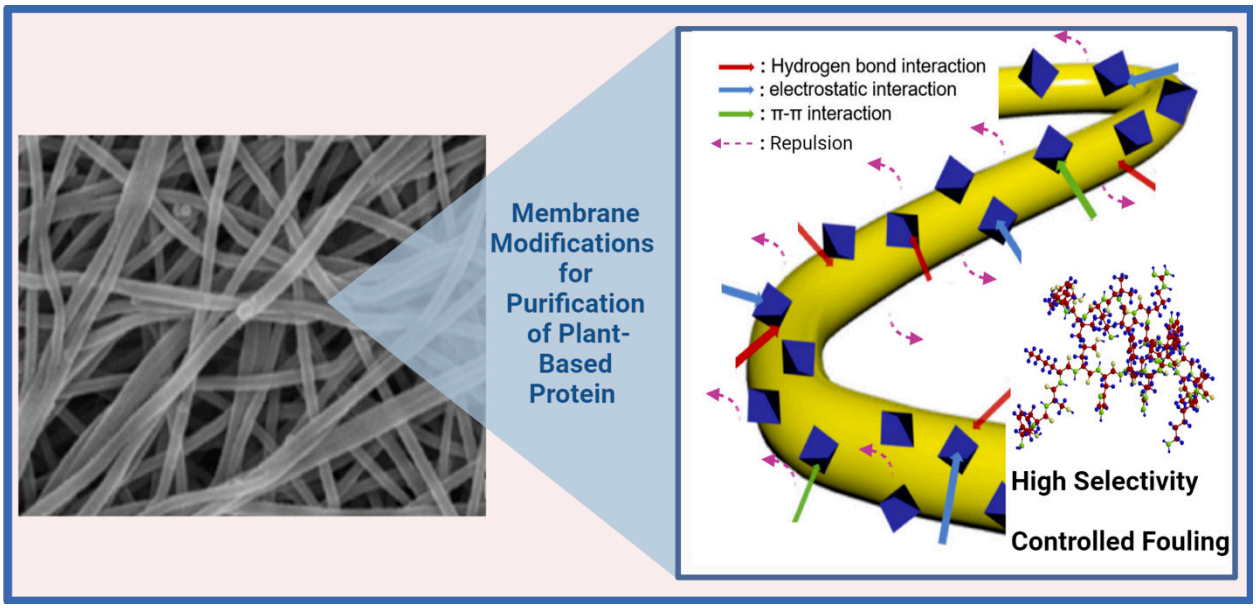

Figure 9. Protein selectivity and fouling resistance improvement through membrane modification.

Finally, since most of the capital investment in industrial production goes towards separation and purification [148], it is necessary to develop economical and high-yield membrane-based technologies for the separation/purification of plant-based proteins/peptides in various industries.

Author Contributions: Writing—Original Draft: M.E. and H.W.; Conceptualization: A.A.; Writing—Review & Editing: H.D., A.L. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (DG).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The information about the data presented in the article is available from the corresponding authors (Huu Doan; H.D. & Amira Abdelrasoul; A.A.) upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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