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# Valorization of fruit and vegetable by-products for protein extraction and their functional applications in food and non-food sectors

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## ABSTRACT

The fruit and vegetable processing sector generates substantial quantities of underutilized by-products, creating both economic losses and environmental challenges. Addressing these issues requires innovative strategies for converting these byproducts into valuable resources. This review explores the potential for recovering proteins from by-products of different fruits and vegetables and highlights their uses in the food and non-food sectors. Various extraction techniques, including conventional methods and emerging green extraction techniques, such as pressurized liquid extraction, enzyme-assisted extraction, and pulsed electric extraction, have been evaluated for their effectiveness in isolating proteins while preserving their functional characteristics. The extracted proteins provide substantial nutritional and functional benefits, making them ideal for use in the development of functional foods, fortification, and nutraceuticals. Non-food applications of the extracted proteins, including animal feed, edible films, bioplastics, and biopolymers, are also discussed. To maximize the potential of proteins, future studies should focus on improving sustainable extraction techniques, as well as their regulatory, economic, and safety aspects, and expand the functional applications of these proteins. The purpose of this review is to contribute to healthier and more sustainable food systems by emphasizing the recovery of proteins from fruit and vegetable processing waste streams.

# 1. Introduction

As consumption habits continue to increase, the yearly intensification of food waste and processing loss is a growing concern. Food by-products are primarily acknowledged as a major issue, posing a threat to the sustainability of the food supply chain. According to the FAO of the United Nations, approximately 1/3rd of global food is wasted annually. Of the 1.5 billion tons of fruits and vegetables grown annually,

an expected 0.5 billion tons are discarded or converted into waste products, such as seeds, pods, shells, peels, and pomace (Zabed et al., 2022). The FUSIONS (Food Use for Social Innovation by Optimizing Waste Prevention Strategies) framework classifies fruit and vegetable waste (FVW) as "any part of fruits and vegetables, whether edible or inedible, that is taken out of the food supply chain for recycling or disposal" (Chatterjee and Mazumder, 2024). FVW is perishable and can cause harmful environmental effects, including the generation of

Abbreviations: ADF, Acid detergent fiber; ASE, Accelerated solvent extraction; BMS, Bitter melon seeds; BMSM, Bitter melon seed meal; CFP, Carbon footprint; DASC, Defatted apple seed cake; DESE, Deep eutectic solvent extraction; EA, Emulsifying activity; EAEP, Enzyme-assisted extraction process; ES, Emulsifying stability; FAO, Food and Agriculture Organization; FVW, Fruit and vegetable waste; HBA/HBD, Hydrogen bond acceptor/ Hydrogen bond donor; HHP, High hydrostatic pressure; HHPE, High hydrostatic pressure extraction; HIC, Hydrophobic interaction chromatography; HPLC, High-performance liquid chromatography; LCA, Life Cycle Assessments; MAE, Microwave-assisted extraction; MPa, Mega Pascal; MWCO, Molecular Weight Cut-Off; NDF, Neutral detergent fiber; OHC, Oil holding capacity; PEFE, Pulsed electric field extraction; PLE, Pressure liquid extraction; PSPIs, Pepper seed protein isolates; ROS, Reactive oxygen species; RTD, Ready to drink; SDF, Soluble dietary fiber; SDGs, Sustainable Development Goals; SFC, Supercritical fluid chromatography; SFE, Supercritical fluid extraction; SPI, Soy protein isolate; SSE, Subcritical solvent extraction; SWE, Subcritical water extraction; UAE, Ultrasound-assisted extraction; UF, Ultrafiltration; UN, United Nations; W/v, weight/volume; WHC, Water holding capacity.

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leachate that contaminates water and soil, the emission of hazardous chemicals that threaten the environment, and the proliferation of pathogenic bacteria, posing a significant threat to human well-being (Wu et al., 2023).

Due to the increasing awareness of food security and sustainability among customers and food producers, the valorization of food crops has attracted the attention of scientists to expand the range, including those prepared from by-products. As stated in the Food Waste Index Report 2021 of the UN Environment Program, food waste exceeds 931 million tons annually. Following the UN SDGs, which seek to decrease food wastage by 50 % by 2030, it is vital to emphasize the reduction of food waste, particularly from perishable fruits and vegetables. Processing these items into stable value-added products is essential to achieve this goal (Tan et al., 2022). The valorization of waste materials has attracted substantial interest in furthering circular economy principles, which concentrate on minimizing waste and optimizing its management. The main target of governments and the food sector is to prevent and reduce food waste. The circular economic concept aims to reduce consumption and waste by recycling and reusing materials to create new products, thus turning waste into a valuable resource for sustainability. Two key challenges are ensuring food security for a growing population and managing food waste that harms the carbon footprint (Chamorro et al., 2022).

FVW contains numerous beneficial substances such as proteins, vitamins, flavonoids, alkaloids, carotenoids, dietary fiber, and polyphenols (Zabed et al., 2022). Beyond proteins, these are rich in numerous valuable compounds. For instance, mangiferin, a xanthone polyphenol found in high concentrations in mango leaves and peels, exhibits strong antioxidant and anti-inflammatory properties. Another example is carminic acid, a natural red pigment derived from cochineal insects on cactus pads, and it is usually used as a food and cosmetic pigment (Castro-Muñoz et al., 2024; Ferreyra-Suarez et al., 2024). Arabinoxylans, which are hemicellulosic fibers present in many cereal and fruit residues, act as prebiotic dietary fibers. These examples highlight the variety of co-products present in fruit and vegetable by-products, whose extraction and utilization have been extensively reviewed (Hernández-Pinto et al., 2024). Fruit peels, seeds, stems, or entire portions that cannot be incorporated into various technological procedures are considered waste because they are not utilized as by-products or converted into valuable yields.

By embracing the circular economy, we can valorize fruit and vegetable remains to reduce the wastage of resources such as water, energy, labor, and land in the agri-food chain. This also helps address environmental concerns related to landfills, carbon footprints, and greenhouse gas emissions, while complying with current regulations (Zabed et al., 2022). In the modern nutritional landscape, it is essential to explore alternate protein sources to meet the growing worldwide demand for protein. Recently, people have become highly conscious of "plant-based or vegan protein." Proteins are the building blocks of life and organic substances found in cells. They also regulate the metabolism in organisms. Depending on the food source, proteins can be divided into plant and animal categories. Animal proteins are mostly present in meat, eggs, and milk; on the other hand, plant proteins are present in beans, grains, seeds, nuts, and other foods (Wang et al., 2023). In general, animal proteins are considered to be superior sources. But animal protein comes with a high energy input ratio of 14:1, meaning it's expensive to produce. Proteins derived from plant sources are an effective and more economical alternative to cope with the increasing protein deficiency issues. Proteins derived from FVW matter are valuable because they can be exploited as sustainable sources of high-quality protein (Zhou et al., 2021). The environmental impact associated with animal protein production is much higher than that associated with plant protein sources, in particular with regard to the carbon footprint. This stems especially from direct livestock production-related greenhouse gas emissions (via enteric fermentation and feed management) (Heusala et al., 2020). The carbon footprint (CFP) measures the CO2 tons generated by human activities, contributing to greenhouse gas emissions. So food waste is also responsible for CFP, which leads to a rise in the temperature of world. Animal protein is also responsible for CFP. Therefore, it is very crucial to decrease food wastage, reduce the demand for animal protein sources, and CFP (Parashar et al., 2020). Furthermore, if the consumption of protein shifts from foods of animal origin to plant origin, that would be beneficial from a health perspective (Heusala et al., 2020). Proteins extracted from plant by-products can be used in the production of many different novel food ingredients, pharmaceuticals and other products with improved functional properties. These extracted proteins are beneficial for several purposes due to their beneficial nutritional profiles, functional qualities, and bioactive properties (Zhou et al., 2021).

Bitter melon seed meal (BMSM) is a byproduct of oil extraction. Besides being rich in glycoprotein-9, BMSM contains a substantial amount of amino acids. It is essential in various cellular and physiological functions, such as immune function, embryonic development, and pathogen recognition. Seed meals can be utilized to create food supplements (Naik et al., 2022). Pumpkin and cactus fruits comprise various underutilized fruit seeds that are rich in protein. A former study has reported that the seeds of cactus fruit have high protein content that can be isolated. Lemon seeds are a protein-rich source, and their protein hydrolysates are promising protein sources for food production due to their stabilizing and emulsifying properties. Therefore, fruit seeds, pomace, and kernels are ideal substrates for valorization due to their rich nutrient and protein content (Lolli et al., 2023).

Vegetable proteins are emerging as safe sources for future diets. RubisCO is a major soluble protein found in vegetables. Their by-products (green leaves, seeds, stems, and peels) constitute the richest sources of protein. Unfortunately, this resource is minimally exploited (Bayomie and Romdhana, 2023). Potato starch wastewater contains a high amount of potato proteins that are beneficial in food and healthcare production. Peas are highly valued for dietary purposes and maintain a prominent position in veggies due to their excellent nutritional profile and high content of protein, and health-beneficial components, including insoluble and soluble dietary fiber, phosphorus, potassium, phenolic compounds, and  $\beta$ -carotene. Pea processing byproducts are useful for extracting proteins (Estivi et al., 2024). The isolated proteins have both functional and techno-functional properties. Therefore, it is essential to seek an alternative, resource-efficient protein source for the food sector and other applications (Barrios et al., 2022).

This comprehensive review explores waste valorization from perishable products, such as fruit and vegetable waste, to extract valuable components, such as proteins, through various extraction techniques. This review distinguishes itself from existing literature by offering a comparative analysis of traditional and innovative green extraction methods. It includes a detailed analysis of the functional characteristics of proteins derived from various underutilized byproducts. This review also presents an extensive discussion about their uses in food production, animal feed, packaging, and bioplastics. This review further explores the environmental sustainability of these valorization processes.

# 2. Bioactive compounds from fruit and vegetable waste

With the ongoing challenge of waste disposal, there is a rising interest in recycling FVW. These waste products are rich in macronutrients (proteins, fats, fiber, and carbohydrates), micronutrients (minerals and vitamins), and bioactive compounds (such as polyphenols, carotenoids, and flavonoids). These phytochemicals possess significant antimicrobial and antioxidant properties. Thus, many studies have focused on utilizing these by-products by incorporating them as ingredients to create functional foods with beneficial health effects for customers (Mateus et al., 2024).

### 2.1. Protein content in fruit and vegetable by-products/waste

Proteins extracted from waste generated by fruits and vegetables are crucial due to their potential as sustainable and eco-friendly sources of high-quality proteins, as illustrated in Fig. 1. The protein percentage present in different FVW is summarized in Table 1. Grapes enclose proteins in the pulp, seeds, and skin. Their protein profiles differ with ripening, environmental, and stress stimuli (Baca-Bocanegra et al., 2021). Around 20 % of pomegranate seeds are made up of proteins, which can be a great alternative to animal proteins and a source of peptides with bioactive properties (Guzmán-Lorite et al., 2022). Muskmelon fruit contains numerous seeds with high protein content (25-37 %) and medicinal properties (Devi and Badwaik, 2022). Muskmelon seeds are abundant in amino acids, so they are comparable to soybeans and an unconventional source of protein. Various alternative protein sources, such as pumpkin seeds, watermelon seeds, and bitter melon seeds, have been utilized. Oil seeds are used as a substitute protein source supplements protein-deficient staple foodstuffs (Pasrija & Sogi, 2022). Bitter melon seeds (BMS) contain abundant oil and proteins. Seed meal contains glycoprotein-9 and is composed of essential amino acids (Naik et al., 2022). Pomace, the solid residue left after tropical fruit processing, is rich in proteins, enzymes, and other functional elements. The content of pumpkin seed meal ranges from 60 % to 65 %, making it a remarkable and advantageous source of plant-based proteins. Besides their extensive use as food components, pumpkin seed proteins possess pharmacological benefits, including anticancer, antioxidant, antidiabetic, and liver-protective properties (Vinayashree and Vasu, 2021). Watermelon seeds are rich in protein, fat, and carbohydrates. Crude and defatted watermelon seeds contained 18.72 % and 54.48 % of protein, respectively. According to WHO recommendations, all essential amino acids are present in sufficient quantities, that are necessary for adults. Watermelon seed proteins exhibit exceptional functional properties, as demonstrated by their application in food products (Gadalkar and Rathod, 2020). Pepper seeds are the residue of pepper industrial output and are high in protein, fiber, and fat. Pepper seed protein extracts recovered from dried pepper meal exhibit high solubility, oil-holding, and water-retaining abilities, along with emulsifying and foaming properties. It can serve as a novel protein substitute for food preparations with improved functional features (Wang et al., 2021).

### 2.2. Bioactive compounds in fruits and vegetable by-products

Phenolic compounds are essential antioxidants that are used in the food, pharmacological, cosmetic, and packaging industries. These compounds exhibit antioxidant, antimicrobial, and anti-inflammatory activities. The peel of pomegranate fruit (*Punica granatum L.*) is known for its remarkable features, as it is rich in polyphenols and various phytochemicals. These compounds exhibit antimicrobial, antioxidant, and antidiabetic properties. Antioxidants and several phenolic compounds are present in sweet cherry pits, grapes, and seeds of date. Sweet cherry pits and date seeds include the primary flavonoids catechin and epicatechin, as well as the most prevalent phenolic acid, vanillic acid (Mateus et al., 2024). Both the seeds and peels of avocado are valuable by-products due to their rich profiles of phytochemicals, including to-copherols, carotenoids, and polyphenols. The primary polyphenols are derivatives of flavonoids and chlorogenic acid (Rojas-García et al., 2022).

Like other cruciferous vegetables, kale and broccoli are also known as nutrient-rich "superfoods" because of their high content of health-beneficial phytochemicals present in them. After processing, these valuable compounds can be extracted from their by-products, which then can be added to food and cosmetic products. Kale contains a rich amount of quercetin and kaempferol, and it is also rich in potent anti-oxidants, like phenolic compounds, that help reduce oxidative stress and the chances of chronic illnesses. Peels of Bottle gourd have triterpenoids, flavone C-glycosides, and cucurbitacin present in them (Sharma et al., 2023). Sweet potato tubers contain numerous bioactive compounds, comprising polyphenols, phenolic acids (e.g., chlorogenic and caffeic acids), and other constituents that have antioxidant properties. Furthermore, onion peels are abundant in flavonoids, specifically anthocyanins and flavones, and are responsible for imparting purple/red and brown colors to several onion varieties (Musilová et al., 2024).

# $2.3. \ \ \textit{Dietary fiber in fruit and vegetable by-products}$

Dietary fiber is a component that human enzymes cannot break down. Fruit and vegetable waste contain lignin, cellulose, and non-cellulosic polysaccharides, which make up the fibers. Dietary fibers have various health-beneficial effects, including prebiotic activities, which facilitate faster transit through the digestive tract by bulking and hydration (Viscusi et al., 2024). Date seeds are rich in dietary fibers (NDF, ADF, lignin, and cellulose). Apple pomace has a higher fiber

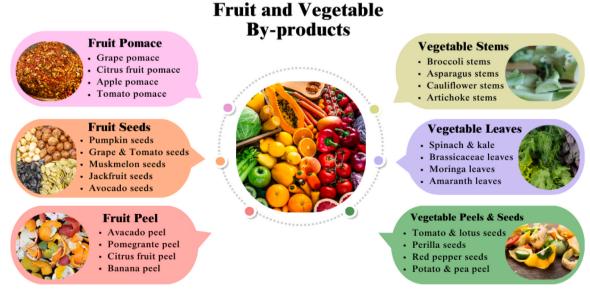


Fig. 1. Sources of protein from fruit and vegetable by-products and waste.

 Table 1

 Emerging green extraction technologies for protein isolation from fruit and vegetable by-products: their extraction conditions and applications.

| Fruit/<br>Vegetable By-<br>product             | Protein<br>Content<br>% | Technology   | Description  | Extraction condition   | Advantages  | Applications  | References  |
|--|-------------------------|--|--|--|---|---|---|
| Tomato seed meal                               | 2835                    | Supercritical<br>fluid extraction<br>(SFE)               | Supercritical $\mathrm{CO}_2$ is employed as a solvent to extract proteins   | Solvent: scCO <sub>2</sub> with the addition of ethanol (as a cosolvent) Pressure: 100–400 bar Temperature: 30–60°C Extraction Time:   | Environmentally<br>friendly<br>Highly selective<br>Rapidly available  | Protein Extraction from<br>tomato seed meal,<br>peach, and avocado peel   | (Mateo-Roque et al.,<br>2024; Rudke et al.,<br>2024)    |
| Grape seed<br>meal<br>Apple<br>pomace          | 10.5–16.3<br>3.8        | Ultrasound-<br>assisted<br>extraction<br>(UAE)           | It uses ultrasonic<br>waves to facilitate the<br>extraction process  | 30–60 min<br>Ultrasound<br>Frequency: 20 kHz<br>Extraction Solvent:<br>saline solution<br>(0.5 M NaCl)<br>Room temperature:<br>25–30° C<br>Extraction Time:<br>30 min<br>Centrifugation:<br>13,000 rpm for<br>10 min     | Economical<br>technique<br>More extraction<br>efficiency<br>Less solvent<br>consumption   | Protein recovery from<br>grape seed meal, apple<br>pomace, avocado seed,<br>pea peel, plum seeds,<br>bitter ground peels, and<br>seed | (Baca-Bocanegra<br>et al., 2021; Chaji<br>et al., 2024) |
| Pomegranate<br>seed<br>Red pepper<br>seed meal | 10–18<br>20–22          | Pressurized<br>liquid<br>extraction<br>(PLE)             | High pressures and<br>temperatures are used<br>to extract proteins   | Solvent: Water or a water-ethanol mixture (e.g., 50 % ethanol) Temperature: 100–220°C Pressure: 100–200 bar Extraction Time: 15–30 min   | Less extraction time<br>Reduce solvent<br>consumption<br>Great extraction<br>yields   | Proteins and bioactive<br>substances extraction<br>from pomegranate seed<br>and peel, red pepper<br>seed meal, and grape<br>pomace    | (Hernández-Corroto<br>et al., 2020)                     |
| Jackfruit<br>leaves                            | 8.69                    | High-<br>hydrostatic<br>pressure<br>extraction<br>(HHPE) | Non-thermal<br>technology conducted<br>between 100 and<br>1200 MPa based on<br>mass transport<br>phenomena                             | Pressure:<br>100–1200 MPa<br>Ambient<br>temperature: 25 °C<br>or up to 40 °C<br>Extraction Time:<br>10–30 min<br>Solvent: Pure water<br>or phosphate buffer<br>(pH 7.0)  | Fast method<br>Environmentally<br>safe<br>Less energy<br>consumption  | Pectin separation from<br>jackfruit leaves, lime,<br>orange, potato peel<br>wastes, and sugar beet                                    | (Ninčević Grassino<br>et al., 2020)                     |
| Persian lime<br>seeds                          | 20.56                   | Enzyme-<br>assisted<br>extraction<br>(EAE)               | In this technique,<br>specific enzymes are<br>used to break down<br>the cell walls, making<br>proteins more<br>accessible for release. | Enzyme: Pectinase, amylase, protease, or cellulase Enzyme concentration: 0.1–5 % (w/v) Temperature: 40–60°C pH: Pectinase 4.0–5.5 Cellulase 4.5–6.0 Extraction time: 30 min to 1 h Centrifugation: 12,000 rpm for 15 min | Less extraction<br>time, less solvent<br>consumption<br>Enhanced quality of<br>extracted<br>compounds.<br>Reduced waste<br>generation | Proteins, phenolic<br>compounds, and<br>anthocyanin extraction<br>from mulberry red<br>dragon fruit, pulses, and<br>nuts              | (Amulya and ul Islam, 2023)                             |
| Watermelon<br>seed powder                      | 1618.7                  | Microwave-<br>assisted<br>extraction<br>(MAE)            | Utilizes microwave energy to enhance extraction efficiency.  | Solvent: Water or buffer solution at pH 6.8 Microwave Power: 200–1000 watts Extraction Time: 5–30 min Temperature: 50–100°C Microwaving Conditions: Continuous or pulsed mode  | Highly powerful<br>Reduce extraction<br>time<br>Less solvent volume<br>Improve the quality<br>of protein                              | Protein recovery from<br>sesame bran, pumpkin<br>seeds, soybean,<br>watermelon seeds, and<br>leafy greens                             | (Behere et al., 2021;<br>Görgüç et al., 2020)           |

(continued on next page)

Table 1 (continued)

| Fruit/<br>Vegetable By-<br>product      | Protein<br>Content<br>% | Technology                                       | Description  | Extraction condition  | Advantages   | Applications   | References                          |
|---|-------------------------|--|--|---|--|--|-------------------------------------|
| Bitter melon<br>seeds                   | 24.8–31                 | Pulsed electric<br>field extraction<br>(PEFE)    | Based on the<br>application of short<br>pulses of high voltage<br>electric field to a food<br>material placed<br>between a set pair of<br>electrodes | Electric Field Strength: 10–40 kv/cm Pulse Duration: 1–100 µs Number of Pulses: 3–100 pulses Total Treatment Time: 30 s at room temperature Buffer: Pure water or phosphate buffer (pH 7.0) | Better extraction<br>yield<br>Decrease energy<br>consumption and<br>utilization of<br>solvents | Extraction of proteins<br>and polyphenols from<br>bitter melon seeds,<br>grape by-products,<br>orange peels, pea pods,<br>and olive pomace   | (Andreou et al., 2020)              |
| Asparagus<br>leafy by-<br>products      | 15                      | Subcritical<br>water<br>extraction<br>(SWE)      | Use of subcritical<br>water to extract<br>compounds  | Temperature:<br>100–200 °C<br>Pressure: 5–30 bar<br>Extraction<br>Time:30–60 min<br>pH: Adjusted to<br>around 8.0   | Environmentally<br>safe<br>Quick method<br>Economical and<br>stable                            | Proteins, amino acids,<br>phenolic and<br>antioxidant compounds<br>extracted from several<br>waste products such as<br>asparagus, coffee<br>residue, peach palm,<br>and rice straw | (Náthia-Neves and<br>Alonso, 2024)  |
| Pomegranate<br>peel<br>Carrot<br>pomace | 4.5–15<br>6             | Deep eutectic<br>solvent<br>extraction<br>(DESE) | Use of different deep<br>eutectic solvents for<br>the extraction of<br>compounds   | DES Composition: Choline chloride and urea or Choline chloride with glycerol Concentration: 10–80 % (w/v) pH: Adjusted to pH 6.0 Temperature: 30–70°C Extraction Time: 30 min- 2 h          | Environmentally<br>safe<br>Non-volatile and<br>non-toxic<br>Highly stable and<br>economical    | Proteins obtained from<br>date by-products, carrot<br>pomace, orange, and<br>pomegranate peel  | (Hernández-Corroto<br>et al., 2020) |

content compared to apples. This quantity varies according to the different apple varieties and extraction techniques used. Fruit peels have sufficient dietary fiber content, which functions as a bulking, binding agent, and fat simulator (Feizy et al., 2020). Pomegranate peel contains approximately 47 % dietary fiber, which is primarily composed of soluble dietary fiber (SDF). SDF possesses remarkable antioxidant properties and can help decrease blood pressure, blood glucose, and lipid levels, thereby alleviating certain health issues, such as hyperglycemia, colon cancer, CVD, and hyperlipidemia (Xiong et al., 2023). Carrot pomace is fiber-rich, with cellulose present in the highest amount, followed by lignin, hemicellulose, and pectin (Feizy et al., 2020). Bottle ground peel has an excellent dietary fiber content. It is used to treat various health concerns, including digestive problems. It is also cardio-protective and has anticancer and antidiabetic activities (Sharma et al., 2023).

# 2.4. Mineral and vitamin content from fruit and vegetable by-products

Lime, orange, and mandarin peels have similar pulps and are favorable sources of mineral components that are often incorporated into dietary products for their medicinal properties. Citrus fruits are potassium-rich. Pomelo and red grapefruit peels contained 20 % more potassium than the pulp. Another essential mineral is zinc, which helps protect the body from oxidative damage and enhances immune function. Its concentration is significantly higher in lemon, orange, and grapefruit peel varieties than in their pulp. Oranges can also supplement the body with K, P, and Mn. In watermelon peel, both calcium and potassium are present in high amounts. Moreover, watermelon peel contains iron, which is important in biological systems (Feizy et al., 2020).

Vitamins are micronutrients that must be obtained from the diet, as they play a protective role. Vitamin K is the most abundant vitamin for blood coagulation in horned melon seeds. They also contained high amounts of B vitamin complexes such as B1, B2, and B9. Vitamin B2 promotes the formation of RBC and helps maintain the health of the skin, eyes, and nervous system. Avocado, pear, and green mango peel, pulp, and seeds contain vitamins A and C, which are important for human well-being. The tubers of sweet potatoes are rich in fiber, vitamins (B1, B2, biotin, B3, and B5), minerals, and other nutrients (Musilová et al., 2024). Citrus fruit peel is the primary source of ascorbic acid, unlike the pulp and seeds. Ascorbic acid is a vital antioxidant that contributes to the antioxidant properties of plant cells by detoxifying ROS and free radicals (Hussain et al., 2023).

## 3. Protein extraction methods

Currently, various extraction procedures, including alkaline extraction, salt-based extraction, dry fractionation, and ultrafiltration, have been applied independently or in combination to obtain plant proteins, as illustrated in Fig. 2 (Osemwota et al., 2021). The most common method for extracting protein is the alkaline method of extraction, and then isoelectric precipitation. This method is well-known for its simplicity, rapidity, and low cost. But the specific conditions required to achieve a greater protein yield result in protein damage and changes, for example, denaturation, racemization, dehydration, and cross-linking of amino acids. These modifications cause changes such as reduced product solubility, decreased nutritional value, and altered functional properties, including foaming and emulsifying ability. The alkaline extraction-isoelectric precipitation method is based on the activation of the proteins, either in an alkaline or acidic medium, after which they are separated at the isoelectric point. It is regarded as a traditional way to separate globulins and albumin proteins and glutelins from flour and oilseed cake (including legumes) (Nouska et al., 2024).

The salt extraction method is the best as it improves the extraction efficiency of protein. The method offers a host of benefits, including

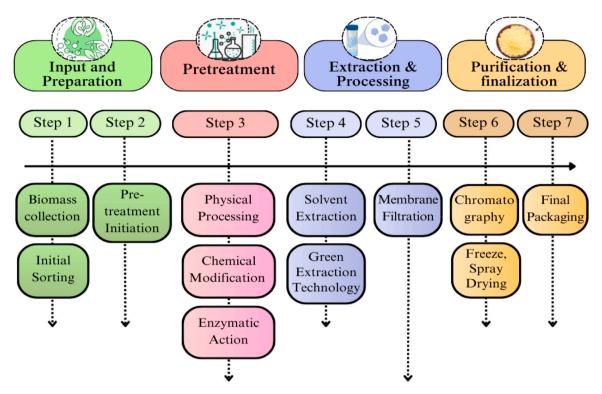


Fig. 2. General flowchart for Protein Extraction from fruits and vegetable waste.

economic reasonableness, simple operation, and mild procedure, which are all known to be beneficial for the quality of proteins (Jiang et al., 2024). This technique can be performed through two forms: low ion concentration salting, where charged protein surfaces prevent aggregation and enhance solubilization of protein molecules; and salting out, an increase in ionic strength to enhance protein aggregation and resultant protein precipitation. Sodium chloride is mostly used for enhancing salting-in, while ammonium sulfate is used to enhance protein salting-out. Furthermore, extracted proteins' physicochemical and structural properties are altered through the salt-assistant technique, thus affecting food extraction processes (Jiang et al., 2021). Most conventional extraction procedures have several drawbacks, for example, low yields of extraction, altered techno-functional protein properties, reduced quality, impaired nutritional properties, undesirable coloration, and high industrial wastages, all of which can pose environmental concerns. Therefore, to overcome the technological and scientific limitations of traditional protein extraction methods, it is essential to develop novel and environmentally friendly extraction techniques (Nouska et al., 2024).

## 4. Emerging green extraction techniques

Advanced green extraction techniques, like enzyme-assisted extraction, microwave-assisted extraction, reverse micelle extraction, and ultrasonic-assisted extraction, have shown positive outcomes in attaining plant proteins with improved techno-functionality, as shown in Table 2. Besides, these modern techniques are also considered economical, safe, and environmentally friendly, so they have the potential to obtain clean-label certifications (Nouska et al., 2024).

# 4.1. Supercritical fluid extraction technique

The traditional methods of oil extraction can be replaced by the supercritical fluid extraction technique using CO<sub>2</sub> (scCO<sub>2</sub>). It is considered a sustainable technique because of its non-toxic and environmentally friendly qualities, as well as the rapid removal process it demands for

food products. The temperature condition required for scCO<sub>2</sub> extraction is nearly at room temperature; maintaining the supercritical state requires a critical temperature (31.1°C) and pressure. This technique is a developing technology that gives several benefits. It is safe for use and also operates without the use of chemicals. It can also be a good alternative to biomass processing. Application of this technique on agroindustrial waste, through which new food products can be formed or current food products can be improved (Mateo-Roque et al., 2024). A study reported that the supercritical fluid extraction process was able to recover 29 % of the peach seed oil (Rudke et al., 2024). Both the protein extraction technology of tomato seeds and their other techno-functional properties were further optimized by supercritical fluid treatment. Such modification leads to pronounced conformational changes and changes in interfacial properties of the protein extracts. These results indicated that scCO2 can provide transformed proteins that will be useful in food production (Mateo-Roque et al., 2024).

## 4.2. Pressurized liquid extraction technique

The pressurized liquid extraction (PLE) method uses higher temperatures and pressures to decrease extraction time, decrease the amount of solvents needed, create high-yield extractions, and use completely harmless solvents. Although PLE has been extensively studied to extract phenolic compounds, this technique was scarcely used for the removal of proteins (Hernández-Corroto et al., 2020). This particular method, also called subcritical solvent extraction (SSE) and accelerated solvent extraction (ASE), is a new and green extraction technology. Commonly, these processes involve the use of solvents and operate under moderately to extremely high temperatures (100-220°C) and high pressures. High pressures, however, can result in high energy expenditures and a considerable budget for extracting the compounds. Also, thermo-labile polyphenols are generally heat-sensitive, and therefore, it would be challenging to extract antioxidant-rich constituents or dietary supplements for food or therapeutic application. With a rapid and effective, high specificity extraction profile over a broad range of chemical polarities, PLE results in a much shorter extraction time and

**Table 2** Functional properties of extracted proteins from by-products of fruits and vegetables.

| Fruit/<br>vegetable | Source of protein by-<br>product | Functional properties   | References                         |
|---------------------|----------------------------------|---|------------------------------------|
| Avocado             | Avocado seed                     | Excellent emulsifying capacity  | (Wang et al., 2022)                |
| Carrot              | Carrot pomace                    | Strong gelling abilities<br>Less WHC<br>Great water/oil-<br>holding capacity<br>High foaming stability<br>Good bulk density | (Luca et al., 2022)                |
| Tomato              | Tomato seed<br>meal              | Thermal stability Significant WHC Excellent foaming properties  | (López-Valdez et al., 2020)        |
| Red pepper          | Red pepper<br>seed meal          | High protein solubility<br>Strong gelling<br>High oil absorption<br>capacity<br>Low solubility<br>Less water-holding        | (Wang et al., 2021)                |
| Soybean             | Soybean seed<br>meal             | capacity Great water retention capacity, Bulk density High foaming stability and capacity Excellent                         | (Rani & Badwaik, 2021)             |
| Peas                | Peapod powder                    | emulsification Moderate gelling Rich foaming capacity, emulsion stability Decent foam stability                             | (Pooja et al., 2024)               |
| Asparagus           | Asparagus leafy<br>by-products   | Strong gelling property Good water holding capacity Moderate OHC  | (dos Santos-Silva<br>et al., 2024) |
| Potato              | Potato protein isolates          | Lower solubility<br>Moderate gelling<br>Poor emulsification   | (Zhao et al., 2022)                |
| Persian lime        | Persian lime<br>Seeds            | Low foaming capacity<br>Good solubility<br>Adequate emulsifying<br>and foaming properties<br>Great water and oil            | (Fathollahy et al., 2021)          |
| Mustard             | Defatted<br>mustard meal         | holding capacity Acceptable oil absorption capacity Good foaming capacity and emulsifying                                   | (Jahan et al., 2022)               |
| Bitter melon        | Bitter melon<br>seed             | activity<br>Strong gelling capacity<br>Good emulsifying<br>properties and foaming<br>capacity                               | (Naik et al., 2022)                |
| Cherimoya           | Cherimoya<br>Seed                | Great foaming stability<br>Sufficient WHC   | (Orellana-Palacios et al., 2022)   |
| Perilla             | Perilla seed<br>meal             | Good oil absorption<br>capacity<br>Emulsifying capacity   | (Zhou et al., 2021)                |
| Banana              | Banana peel                      | High foaming capacity<br>Significant emulsion<br>capacity<br>Strong oil-holding<br>capability                               | (Devi and Badwaik, 2022)           |
| Citrus fruits       | Citrus peel                      | Excellent water-<br>holding and oil-<br>holding capacity<br>High foaming capacity   | (Fathollahy et al., 2021)          |
| Pumpkin             | Pumpkin seed<br>meal             | Thermal stability Better solubility, water absorption, Moderate foaming capacity High oil-holding capacities                | (Sá et al., 2023)                  |

Table 2 (continued)

| Fruit/<br>vegetable | Source of protein by-<br>product | Functional properties  | References                  |
|---------------------|----------------------------------|--|-----------------------------|
| Muskmelon           | Muskmelon<br>seeds               | Significant foaming<br>and<br>emulsifying properties<br>Thermal stability<br>High Protein solubility | (Pasrija and Sogi,<br>2022) |

lower solvent consumption compared to standard solid-liquid extraction procedures (García et al., 2021). The functional properties of peach seeds and pomegranate proteins can be considerably enhanced by the pressurized liquid method. Although the protein content of peach seed cake is 29 %, PLE extraction can increase it to 95 %. The resultant PLE protein fraction exhibited excellent functional properties, including a high foaming capacity, hydrophobicity, and a high whiteness index. Moreover, it is rich in amino acids like valine, leucine, and phenylalanine. Therefore, PLE is highly recommended for protein extraction (Rudke et al., 2024).

## 4.3. High hydrostatic pressure technology

High Hydrostatic Pressure Extraction (HHPE) is a non-thermal process typically performed in the 100-1200 MPa range established for mass transport phenomena. Based on phase behavior principles, increasing the applied pressure enhances plant cell permeability, which, in turn, increases the diffusivity of cell components; thus, the solubility of components increases with higher pressure. This method is often used to preserve and process various food products, including pectin extraction from lime, orange, and potato peel waste, as well as sugar beets (Ninčević Grassino et al., 2020). The HHPE enhances protein hydrophobicity while decreasing solubility. This effect occurs when buried sulfhydryl groups are exposed after protein unfolding, resulting in denaturation, aggregation, and coagulation. As a result, functional properties were enhanced. Specifically, structural changes, including improved surface hydrophobicity, the introduction of sulfhydryl groups, and alterations in secondary structure, contribute to the enhanced thermal stability and protein emulsifying property. The emulsifying activity and foaming stability of pea proteins are considerably improved by HHP supercritical CO<sub>2</sub> at specific pH levels. In contrast, its solubility does not increase considerably. Using the HHP method, protein extraction yields from plant sources such as bitter melon seeds, pumpkin seeds, and soybeans have significantly surpassed 30 % (Moreno-Nájera et al.,

### 4.4. Ultrasonic-assisted extraction technology

Ultrasonic-assisted extraction (UAE) is a simple, economical, and effective method that enables reduced solvent use, lower process temperatures, shorter extraction times, and improved extraction productivity. Therefore, it has applications in a variety of sectors, including food and chemicals. The UAE has been efficiently employed to get bioactive compounds from numerous fruits and vegetables that include medicinally beneficial components, for example, purple sweet potatoes, blackberries, Stevia rebaudiana Bertoni leaves, false daisy, grape pomace, Momordica charantia, Cassia auriculata leaves, Rheum moorcroftianum, and Melissa officinalis L (Chakraborty et al., 2020). UAE is helpful for protein extraction from Manila tamarind and plum seeds (Rudke et al., 2024). Ultrasound has been examined as an eco-friendly and sustainable treatment method. UAE is an inexpensive and simple method, which is economical for industrial and commercial production on a large scale. This decreases the extraction time, mass transfer, the use of the solvent, temperature, and energy needs. Also, the UAE has a lot of advantages for the environment over the old extraction process. Comparative experimental study with the present extraction process and with the conventional alkaline extraction technique demonstrated that ultrasonification followed by alkaline extraction improved the yield and protein percentage of the cherimoya-seed protein by 6 % and 12 %, respectively. Cherimoya-seed proteins' structural characteristics and heat stability were also improved (Orellana-Palacios et al., 2022).

### 4.5. Enzyme-assisted extraction technology

One of the most widely applied methods in the removal of macromolecules and micronutrients from plant cells is termed as enzymeassisted extraction process (EAEP) (Görgüç et al., 2020). EAEP utilizes enzymes to assist in the breaking down of plant cell wall structures, helping to release the elements of the plant that are desirable. The enzymes generally used in this method are proteases, cellulases, hemicellulases, a-amylases, xylanases, and pectinases. This process helps to increase the quality and the yield of anthocyanins obtained from fruits and vegetables (Amulya and ul Islam, 2023). Among the various enzymes employed in this technique, alkylase (a protease) has been recognized as an effective enzyme to extract proteins (Görgüc et al., 2020). Use of the protease enzyme during extraction yields a protein extract with greater protein content and an enriched amino acid profile. Proteins isolated via EAEP show higher water retaining capacity, zeta potential, and net charge. Most of the time, its use has been successful in the extraction of oil from oil-containing parts of plants, like sunflower seeds, soybeans, rapeseed, and almonds. Furthermore, by hydrolyzing cell walls with carbohydrates and breaking up lipid-containing cell membranes with proteases, the protein content can be improved along with oil. Finally, this technique causes an increase in the porosity of the materials when the diffusive behavior of the intracellular components into the liquid phase is evaluated (Yang et al., 2024). Compared with conventional extraction, EAEP has many merits, including shorter extraction time, lower solvent consumption, and high quality of extracted compounds. Moreover, the mentioned process is sustainable, produces few wastes, and occurs under mild extraction conditions (Amulya and ul Islam, 2023).

## 4.6. Microwave-assisted extraction technology

The use of microwave for the extraction and removal of components from plant products has been extensively studied in the food production industry (Varghese & Pare, 2019). Microwave-assisted extraction (MAE) is a rapid method that reduces the degradation rate of compounds. By means of microwave irradiation of the food, this process overall induces mass and heat transfer from the center of the solid mass towards the extraction medium by exciting the mass's molecular water molecules. Many researchers have investigated the use of MAE as a purification method for enzymes, proteins, carbohydrates, and polyphenols (Görgüç et al., 2020). It has led to enhanced solubilization of the polymeric carbohydrates of the cell walls of the soybean. This helped to increase protein content, soymilk yield, and solubility (Varghese and Pare, 2019). Polypeptides from pumpkin seeds, peaches, and sesame bran have also been isolated using MAE. Watermelon seed proteins extracted by MAE show better functional properties than those obtained using conventional extraction practices, suggesting their possible applications in or for food products (Behere et al., 2021).

## 4.7. Deep eutectic solvent extraction (DESE)

DESs have been developed to be one of the alternative green approaches to traditional organic solvents for protein extraction applications. A DES is generated when a hydrogen bond acceptor (HBA, such as choline chloride) is combined with a hydrogen bond donor (HBD, such as urea, glycerol, and organic acids). The eutectic mixture exhibits a much lower melting point than both components independently (Hernández-Corroto et al., 2020).

The efficacy of DESE of protein is basically related to the choice of

the components for HBA and HBD, which collectively define the essential solvent properties such as polarity, viscosity, hydrogen bonding ability, and pH. Adding water is a good compromise to decrease viscosity and extractant efficiency, without significantly compromising the green character of the process, since high viscosity can lead to decreased mass transfer (extraction efficiency). The DES polarity must agree with the compound to target. Generally, hydrophilic DESs are more suitable for the extraction of phenolic compounds and polar proteins. The hydrogen bond network widely present on DESs is a key factor for breaking down the hydrogen bonds in the plant cell wall matrix and allows separation of intracellular proteins (Dheyab et al., 2021; Jablonský et al., 2018).

In addition, acidic DESs are capable of hydrolysis of cell wall poly-saccharides, increasing permeability and release of proteins. Having the characterized ability to denature proteins during extraction, DESs have been demonstrated to have utility in intracellular protein extraction, through hydrogen bond breaking within the cell wall and solubilization of protein molecules by generating new hydrogen bonds between the target protein and components of DES. The recovery rate is highly related to the water and HBA/HBD ratio. Due to the high solvating power of DESs, the extracted proteins are stable easily, which is often enhanced owing to the extraction (Hernández-Corroto et al., 2020).

## 5. Purification and isolation techniques for proteins

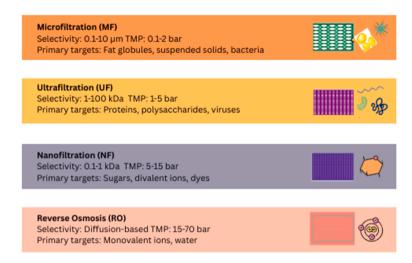
Proteins from agro-food materials such as fruit and vegetable waste can be extracted by a systematic multi-phase approach. In general, the processing will start off with initial preparative steps, such as drying and grinding, and then extraction using alkaline medium, or saline medium, or using more eco-friendly solvents to dissolve the proteins. The following critical isolation and purification step removes the proteins of interest from other dissolved compounds such as sugars, colorants, and mineral complexes. Although methods of isolating proteins using conventional techniques, such as isoelectric precipitation and salting-out procedures, are relatively common, they often lead to cleavage of protein structure and poor purity levels. It is well-known that membrane-based filtration technologies have emerged as a modern, temperature-controlled option to this purification step, offering higher recovery yields, lower structural degradation, and excellent preservation of biological functionality (Verma et al., 2021).

## 5.1. Membrane filtration

Ultrafiltration and microfiltration are some of the most common techniques used within a diverse range of industries, including the food and beverage industry. Their main focus is the application of semipermeable membranes to separate and retain various components of a fluid stream on the basis of the size and weight of the molecules (Castro-Muñoz et al., 2020). The application of pressure-driven membrane processes is mainly dependent on membrane pore sizes or molecular cut-off values. As shown in Fig. 3, for example, microfiltration (MF) with pore sizes of 0.1-10 microns is used to remove and isolate fats, suspended particles, and certain microbes, particularly bacteria. Ultrafiltration (UF) with a molecular weight cut-off (MWCO) of 1-100 kDa is increasingly used for concentration and fractionation of certain sugars, proteins, and other macromolecules. Nanofiltration (NF) with 0.1-1 kDa MWCO and Reverse Osmosis (RO) membranes have increasing applications in the removal of salts and other organic molecules, demineralization, and concentrated demineralized water, as well as in the isolation of some smaller organic molecules and ions (Verma et al., 2021).

Membrane technologies have considerable application in the removal of salts and purification of water, as well as in the removal of certain pollutants from industrial and textile wastewater. Food processing operations employ membranes for concentration, clarification, and de-alcoholization (Castro-Muñoz et al., 2020). In the food industry,

## Part A: Classification of Membrane Processes



PART B: Simplified UF Process Flow for Protein Recovery

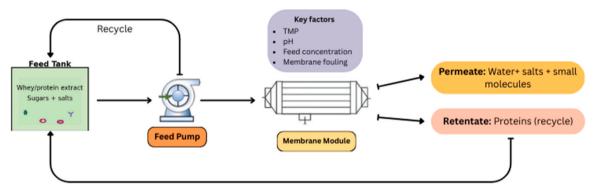


Fig. 3. Schematic overview of pressure-driven membrane processes used for recovering proteins from agro-food by-products.

ultrafiltration is used to concentrate certain proteins and biopolymers (whey protein isolation, fruit juice clarification) while fats and salts are removed (Verma et al., 2021). Nanofiltration can be used to demineralize whey streams or to fractionate sugars. Due to the antimicrobial activity, UF/NF membranes can be utilized in wastewater for filtration of effluents as a detoxification treatment from pathogens, pollutants (organics), and toxic substances (heavy metals) (Castro-Muñoz et al., 2021). In the drug industry, for the separation and purification of bioactive peptides and the concentration of the extract, membranes are used. Besides, membrane processes are one of the most effective extraction and purification methods for bioactive compounds from agricultural and food wastes. After all, compared to the extraction method, they represent a sustainable option, thanks to their energy-saving requirement as well as the absence of phase transformations or chemical additives (Castro-Muñoz et al., 2020; Castro-Muñoz et al., 2016).

Due to the large amounts of fruit and vegetable by-products available, separations based on molecular sizes are a widely employed method in protein extraction. For example, high-protein corn gluten meal (containing 67 % protein), high-fat corn germ, and high-fructose corn syrup are all membrane filtration products obtained by separating the solid fraction of corn. Membranes are used in agro food by-products for the concentration of protein-rich retentate after juice pressing or primary extraction (Verma et al., 2021). For example, UF is applied for Patatin and other proteins recovery from potato fruit juice (PFJ). Fruit pomaces (e.g., grape and apple) and seedcakes are so far commonly pre-solubilized before being filtered for materials containing solids and then UF/NFd to enrich the protein fraction (Schmidt et al.,

2016). The scope for membrane technology in protein recovery is much wider than fruit and vegetable by-products. Ultrafiltration has been shown to be a method for the extraction of juice proteins from potato fruit juice while maintaining high yields and retaining functional properties. The dairy sector routinely utilizes this technology as a standard approach for manufacturing whey protein concentrates and isolates. Moreover, they have been effectively utilized to separate proteins from different sources, like legume processing effluents, marine processing by-products, and alfalfa green juice. These diverse applications highlight the adaptability and commercial viability of membrane technology throughout the broader agro-food industry (Castro-Muñoz et al., 2021).

Membrane processes have the advantage of preserving protein functionality by operating at low temperature and neutral pH. Membrane-filtrated proteins usually exhibit enhanced functional characteristics. This improvement will occur because membrane filtration effectively concentrates proteins while leaving non-protein low-molecular-weight salts, sugars, and anti-nutritional factors. This selective purification lessens the need for harsh extraction conditions and reduces exposure to potential denaturing environments linked to isoelectric precipitation (Osemwota et al., 2021). The UF membrane is a selective membrane process in which the solutes are filtered using pressure or centrifugal force. UF is currently largely used for fruit and vegetable by-products as a downstream purification and concentration stage. For example, UF can effectively concentrate a solution of proteins after they have been extracted, while at the same time removing small molecular solutes such as salts, sugars, and some anti-nutritional factors via the permeate stream. This process is referred to as diafiltration (dialysis)

and can greatly enhance the overall purity and potency of the resulting protein isolate. In addition, because UF prevents drastic pH shifts and denaturation of protein, it's an excellent technique to obtain protein isolates with relatively better characteristics (in terms of solubility, emulsifying, and foaming capacities) as compared to isoelectric precipitation (Thammasena et al., 2020).

## 5.1.1. Factors influencing pressure-driven membrane technologies

The performance of pressure-driven membrane processes for protein recovery is affected by several critical factors. Transmembrane pressure (TMP) is an important factor, while flux increases with an initial increase in TMP. But too much TMP can cause fouling layers to compact together and stick damaged proteins, decreasing performance and increasing the amount of energy consumed. The membrane process is also highly affected by the feed composition. Molecules such as polysaccharides, polyphenols, and lipids can induce membrane fouling or form protein complexes that impact separation behavior. On the one hand, other process variables such as operating pH and temperature are equally important. pH can influence protein charge and solubility and has an impact on membrane surface properties and electrostatic interactions, especially close to the isoelectric point (Castro-Muñoz et al., 2020; Castro-Muñoz and Fíla, 2018).

Temperature affects viscosity and diffusion rates, though excessive heat can denature proteins (Verma et al., 2021). It has been shown that, under the influence of electrostatically repulsive forces between molecules and between the protein and the membrane, adsorption and hence fouling are minimized if the pH is changed significantly from the isoelectric point of the protein. This concept is directly translatable to food protein purification and is well established in the purification of sensitive biopharmaceuticals (mAbs) (Castro-Muñoz et al., 2022; Vollet Marson et al., 2021).

One of the major obstacles in the specific operation of membrane processes is fouling, which corresponds to the accumulation and deposition of feed components on the membrane's surface or inside its pores. Fouling means a reduction in permeate flux, increased energy demand, more frequent cleaning needs, and shorter membrane life. The main mitigation strategies for fouling are optimizing the hydrodynamic conditions, adopting periodic cleaning methods such as back flushing and chemical cleaning, and, more recently, ultrasonic cleaning or pulsed electric field cleaning. To optimize performance and extend membrane lifetime, it is recommended to design antifouling membrane surfaces (i. e., hydrophilic or charged coatings) and limit operation below critical flux while maximizing pH and ionic conditions (Castro-Muñoz et al., 2016; Pichardo-Romero et al., 2020).

# 5.2. Centrifugation

Centrifugation is a density-based separation technology that we use to not only isolate proteins and sugars, but also filtrate and purify them from complex compounds found in fruit and vegetable by-products. This technique provides for the isolation and separation of selected regions from proteins depending upon different densities and sedimentation factors of the different biomaterials (Prandi et al., 2023). This is an efficient and straightforward approach to obtaining purified protein isolates from a range of FVW. Functional and structural properties of the isolated proteins can be maintained by applying this technique. In view of this, centrifugation represents an important strategy that allows the technically manageable and sustainable use of FVW and contributes to the conception of becoming more sustainable and resource-effective techniques for protein extraction (Prandi et al., 2023).

# 5.3. Freeze drying

Another cost-effective way of stabilizing the structure and activity of proteins recovered from FVW by-products is Freeze-drying (Lyophilization). This treatment can be accomplished with the removal of water

from the protein-rich material in conditions of low temperature and under vacuum processes. Thereby, the dry powder concentrate of protein is obtained. Freeze-drying is accomplished at low temperatures and with proper isolation from the environment and exposure to air and other hostile conditions. This ensures that natural conformation and functionality of the extracted proteins are retained (Mutukuri et al., 2021). Freeze drying can prevent the significant denaturation of the protein and reduce the occurrence of microbiological reactions, but the shortcoming of the freeze drying method is the expensive and slow drying process (Shen et al., 2021). Compared with spray drying, it has certain shortcomings in efficacy and cost, which will inevitably affect the production time and equipment weight, which is restricting the application of industrial production (Li et al., 2023).

## 5.4. Spray drying

Spray drying is yet another process that is also used to dry and stabilize proteins isolated from fruit and vegetable wastes. This technology is basically a dehydration process that atomizes a liquid feed, rich in protein, into microscopic particles and quickly dries the particles with hot air to provide a dry protein concentrate. Spray drying has also been utilized to yield solid protein formulations. Spray drying has excellent output, and the properties of the spray-dried elements can be influenced to attain the preferred flow ability (Mutukuri et al., 2021). Spray drying is commonly used because it has lower operational costs and greater versatility than freeze drying. Nonetheless, elevated temperatures used in spray drying can result in increased losses. The fruit powder obtained through spray drying exhibited the highest yields of minerals and soluble sugars but showed a reduced total phenolic and flavonoid content (Li et al., 2023).

### 5.5. Chromatography

Protein isolation and purification techniques involve one or more chromatographic stages for final isolation and purification purposes. The primary stage involved moving the protein-containing solution over a column with several materials under the action of an external force. Different proteins interact distinctively with the chromatographic column constituents, resulting in different retention times. This step permits the isolation and purification of proteins based on their precise retention times. Usually, the absorbance at 280 nm is used to identify proteins. The chromatography method is divided into several categories, including hydrophobic interaction chromatography (HIC), gel chromatography, supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC) (Fărcaș et al., 2022).

## 6. Functional properties of extracted proteins

Protein functional properties are influenced by their functional groups and conformation, as well as their interactions in complex food structures. The physical and chemical properties of foods, like texture and sensory qualities, are affected by these factors during processing, packing, and consumption. The functional protein applications are based on several physical and chemical properties, including hydrophilicity, configuration, and conformation (Table 2). The properties that are discussed above are also influenced by the interactions with other components and production conditions like temperature, pressure, pH, and ion strength (Ma et al., 2024).

# 6.1. Functional solubility of proteins

Protein solubility in varying conditions is fundamental to understanding the suitability of a variety of protein isolates for their application in food production. Protein solubility is strongly affected by the relative surface abundance of hydrophilic and hydrophobic amino acids, and by the protein-solvent interactions. At both ionic pH values above

and below the isoelectric point, an increase in ionic hydration, charge, and a decrease in hydrophobic residues correspond to increased solubility (Vinayashree and Vasu, 2021). The quinoa protein extracted at pH 9 was more soluble at the isoelectric point compared with the protein extracted at pH 11. The water retention properties and the amino acid composition of the protein isolated using both extraction methods are similar (Osemwota et al., 2021). It is reported that pumpkin seed proteins have a high solubility (Xu et al., 2020).

## 6.2. Water and oil holding capacity of proteins

Water holding capacity (WHC) refers to a property that can retain water against the force of gravity (Vinayashree and Vasu, 2021). Water and oil holding capacity is a functional property of proteins, and is very important in food formulation to determine the flavor, texture, retention, mouthfeel, and shelf life of the finished product. When a protein is incapable of retaining water, it may lead to tough and dry food product formulations (Osemwota et al., 2021). WHC of proteins depends on their arrangement and conformation of the molecules. Proteins with the desired WHC can be utilized in ground meats, sausages, baked doughs, bakery goods, and gel formations (Vinavashree and Vasu, 2021), Ouinoa protein has improved WHC in contrast with some legume proteins, even though the properties vary between various quinoa varieties (Shen et al., 2021). Defatted apricot kernel flour had great water-binding properties, whereas the flour of defatted mango kernel had the lowest. The significant amount of protein in apricot flour, which has polar amino acid residues, increases the water-holding capacity (Sorour et al., 2021).

Proteins with great OHC can be utilized in food production for minced meat preparation, meat alternatives and baked foods, cake batters, broths, mayonnaise, and salad toppings (Vinayashree and Vasu, 2021). The main features that determine protein OHC are the amount and type of protein and surface hydrophobicity. Pomegranate seed protein isolate showed a high OHC value. Therefore, it can be used in products such as sausages, cake batters, and pastes with a high-fat content (Oroumei et al., 2024). A previous study established that defatted apricot kernel flour had the maximum oil-holding capacity next to defatted peach kernel flour. This suggests that both flours benefit bakery products like biscuits and cakes, which require high oil absorption (Sorour et al., 2021).

## 6.3. Emulsification and foaming properties of proteins

Emulsifying capacity includes emulsifying activity (EA), which indicates a protein's ability to form an emulsion, and emulsion stability (ES), which refers to the capacity of emulsion droplets to stay dispersed without clumping or rising to the surface (Vinayashree and Vasu, 2021). In emulsification, proteins travel to the oil-water interface and create viscoelastic films around oil droplets, aligning their hydrophobic groups near the oil and hydrophilic groups near the water (Osemwota et al., 2021). Proteins have the ability to be utilized as emulsifiers in several foodstuffs, such as creams, drinks, broths, cake batters, pastes, and ice cream.

Foaming ability relates to the rate at which surface tension decreases at the air/water interface, which is triggered by the adsorption of protein particles (Ogunbusola et al., 2022). The source, processing techniques, concentration, pH, temperature, mixing time, and foaming skill affect the protein foaming capacities. Food foams are mainly formed by trapping air in protein films, and their firmness is crucial for foodstuffs like whipped toppings, meringues, mousses, and angel cakes (Vinayashree and Vasu, 2021). White melon protein isolates exhibited low foaming capacity and stability. These isolates consist of well-structured globular proteins that display a relatively low degree of surface denaturation (Ogunbusola et al., 2022). Under acidic and alkaline conditions, the pomegranate seed protein isolate displayed better foaming properties owing to its high protein solubility. This property results from a rise in the protein net charge in an aqueous medium and

improved protein solubility (Oroumei et al., 2024).

## 6.4. Gelation property of proteins

Protein-based gels provide improved textures and structures in food preparations. The protein recovered from avocado seed displayed the desired emulsification and gelation features. These properties allow them to be used as functional materials for a variety of food and nonfood applications (Wang et al., 2022). Pea proteins are isolated by micellar precipitation or ultrafiltration using salt or alkaline extraction from gels with great strength. Quinoa protein forms solid and steady gels at pH 3.5 when heated between 70 and  $90^{\circ}\text{C}$  (Shen et al., 2021). Potato peels can be used to enhance the texture and gelation of foodstuffs. They are beneficial for producing structured items, for example, meat analogs and plant-based substitutes, as they improve mouthfeel and create stable gels.

# 7. Functional applications of proteins in food and non-food sectors

Proteins removed from FVW have various uses in food and non-food production owing to their varied functional properties, as listed in Table 3 (Goenaga et al., 2023).

## 7.1. Application of extracted proteins in the food industry

Consumption of fruits and vegetables is essential for health. They are enriched in nutrients and bioactive substances. Proteins are vital to an individual's diet to support health in all age groups. Plant sources like cereals, legumes, seeds, fruits, and vegetable by-products, including peels, leaves, stems, and pomace, offer valuable protein addition. Additionally, FVW processing provides a great source of proteins for producing unique protein-rich foods (Hayrapetyan et al., 2020). Banana peels, frequently thrown away or composted, are high in protein, fiber, PFA, and essential amino acids. A study was designed to create a functional energy bar utilizing oats, amaranth grains, and banana peel powder. These results indicate that adding banana peel powder enhances the protein, fiber, and phenolic amount of the bar, which can help fight protein-energy malnutrition (Singh et al., 2022).

Apple seeds are protein and fiber-rich sources. A study showed that adding 5 % and 20 % defatted apple seed cakes (DASC) to the total quantity of wheat flour improved insoluble fiber and protein amounts considerably in bread samples. Hydrolyzed proteins from grape seeds improve the nutritional and physicochemical characteristics of vogurt. The commercial production of yogurt with hydrolyzed protein as a functional food, with a desirable flavor and high dietary profile, promises to be practicable and valuable (Varedesara et al., 2021). Plant-based proteins are functional foods that serve various purposes in food preparation, including acting as thickening agents and stabilizers for emulsions, foaming agents, and binders for fats and water. Proteins extracted from potatoes, rice, and peas have demonstrated effective emulsion stabilization by encapsulating a film around the oil droplet. Bitter melon seed protein isolates can be used as gelling agents in the production of several foods. It has a great foaming capacity; thus, it can be used to make ice cream, bakery items, and sweets (Naik et al., 2022).

Hydrolyzed proteins and peptides featuring specific bioactive sequences can reduce the potential of various disorders such as diabetes, cancer, and CVD, while also boosting the immune response to infections. A number of studies have assessed the antioxidant effects of protein or peptide hydrolysates sourced from plants, including tomato seeds, mung beans, rice bran, olive pomace, and pomegranate peel. Pomegranate seed protein, which is waste material remaining after pomegranate seed oil production, is an excellent source of bioactive peptides with antioxidant activity. Trypsin-treated hydrolysate of pomegranate seed protein has demonstrated excellent antioxidant potential (Hernández-Corroto et al., 2020; Rahimipanah et al., 2023). Individual

 Table 3

 Functional applications of extracted protein from fruit and vegetable by-products.

| Functional application | Source of by-product  | Product name   | Results  | References                            |
|------------------------|---|--|--|---------------------------------------|
| Protein enrichment     | Blueberry pomaces, Muscadine grape, chickpea, or pea protein  | Protein-rich plant-based RTD beverage  | Protein-polyphenol particles derived from industrial fruit pomaces were utilized to develop a flavorful, protein-enriched, plant-based ready-to-drink beverage.                            | (Hoskin et al., 2022)                 |
|                        | Banana peel powder  | Functional snack bar with amaranth, oats, and unripe banana peel powder            | Banana peel powder enhanced the protein, minerals, fiber, essential amino acids, phenolic compounds, and antioxidant properties of the functional snack bar                                | (Singh et al., 2022)                  |
|                        | Brewer's spent grain, sugar beet pulp, and apple pomace   | Corn snack products  | Incorporating by-products can increase the nutritional profile of corn snacks by increasing the levels of protein, dietary fiber, and polyphenols  | (Jozinović et al.,<br>2021)           |
|                        | Protein maize, Irish potatoes, and avocado seeds flour  | Weaning food   | A blend of flour incorporating 65 % high-quality protein maize, 15 % Irish potato peel, and 20 % avocado seed flour demonstrated an increase in protein, carbohydrate, and fiber content   | (Olaleye et al.,<br>2020)             |
| Protein fortification  | Bell pepper seeds   | Bell pepper and tomato for pasta   | Tomato waste and pepper seeds by-products were added at 10–30 % to make pasta,   | (Teterycz and                         |
| Protein                | Tomato waste Papaya seeds and peel  | fortification Biscuits supplemented with papaya seed                               | resulting in a 27 % increase in protein and improved amino acid composition<br>Adding papaya seed and peel in amounts ranging from 2 % to 10 % notably                                     | Sobota, 2023)<br>(Jiang et al., 2022) |
| supplementation        | rapaya seeus anu peer   | and peel   | enhanced the protein content, polyphenol compounds, and antioxidant activities   | (Jiang et al., 2022)                  |
| Protein additives      | Grape seed protein  | Grape seed protein hydrolysate in stirred  | Grape seed protein hydrolysate enhances yogurt by increasing pH, viscosity, and  | (Varedesara et al.,                   |
| Trotom unutives        | onpe seed protein   | yogurt   | texture firmness while reducing acidity, offering consumers an enjoyable flavor and nutritional benefits   | 2021)                                 |
|                        | Potato juice protein  | Potato protein-based vegan burgers   | Potato protein-based plant burgers containing 20-22 g of high-quality protein and  | (Kowalczewski                         |
| Meat alternatives      |   |  | good digestibility were developed, offering a solid essential amino acid profile   | et al., 2024)                         |
|                        | Pea, soy, and oat protein   | Meat analogs from pea and oat protein  | Pea protein, soy protein isolated, and oat protein were mixed to create meat analogs.<br>Dry-fractionated protein enhances the sustainable production of plant-based meat alternatives     | (de Angelis et al., 2020)             |
| Animal feeds           | Vegetable by-products (beans, peas, broccoli, carrots, chickpea, green beans, cauliflower, pepper, potato, and spinach) | Ruminant feed  | Vegetable by-products showed higher nutritional benefits and had a greater crude protein level compared to corn silage, making them suitable for inclusion in cattle diets                 | (Goenaga et al., 2023)                |
|                        | Potato protein  | Fish meal made with the addition of potato   | Potato protein concentrate may substitute for as much as 20 % of fish meal in the diet   | (Takakuwa et al.,                     |
|                        |   | protein concentrate  | of greater amberjack without negatively influencing growth performance or feed efficacy  | 2020)                                 |
|                        | Broccoli florets  | Broccoli waste as a potential feed for   | Dried broccoli has the potential to substitute for as much as 24 % of grains and   | (de Evan et al.,                      |
|                        |   | ruminants  | protein ingredients in a concentrate while having no negative impact on rumen fermentation   | 2020)                                 |
| Bioactive peptides     | Pomegranate seed protein  | Antioxidant peptide production from pomegranate seed protein                       | Enzymatic hydrolysis of pomegranate seed protein produces antioxidant peptides, with trypsin-hydrolyzed protein showing strong antioxidant capacity  | (Rahimipanah et al., 2023)            |
| Edible films           | Pea starch  | Protein-pea starch edible film with  | A composite film from a suitable ratio of pumpkin seeds and pea protein starch can   | (Xu et al., 2020)                     |
|                        | Pumpkin seeds protein   | pumpkin seeds  | package oily foods   |                                       |
|                        | Mung bean protein and pomegranate peel  | Antioxidant edible films with mung bean protein supplemented with pomegranate peel | The results highlighted the potential to make bio-functional edible films for food packaging using mung bean protein and peel of pomegranate, which are by-products from the food industry | (Moghadam et al., 2020)               |
| Biopolymers            | Potato protein  | Chitosan/potato protein/linseed oil based biopolymer                               | Biopolymer films were designed to improve storage quality and shelf life of raw meat   | (Wang et al., 2020)                   |
| Nano-emulsions         | Pea protein   | Pea protein Nano-emulsion in food products   | Pea protein Nano-emulsion is an effective carrier and stabilizer of vitamin D in food products with a slight impact on taste and appearance  | (Akkam et al.,<br>2021)               |

bioactive peptides alone have great applications in the dietary, therapeutic, and cosmetic industries. Apart from being a good source of protein, apricot kernel flour is also high in bioactive compounds, minerals, and fiber. Most of the time is used in the bakery industry. In one study, yogurt and ice cream were prepared with defatted apricot kernel powder as a source of protein in a ratio of 10-40 % and 10-50 %, respectively (Hyun et al., 2019). Muskmelon seeds contain tons of proteins, minerals, vitamins, and amino acids. These seeds are a natural source for adding beneficial compounds to different food products; for example, muskmelon seed flour added to biscuits increases the fiber, the protein content, the TPC, and the TFC (Masoud et al., 2024). This is complemented by the nutritive content of food items obtained through the use of high-protein by-products derived from the plant food region. The residual generated from the processing of tomatoes and peppers, such as waste from tomatoes, defatted pepper seeds, and the placenta of peppers, can be incorporated into pasta in proportions ranging from 10 % to 30 %. Pasta that includes at least 20 % pepper placenta, 30 % defatted pepper seeds, and 10 % tomato waste provides protein and fiber while maintaining good cooking characteristics (Teterycz and Sobota, 2023).

### 7.2. Application of proteins in the animal and aqua feed sector

Using fruit and vegetable waste as animal feed can help overcome feed shortages in developing countries. These by-products, including skin, seeds, rinds, and pomace, are rich in valuable phytochemicals, proteins, fibers, and vitamins. They can be fed as they are or processed through drying or ensiling with lower-quality feeds. Some types of vegetable waste, such as broccoli, are high in protein and fiber, making them appropriate for feeding ruminants. Broccoli florets' dry matter content is more than stems, and both parts are high in sugars and degradable proteins and have low levels of lignin fiber. Dried broccoli can substitute as much as 24 % of cereals and high-protein components in ruminant diets without negatively affecting rumen fermentation (de Evan et al., 2020). Fresh broccoli by-products are abundant in crude protein and have been researched as an advantageous feed for dairy cattle, sheep, goats, and young lambs (Aziz et al., 2023). Research has shown that fermented soybean meal, which is high in protein, enhances animal nutrient utilization, digestibility, and absorption. This leads to improved growth, higher feed intake, better gut health, and improved quality of livestock products in ruminants, pigs, and poultry. Rapeseed protein concentrate and yellow pea protein isolate have been utilized in the animal feed and aquaculture industries (Zahari et al., 2021).

# 7.3. Applications of proteins in the non-food sector

The increasing consumer demand for nutritious foods and concerns regarding non-biodegradable packaging have led to the innovation of edible packaging crafted from plant-derived proteins. Proteins are preferred among biopolymers for creating biodegradable films owing to their greater film-forming capabilities and excellent gas barrier properties, which are crucial for preventing food oxidation. However, their inherent hydrophilicity often results in poor moisture barrier properties. This limitation is frequently overcome by creating composite or blended films (Siddiqui et al., 2024). For instance, plant proteins can be blended with nano-reinforcements like cellulose nanocrystals or chitosan to increase mechanical strength, thermal stability, and barrier properties, making these reinforced protein-based bioplastics increasingly viable for flexible packaging applications. Edible films frequently utilize synergistic relationships between proteins and polysaccharides. For instance, in films made from gelatin and chitosan, the positively charged amino groups in chitosan can engage with the negatively charged groups in gelatin. Polyelectrolyte complexes are produced by this interaction, which support mechanical durability, decrease water solubility. They also enhance the gradual release of active compounds used for preservation (Eranda et al., 2024).

Furthermore, proteins can be enzymatically, chemically, or physically cross-linked to create a stronger and water-resistant network, increasing the packaging material's practicality. Proteins like soy, corn zein, and wheat gluten are used in coatings, while yellow pea protein and whey protein isolates are utilized in wet processing scenarios. The surfaces of yellow pea protein films are low-hydrophilic and possess strong thermal and mechanical characteristics (Acquah et al., 2020). Biopolymer films that are eco-friendly, compostable, naturally derived, and safe are frequently made from natural materials, such as proteins, lipids, and carbohydrates. Proteins are favored among biopolymers for making biodegradable films owing to their greater film-forming capabilities (Wang et al., 2020).

Mung bean, a novel plant protein, has the potential to be an effective biopolymer that forms film. A cheap by-product of the food industry is pomegranate peel. According to one study, pomegranate peel improves the bio-functional abilities of mung bean protein films (Moghadam et al., 2020). Cottonseed protein is a sustainable substitute for formaldehyde-based adhesives as it is a renewable resource that does not harm the environment. Lately, cottonseed protein or water-extracted cottonseed meal has been used as a sustainable adhesive in the manufacture of wood, plywood, and particleboard. Another research on soy protein isolate (SPI) coating with honey for fresh-cut pineapple packaging revealed that it extended pineapple shelf-life by 16 days at 4°C and enhanced the retention of phenolic substances. SPI coatings also hinder microbial growth (Yousuf and Srivastava, 2019).

Bioplastics derived from renewable and natural resources offer a promising approach to combating plastic pollution. They break down more readily than standard plastics. Bioplastics, which foster the circular economy, can be produced from food scraps. Bioplastics made from potato peel proteins are solid and flexible, making them suitable for packaging options like bags and containers. In summary, these bioplastics have a minor environmental impact and contribute to lower greenhouse gas emissions and harmful by-products than traditional plastics (Miescher et al., 2024). Plant protein nanocarriers offer a novel and economical approach to drug delivery because of their hydrophobic characteristics that facilitate extended drug release. The variety of functional groups allows modifications to regulate their properties and attach to targeting agents. Soy protein isolate (SPI) is exceptionally adaptable, and techniques such as desolvation or coacervation can be used to produce nanoparticles, with legumin serving as an essential storage protein in soybeans (Fan et al., 2021). Bioactive peptides derived from FVW are achieving approval in the food, cosmetic, and therapeutic sectors as they provide various health-promoting benefits, including antithrombotic, antimicrobial, cholesterol-lowering, and antioxidant properties. Peptides obtained from apricot, plum, olive, pomegranate, and peach seeds may serve as valuable components in anti-aging skincare products (Guzmán-Lorite et al., 2022).

# 8. Environmental sustainability of protein recovery technologies

The successful implementation of a circular economy demands environmental sustainability in addition to technological efficiency. Valorizing FVW aligns with a circular-economy approach that preserves resources and lessens environmental burden. For example, upcycling agro-residues can help avoid landfill disposal and save water and land that would otherwise be used to grow new crops (Lee et al., 2024). Additionally, as we know that plant-based proteins usually have a smaller CFP than animal proteins, so their extraction from waste streams contributes to greater sustainability. The environmental sustainability of isolating proteins from FVW processing is essential when assessing the overall worth of these valorization techniques (Scapini et al., 2023). Even though conventional extraction methods (e.g., alkaline extraction and isoelectric precipitation) are well-established, they frequently use large amounts of water and chemicals, produce significant wastewater requiring treatment, and can be energy-intensive. All these factors

contribute to a larger environmental footprint (Usman et al., 2022).

Emerging green extraction technologies like PLE, UAE, and MAE with reduced solvent usage and energy consumption can offer environmental advantages. Supercritical CO2 offers a non-toxic, recyclable solvent substitute. PEFE and UAE function at room temperatures and minimize heating energy (Athanasiadis et al., 2024). Even though PLE and MAE use elevated temperatures but shorter processing times frequently result in lower net energy use. DESE employs biodegradable solvents, reducing hazard (Nouska et al., 2024). However, life cycle assessments (LCA) are vital for comparison, as green techniques may have higher upfront energy costs due to specialized equipment (e.g., high-pressure pumps for HHP or SFE), but their overall environmental impact, considering all inputs and outputs, is often favorable (Lee et al., 2024). Future efforts should focus on optimizing energy efficiency, industrial scaling, and comprehensive LCAs to validate benefits. Integrating these processes is key to sustainable fruit and vegetable by-product valorization. This approach ensures the maximal recovery of valuable compounds while simultaneously mitigating the environmental impact associated with food processing waste

# 9. Challenges and limitations in valorization

Although by-products of fruits and vegetables are plentiful and constitute an underexploited source of valuable proteins, their practical use has numerous limitations, as illustrated in Fig. 4 (Miller et al., 2020). The differences in the composition and characteristics of the by-products, which are affected by the types of fruits and vegetables, growing conditions, and processing techniques, make it difficult to establish uniform extraction and purification methods. Compounds that interfere with proteins, including pigments, polyphenols, and enzymes, also pose challenges during protein isolation and require additional purification stages. Proteins extracted from vegetables and fruits have anti-nutrients, referred to as plant complexes, that protect against pathogens and pests. Some modification processes can be used to reduce or remove the negative effects of the anti-nutrients (Salem, 2020). The other significant limitation is the low protein content of most vegetable and fruit by-products, which involves processing huge quantities of raw materials to obtain sufficient quantities of target proteins (Orellana-Palacios et al., 2022). Specific isolated proteins have narrow applications in food products due to their unpleasant flavors, such as

bitterness, which can be addressed by various modulation strategies. The isolated plant proteins are usually bitter in taste because they contain anti-nutritional factors (Salem, 2020). Furthermore, the isolated proteins may need improvement as they have unfavorable functional properties (solubility and gelling capacity) that make them unsuitable for the desired food or non-food uses. Additionally, beyond the economically risky demands required to utilize fruit and vegetable by-products as a protein source, there are considerable technical, regulatory, and economic obstacles. This includes the absence of contaminants, allergens, and sources of toxic factors. Safety assessment and toxicology studies are essential tasks in approval for novel protein sources (which can be time-consuming and expensive) and, therefore, in designing a novel protein source (Cui et al., 2021). Purification of protein from by-products is quite costly. The implementation of special equipment, energy-consuming, and different purification techniques leads to higher production costs. Thus, for future research directions, the new, effective, and scalable techniques of protein extraction and purification from food waste should be designed that combine efficiency with environmental and economic sustainability. This can help the circular economy and maximize the use of fruit and vegetable by-products as a source of protein (Fuso et al., 2022).

## 10. Conclusion

Proteins from food by-products offer great potential to improve food industry sustainability. In this review, we have discussed the tremendous potential of these unexploited resources, the advances in extraction and purification technologies, and the various functional applications of the proteins extracted. Despite the positive outlook, important challenges remain, including techno-economic barriers such as the high cost of upscaling new green technologies, technical constraints such as membrane fouling, functionality, and sensory performance of some extracted proteins, and regulatory and safety gaps for new protein sources and solvents. Future research should focus on developing hybrid processes to improve yield and reduce energy consumption, intensifying research on next-generation anti-fouling membranes, systematically investigating the relationship between HBA/HBD structure and protein stability for DESs, and employing modification techniques to improve the techno-functional properties, environmental sustainability, and affordability of the extracted protein. Conducting comprehensive Life



Fig. 4. Limitations in Protein Extraction from Fruit and Vegetable By-Products.

Cycle Assessments to quantitatively validate the environmental benefits of these valorization chains is also essential. The discussion on the environmental advantages of green extraction techniques underscores their potential to reduce the ecological footprint of the process. Better extraction processes will lead to healthier diets, boost the circular economy, and be in line with global sustainability objectives. Further research is required on bioprocessing and processing techniques to modify these proteins and improve their functionality. Successful commercialization requires strong collaboration between academic research and industry, focusing on upscaling production processes and addressing cost and nutritional considerations. The safety, economic, and regulatory aspects of these extracted proteins must be addressed to ensure their successful integration into the food supply chain. By addressing these challenges and focusing on these future directions, the scientific community will achieve the full potential of agro-food byproducts, converting waste into useful resources and making substantial contributions to global food security and the sustainability of the environment.

## CRediT authorship contribution statement

Tanveer Ahmad: Writing – review & editing, Methodology, Conceptualization. Muhammad Inam-ur Raheem: Writing – review & editing, Supervision, Methodology, Data curation. Arashi Shahid: Resources, Methodology, Formal analysis. Teresa Cirillo: Supervision, Methodology, Investigation. Francesco Esposito: Writing – review & editing, Conceptualization. Laiba Khalid: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. Ifrah Jabeen: Writing – review & editing, Writing – original draft, Methodology.

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# **Data Availability**

The data will be available upon request.

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