Extraction of Protein from Agricultural Waste

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Protein is the link of amino acids bonded by peptide bonds forming the primary, secondary, tertiary, and quaternary structure of the protein. Regardless of the superiority of the protein quantity in animals compared to plants, plant-derived protein has become one of the alternative options in overcoming the increasing environmental issue due to the mass production in the agricultural sector. Plant-derived protein is now receiving attention for its functionality as a sustainable protein source. In recent years, downstream bioprocessing industries are venturing into less tedious, simple, and high-efficiency separation by implementing advanced purification and extraction methods. The separation of proteins, with the main focus on amylase as an enzyme from industrial agriculture waste using various techniques of extraction, is being discussed further.

agricultural waste protein

1. Characteristics of Agricultural Waste and Protein

The agricultural and industrial waste can be separated into two categories: agricultural and industrial residues. Agricultural residues include stems, stalks, leaves, seedpods and husks, seeds, roots, bagasse, and molasses, as well as field and processing residues. Meanwhile, industrial residues are mostly from the food-processing industries, such as potato peel, orange peel, soybean cake, cassava peel, and other organic residues.

Industrial residues are expected to multiply in tandem with the increasing population and demand in food supply [1]. This corresponds to the development of the high-input agricultural trend, which will improve the overall residue production, including agricultural waste, by 1.3 Pg dry matter per year [2]. However, these protein-rich residues have started to gain interest for their economically attractive value and capability to be recovered. The residues are now mostly used for the extraction and utilization of usable protein and applied in foods and supplements [3]. This agricultural waste should be significantly regarded as a potential resource to cope with the modern food-technology process and in line with a complete life cycle analysis system [4].

Protein is naturally synthesized in plants and animals; generally, protein is abundant in animals compared to plants [5]. Hicks and Verbeek (2016) stated that the growing worldwide demand for animal-based products necessitates a significant rise in plants and other feed resources, resulting in a much higher amount of protein-rich materials being generated as waste than the protein supplied for consumption. The major facet of this occurrence is to convert these agricultural wastes into usable protein [3]. The discovery of usable protein from these wastes will be feasible along with the technology available for recovering nutrient-rich protein. Membrane separation, adsorption, microbe-assisted protein recovery, and other conventional extraction methods have been presented as potential strategies

for protein recovery from waste [6][7][8]. The recovery of enzyme protein is one of the concerted efforts for converting these wastes into usable protein in the industry [9][10][11].

Amylase is recognized as a crucial industrial enzyme protein, comprising approximately 30% of the world enzyme market [12][13]. It is eminent for the food, fermentation, and pharmaceutical industries. Amylase can be found in animals, bacteria, and plant cells. Despite various sources of amylase, only fungi and bacterial amylase dominate the industrial sector. Previously, large-scale production was limited to only certain strains of bacteria and fungi (extracellular protein), making them the only resources susceptible to meet the huge demand of the industries [14]. However, the discovery of biotechnologies has found that plants (intracellular protein) can suffice as a rich source of plant-derived enzymes for biotechnological and industrial purposes at lower cost and toxicity [9].

2. Methods in Extraction and Purification of Protein from Agricultural Waste

The huge amount of agricultural waste generated per year eventually leads to pollution and economic loss. However, these waste streams are rich in bioactive compounds, including proteins that have the potential to be recovered and re-utilized as functional foods, medications, cosmetics, and bio packaging [15]. Hence, much research has been completed on the recovery and utilization of these agricultural wastes [15][16][17][18][19].

2.1. Membrane Extraction

Membrane extraction is a bioseparation method that uses a membrane, which is an interphase that is usually heterogeneous. The membrane acts as an obstacle to the flow of molecular and ionic groups present in the liquid or vapor phase. Membranes have traditionally been used for size-based separation with high throughput but low-resolution requirements. This method includes microfiltration, ultrafiltration, and nanofiltration techniques for protein concentration and buffer interchange [8]. Separation using membrane extraction has been proposed for enhancing processes due to the absence of chemical usage, reduced energy consumption, and simpler scale-up of operations [20].

Membrane technology has been demonstrated to be excellent in recovering proteins from waste sources [6][21]. The ultrafiltration method was applied to extract valuable protein from poultry-handling industry waste [6]. The result obtained from this experiment demonstrated the highly relevant and ideal application of ultrafiltration to extract protein from the poultry waste stream.

Amylase separation via microfiltration was reported by Rodrigues et al. (2017) [22]. Crude enzyme extract was subjected to a microfiltration process to separate enzymes of different molecular weights from other low-molecular-weight compounds of the previous fermentation process. It was determined that the enzymatic activity of the retentate increased by 38% [22]. This is due to the removal of lower-molecular-weight compounds from the retentate, which could affect amylase activity [22]. The efficiency of a two-stage membrane technique for purifying protease from a pineapple waste mixture was evaluated [23]. Two-stage ultrafiltration, enzymatic pretreatment, and

diafiltration were included in this system. During membrane filtration, the enzymatic pretreatment improved flux performance. The excellent membrane selectivity of the diafiltration procedure helped this system to achieve a 4.4-fold increase in enzyme purity [23].

Protease purification was conducted via a one-step ultrafiltration process of feather meal [24]. Keratinolytic proteases were produced by *Bacillus* sp. P45 with chicken feather meal as the substrate. The optimized ultrafiltration process recorded an enzyme recovery of 87.8% with a 4.1-fold purity increment. The limitation of this membrane-based extraction is flooding and loading limits while passing through the membrane, emulsification on the membrane, and large solvent inventory and high investment in the machinery [25][26]. On the other hand, the benefit of membrane separation techniques is energy efficiency, where most of the membrane-based extraction techniques require low energy consumption and possess small dimensions that can reduce space consumption [26].

2.2. Precipitation

One of the most frequent methods used for separating enzymes and proteins is via precipitation. The idea behind this procedure is to introduce salts, polar and non-polar solvents, and organic polymers into cell extracts by changing the temperature or pH [27]. The concept of precipitation is the phenomenon whereby a substance is dissolved in a solution, which will then emerge, and the emerging precipitate will be separated from the solution [28]. For example, for the precipitation of protein by acid, the changes in the pH of the medium affect protein structure. When acid is added, the hydration sphere surrounding the protein is disrupted, leading to precipitation [29].

Haslaniza et al. (2014) applied the precipitation method to optimize the production of protein hydrolysate from cockle meat wash water [30]. This experiment demonstrated precipitation as a reliable technique in recovering protein from waste as the objective of this experiment was achieved. Furthermore, the optimized parameter was successfully identified [30].

A study reported the separation of amylase from agricultural waste by precipitation. Ammonium sulfate precipitation was used to separate amylase from cultivated dhal industrial waste with 40% activity [31]. The same technique was also used to purify amylase from cultivated tapioca liquid waste, which managed to obtain the enzyme-specific activity of 37.56 ± 0.38 U/mg [32].

The advantages of precipitation include the possibility to be applied in a continuous process due to its simplicity, scalability, proven usage in bioprocessing, as well as good biocompatibility and storage stability [28]. Meanwhile, the drawbacks of precipitation are the possible introduction of additional impurities, and quality control is needed because the yield of separation may be significantly influenced by pollutant ions, such as divalent ions [28].

2.3. Ultrasonication

Ultrasound-assisted extraction involves several mechanisms, including fragmentation, erosion, capillarity, texturization, and sonoporation [33]. This technique can be performed by bath or probe modes. A comparison between ultrasound-assisted extraction and conventional solvent extraction was made to purify carotenoids from pomegranate peel [34]. They concluded that ultrasound-assisted extraction exhibited superior attributes in terms of a green process, low energy consumption, and safer processing with a higher yield of carotenoids.

Ultrasonication is regarded as one of the green technologies for the extraction of plant-based proteins [35]. Ultrasound-assisted extraction was conducted on wampee seed to extract protein, which is regarded as an advantageous nutraceutical and food ingredient. The finding from this experiment proved that ultrasonication is feasible in recovering protein from wampee seed [36].

Jain and Anal (2016) carried out ultrasound-assisted extraction of functional protein hydrolysates from the membrane of chicken eggshells [21]. According to this study, ultrasonic treatment is an effective method for separating protein hydrolysates.

Another study on ultrasonication was performed for the extraction of bioactive compounds from amaranth [37]. Amaranth is an underutilized plant with numerous precious bioactive compounds, such as polyphenols, betaxanthins, and betacyanins, that can inhibit fatal diseases [38]. Based on the study performed by Ahmed et al. (2020), ultrasound-assisted extraction enhanced the extraction of bioactive compounds and the antioxidant activities of amaranth [37]. This method also has the potential to serve as an alternative to the conventional extraction technique [37].

The application of ultrasound-assisted extraction is reported to be more efficient and offers a shorter extraction time with lower solvent utilization; furthermore, there is a huge potential for the automation of this extraction process [37][39]. Meanwhile, the drawback of ultrasound-assisted extraction is the presence of oxidation that can cause negative impacts on bioactive compounds, the decrease in nutritional content, and the occurrence of cell rupture on the extracted bioactive compounds [37].

2.4. Chromatography

Chromatography can be classified into four separation techniques: ionic exchange, surface adsorption, partition, and size exclusion [40]. Theoretically, the separation of molecules occurs between the movement of the mobile phase and the stationary phase.

Melnichuk et al. (2020) reported the valorization of two types of agricultural waste (i.e., soybean and wheat) by solid-state fermentation [11]. Size-exclusion chromatography was used to purify the enzyme protein, α-amylase, for the fermented samples. The recovery and purification factors of 83% and 6 were achieved, respectively.

Other research was conducted using chromatography for the purification of lithium [41]. Lithium is an alkali metal that is highly reactive, contains highly soluble salts, and possesses a low ionic charge [42]. Chromatography

recovered lithium at a higher efficiency than extraction using the conventional liquid–liquid extraction (LLE) method [41].

Chromatography solves the drawbacks of LLE, such as the high consumption of solvent and complex extraction stages, by achieving higher purification of lithium [41]. Nevertheless, research on extraction using chromatography is still in premature development and operated on a small laboratory scale [41].

2.5. Liquid-Liquid Extraction

When two immiscible or partially soluble liquid phases are stacked together, LLE is used to separate components from one phase to the next. Some of the most common varieties of this bio-separation technology principles include an aqueous two-phase micellar system, an aqueous two-phase polymer system, and a two-phase reverse micellar system [43][44]. When water-soluble polymers are mixed with other polymers, solvents, or inorganic salts at quantities above their critical concentrations, a two-phase system emerges. This principle is used to separate, concentrate, and fractionate biological solutes and particles, such as proteins, enzymes, and nucleic acids.

The LLE process is based on the movement of the targeted analyte from one phase to another when two immiscible liquids come into contact. This method is theorized to be dependent on preferential mass transfer and dissolution of target biomolecules in a complex aqueous matrix. The thermodynamic forces will drive the transfer of chemical species across the phase. The traditional method made use of a separatory funnel to quantitatively transfer the liquid out. Hence, relative density plays an important role, where the less-dense organic phase will reside in the top phase and the aqueous sample in the bottom phase [45].

Liquid-liquid extraction is utilized in the industry, particularly in the chemical and mining industries, as well as in the recovery of fermentation products at the end of the process [46]. Along with its simplicity, low cost, and ease of scaling up, LLE has been used to purify biomolecules on a large industrial scale for more than a decade [43]. The advantages of employing LLE include low viscosity, lower mechanical cost, and shorter time of phase separation. Most crucially for industrial applications, LLE has been certified by industry regulatory bodies [47].

By considering the benefits offered by this technique, researchers have explored its potential for improvements in terms of separation mechanism and setup. Hence, advanced separation techniques have been introduced by following the basic principles of LLE.

2.6 Advanced Liquid-Liquid Extraction Techniques

Several advanced techniques have been developed based on the separation principles of LLE. These techniques aim to overcome some drawbacks of the conventional LLE method by integrating several technologies to improve separation efficiency and process feasibility. The advanced LLE extraction includes Liquid Biphasic System (LBS), Liquid Biphasic Flotation (LBF), Thermoseparation (TMP), Three-Phase Partitioning (TPP), microwave-assisted LBS, ultrasound-assisted LBS, and electrically assisted LBS. These techniques have been reported to be successfully applied for the separation of proteins from agricultural waste. Advanced LLE techniques have received

positive feedback for the application of LBS, LBF, TMP, and TPP on the extraction of proteins from agricultural waste [48][49][50][51]. The methods are effective and superior in obtaining the targeted biomolecules compared to the conventional method. Furthermore, the drawback of conventional methods for extraction and purification in terms of economic and complexity of the operation has been improved.

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