

Application of a Liquid Biphasic Flotation (LBF) System for Protein Extraction from *Persiscaria Tenulla* Leaf

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Keywords: protein, polygonum, liquid biphasic flotation, leaf, Extraction

Abstract:

Persiscaria tenulla, commonly known as Polygonum, is a plant belonging to the family Polygonaceae, which originated from and is widely found in Southeast Asia countries, such as Indonesia, Malaysia, Thailand, and Vietnam. The leaf of the plant is believed to have active ingredients that are responsible for therapeutic effects. In order to take full advantage of a natural medicinal plant for the application in the pharmaceutical and food industries, extraction and separation techniques are essential. In this study, an emerging and rapid extraction approach known as liquid biphasic flotation (LBF) is proposed for the extraction of protein from *Persiscaria tenulla* leaves. The scope of this study is to establish an efficient, environmentally friendly, and cost-effective technology for the extraction of protein from therapeutic leaves. Based on the ideal conditions of the small LBF system, a 98.36% protein recovery yield and a 79.12% separation efficiency were achieved. The upscaling study of this system exhibited the reliability of this technology for large-scale applications with a protein recovery yield of 99.44% and a separation efficiency of 93.28%. This technology demonstrated a simple approach with an effective protein recovery yield and separation that can be applied for the extraction of bioactive compounds from various medicinal-value plants.

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Article

Application of a Liquid Biphasic Flotation (LBF) System for Protein Extraction from *Persiscaria Tenulla* Leaf

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Abstract: *Persiscaria tenulla*, commonly known as *Polygonum*, is a plant belonging to the family Polygonaceae, which originated from and is widely found in Southeast Asia countries, such as Indonesia, Malaysia, Thailand, and Vietnam. The leaf of the plant is believed to have active ingredients that are responsible for therapeutic effects. In order to take full advantage of a natural medicinal plant for the application in the pharmaceutical and food industries, extraction and separation techniques are essential. In this study, an emerging and rapid extraction approach known as liquid biphasic flotation (LBF) is proposed for the extraction of protein from *Persiscaria tenulla* leaves. The scope of this study is to establish an efficient, environmentally friendly, and cost-effective technology for the extraction of protein from therapeutic leaves. Based on the ideal conditions of the small LBF system, a 98.36% protein recovery yield and a 79.12% separation efficiency were achieved. The upscaling study of this system exhibited the reliability of this technology for large-scale applications with a protein recovery yield of 99.44% and a separation efficiency of 93.28%. This technology demonstrated a simple approach with an effective protein recovery yield and separation that can be applied for the extraction of bioactive compounds from various medicinal-value plants.

Keywords: extraction; leaf; liquid biphasic flotation; polygonum; protein

1. Introduction

The usage of traditional herbs for preventive health care is widespread, and plants are the source of numerous natural antioxidants that could be utilized for the development of novel medicines. Natural antioxidants and bioactive compounds that originate from traditional herbal medicines have received escalating attention for their ability in treating specific human diseases. For example, traditional herbal medicine has been used widely in treating cancer patients [1] and to treat neurodegenerative disorders [2]. Plants comprising high medicinal value are currently screened for a variety of pharmacological properties.

Persicaria tenulla (formerly known as *Polygonum (P. minus)*) or frequently recognized as “kesum” in Malaysia, have been used as a flavoring ingredient and food additive in Malaysia. Polygonum plant has been reported to contain a wide range of pharmacological properties and many studies have been conducted to evaluate the phytochemical and pharmacological aspects of the plant, which include anti-inflammatory activity [3], antiproliferative effects [4], anti-microbial activity [5], cytotoxic activity [6], gastric cytoprotective activity [7], and antiviral activity [8]. It has been proven that *P. minus* comprises many high-value components that include protein, flavonoids, and antioxidant vitamins, such as carotenes, retinol equivalents, and vitamin C. However, there are limited studies concerning the effective extraction techniques of the biomolecules from the plant extract.

The major challenge in the extraction of the high-value components from herbal plants is the downstream processing. Up to date, there is a lack of effective and efficient techniques for high yield and cost-effective biomolecule extraction. In this study, a novel method known as liquid biphasic flotation (LBF) system is introduced to extract protein from *P. tenulla* leaves. The LBF process comprises the incorporation of two processes, which are an aqueous two-phase system (ATPS) and a solvent sublation (SS). The conventional flotation system that is commonly known as SS was first introduced by Sebba [3,9]. The SS process is a type of non-foaming adsorptive bubble separation technique in which the surface-active or hydrophobic compounds in aqueous phase are adsorbed on the bubble surfaces of an ascending gas stream and then collected in an immiscible apolar organic solution layer placed on top of the aqueous phase. The mass transfer of SS involves the air bubbles that are produced from the sublation column. The air bubbles drag a sheath of water into the top organic solvent, which eventually drains as water droplets, depleted of solute, and descend back into the bottom aqueous phase via the gravitational force [5,10]. As for the LBF process, the mass transfer comprises the integration of ATPS and SS, which are the utilization of aqueous two-phase systems as a liquid medium for facilitating the mass transfer of biomolecules and the involvement of mass transport from SS. The LBF system is known to be a newly developing separation process that has many benefits over conventional processes. LBF has several advantages, such as a high separation efficiency, high yield, simple separation, and is an economical technique [11]. Recently, the LBF process has gained much attention and many studies have been conducted using this technique for various biomolecules extraction, which include lipase from bacteria, penicillin G, puerarin, α -lactalbumin, lincomycin, C-phycoyanin, polyphenols, betacyanins, and protein from microalgae [12].

In this current study, the LBF method is utilized to extract protein from *P. tenulla* and the optimization of the technique is performed to obtain optimal operating conditions for protein recovery. Parameters that were evaluated in this research were the effect of types of alcohol, types of salt, the concentration of alcohol and salt, amount of kesum biomass, pH, flotation time, and scale-up capability. This study aimed to assess the feasibility of LBF in protein extraction from kesum leaf and to demonstrate that the LBF system is an effective method that has a high possibility to be employed in large scale productions.

2. Materials and Methods

2.1. Materials

Ammonium sulphate, dipotassium hydrogen phosphate, magnesium sulphate, disodium hydrogen phosphate, sodium hydroxide, hydrochloric acid, and Bradford reagent were purchased from R&M Chemicals, (Selangor, Malaysia). Food grade 99.8% ethanol and 2-propanol were acquired from R&M Chemicals, (Selangor, Malaysia). Fresh Kesum leaves were obtained from Aeon supermarket (Selangor, Malaysia, distributed by Edsam Trading Sdn. Bhd.).

2.2. Equipment

The LBF equipment was made up of a glass column, where the small LBF column had an internal diameter of 2.4 cm, height of 15 cm, and could accommodate a solution with a maximum volume of 50 mL. For the large-scale LBF system, the internal diameter was 7 cm, 15 cm in height, and the capacity of the system was 500 mL. The base of the column was built using a G4 sintered disk with a pore size of 10 μm manufactured by DONEWELL RESOURCES SDN. BHD, (Selangor Malaysia) and the base was connected to an air pump for air bubble generation. A Dwyer flowmeter (model RMA-26-SSV) with the range of 25–250 cc/min was used to measure the flow rate of air supplied to the column. Figure 1 displays the experimental framework of the LBF method that was used in this study.

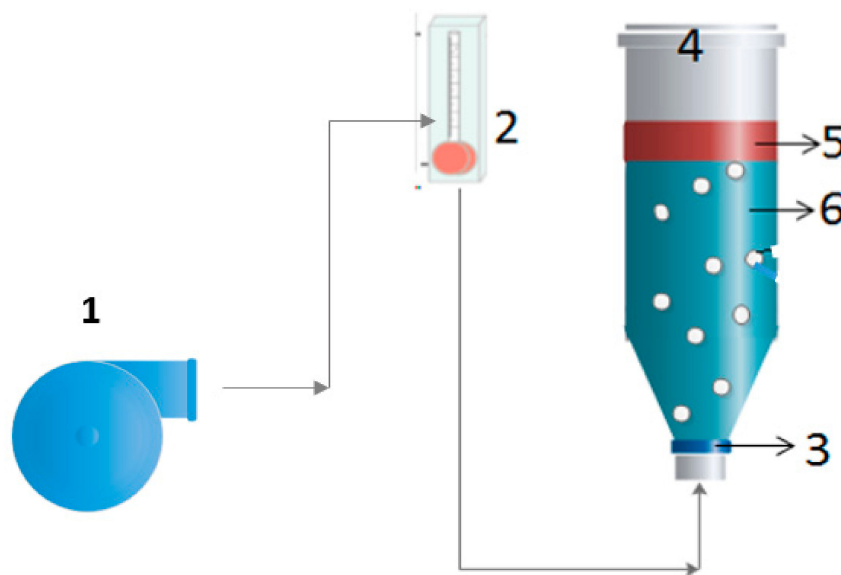


Figure 1. Schematic diagram illustrating the apparatus set-up of liquid biphasic flotation (LBF) system for protein extraction. 1: Air pump; 2: flowmeter; 3: sintered disk; 4: LBF column; 5: top alcohol phase; 6: bottom salt phase.

2.3. Methodology

2.3.1. Preparation of Kesum Leaf Powder

Fresh *Persicaria tenella* obtained from a supermarket were used in this study. The leaves with petiole attached were removed from the stem and were cleaned. The leaves were cut into smaller pieces and were desiccated in a silica gel box overnight. The dried leaves were then ground with a mortar and pestle into powder form.

2.3.2. LBF Extraction

This study was performed in batches and was repeated thrice. A one variable at a time (OVAT) method was used in this study to assess the effect of different parameters on the protein recovery.

The initial condition of 250 g/L of ammonium sulphate was dissolved in 15 mL of distilled water. Fifteen milliliters of salt solution, which served as the bottom phase, was pipetted into the flotation system. Fifteen milliliters of 100% ethanol was added to 300 mg of ground leaves and vortexed. The top phase was then poured into the LBF tube gently from the edge. The system was capped with a lid and then immediately timed using a stopwatch. The amount of bubbles that formed was maintained by adjusting the pressure of the flotation system. Adjustment of the pH was made via the addition of hydrochloric acid (1 M) or sodium hydroxide (1 M). After the system had settled for 10 min, the top phase was pipetted into a tube and the remaining bottom phase was poured into another tube. The volumes of the top and bottom phases were measured.

2.3.3. Protein Assay

The protein concentration was obtained by applying the Bradford method. An extracted protein sample with a volume of 0.25 mL was mixed with 2.5 mL of Bradford reagent in a cuvette. After 10 min of reaction time, the absorbance was measured using a UV-Vis spectrophotometer at the wavelength of 595 nm. The absorbance reading obtained was converted to a protein concentration by using a standard calibration curve that was established using a standard protein, namely BSA. The results were expressed as a mean of triplicate readings.

2.3.4. Calculation of the Separation Efficiency (E) and Recovery Yield (R)

The separation efficiency (E) describes the concentration of protein being extracted in the alcohol phase (top phase). The efficiency is obtained by calculating the concentration of protein present in the bottom phase before and after the flotation process and it was evaluated by employing Equation (1):

$$E = \left(1 - \frac{C_B}{C_{Bi}}\right) \times 100\%, \quad (1)$$

where c_B represents protein concentration in bottom phase after flotation, while c_{Bi} signifies protein concentration in bottom phase before flotation. The E value determines the concentration of protein being successfully recovered in the alcohol-rich top phase.

The total recovery yield (R) of protein was assessed by applying Equation (2). The C_T describes the protein concentration that is recovered in the top phase, while V_T defines the volume of the top phase. Based on the protein concentration obtained from the top phase, the amount is compared with the theoretical protein content in mg to obtain the recovery yield. The amount of protein present in the leaf is based on theoretical value obtained from Revathy Sankaran et al. [11].

$$R (\%) = (C_T \times V_T) / (\text{Amount of protein content based on theory}) \times 100\% \quad (2)$$

3. Results and Discussion

3.1. Effect of Alcohol Types on the Protein Recovery and Separation Efficiency

In this study, water-miscible pure alcohols (100%), namely ethanol and 2-propanol, demonstrated the ability to form LBF with 250 g/L of ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$. Based on the results, LBF formed using $(\text{NH}_4)_2\text{SO}_4$ /ethanol showed a 74.93% separation efficiency and a 32.35% protein recovery. In contrast, the separation efficiency and recovery yield achieved using LBF containing $(\text{NH}_4)_2\text{SO}_4$ /2-propanol were 57.95% and 28.2%, respectively. The results clearly show that LBF formed using $(\text{NH}_4)_2\text{SO}_4$ /ethanol was more efficient in recovering protein from kesum leaves than LBF containing $(\text{NH}_4)_2\text{SO}_4$ /2-propanol. Ethanol exhibited a better performance possibly due to its property of high solute solubility that can assist in the desorption of the solute from the substrate [12]. Additionally, by comparing both solvents, ethanol is more environmentally friendly compared to propanol [12]. From the industrial point of view, ethanol is a better selection for large-scale production as it can be easily reused [13]. The findings suggest that the efficiency of LBF in protein separation

is dependent on the type of alcohol used in the system. In this case, LBF formed using 250 g/L of $(\text{NH}_4)_2\text{SO}_4$ and 100% of ethanol was chosen for the subsequent studies.

3.2. Effect of Types of Salt on Protein Recovery and Separation Efficiency

Selecting a proper phase-separation salt with a high salting-out ability is a key step in developing an efficient LBF system for maximum protein recovery from kesum leaves. The type of salt selected is considered to be an important variable to take into account when designing an LBF for protein separation owing to their strong effects on the salting-out effect and the partition coefficient of protein [14]. While keeping the other variables constant, such as alcohol type, alcohol concentration, salt concentration, flotation time, and the amount of starting material constant, the relative salting-out effectiveness of salt types was investigated in this study. The salts used were ammonium sulphate, di-potassium hydrogen phosphate, magnesium sulphate, and disodium hydrogen phosphate.

In this study, the protein separation efficiency was found to be varied with the types of salts used. There was no value for the separation efficiency in the LBF formed using magnesium sulphate/ethanol and disodium hydrogen phosphate/ethanol due to the precipitation that occurred in these two systems (Figure 2b). A similar conclusion was also reached by Phong et al. [14], who reported that the protein separation efficiency as a result of the salting-out effect is greatly influenced by the types and complexation of the cations and anions of salt in the LBF system.

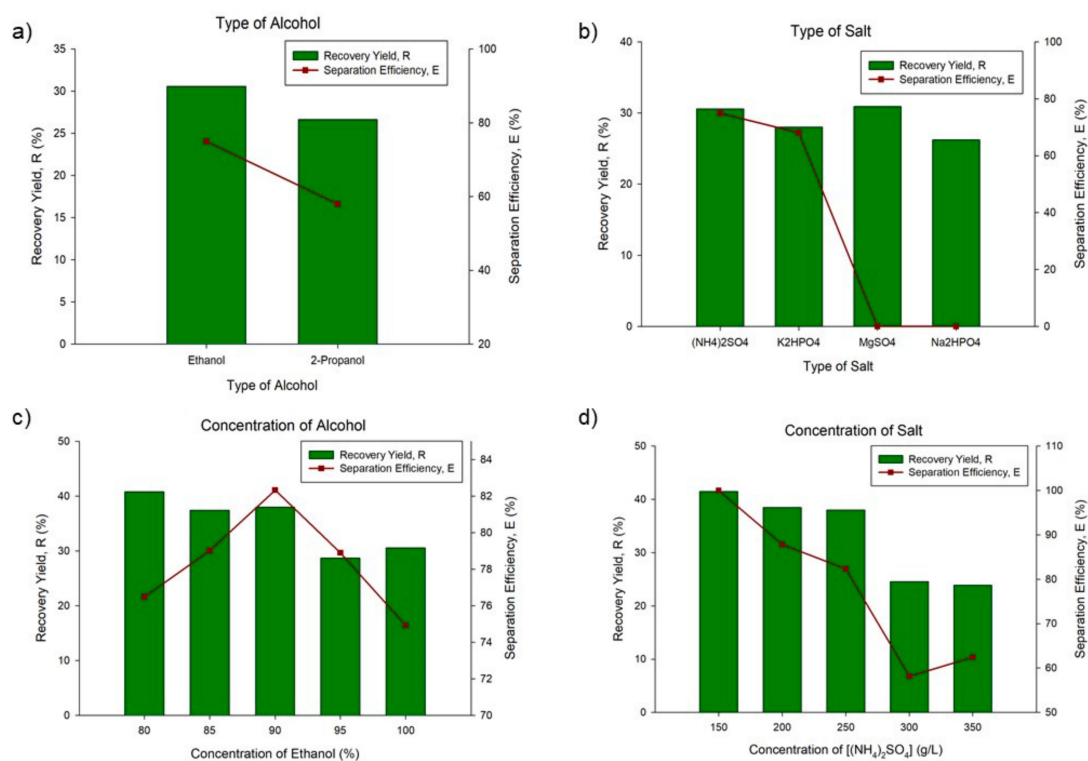


Figure 2. Effect of different conditions on the protein recovery and separation efficiency: (a) Effect of the alcohol type, (b) Effect of the types of salt, (c) Effect of the ethanol concentration, and (d) Effect of the ammonium salt concentration.

Among all the salts, LBF formed using ammonium sulphate achieved the highest separation efficiency and recovery yield, with the values of 74.93% and 32.35%, respectively, as shown in Figure 2b. This trend supports previous findings in the literature. The relative effectiveness of salt types was found to follow the well-known Hofmeister series [14], in which ammonium sulphate forms two ions at the ends of their respective Hofmeister series [15]. Apart from this, ammonium sulphate has been reported as the most commonly used salt for salting out of proteins due to its effectiveness, high solubility,

cheapness, availability of pure material, lack of toxicity, and their ions possess the stabilizing effect on protein structure and bioactivity [15]. All these characteristics have made ammonium sulphate a popular choice for use in protein precipitation [16]. As such, LBF formed using 250 g/L of $(\text{NH}_4)_2\text{SO}_4$ and 100% ethanol was selected for the next experiment.

3.3. Effect of Concentration of Alcohol on Protein Recovery and Separation Efficiency

While keeping the other variables constant, the relative effectiveness of salting-out at different concentrations of ethanol was investigated in this study. It is stated that a high extraction yield can be obtained by using ethanol-water compared to pure ethanol [17]. In this research, the influence of ethanol-water with several different concentrations and pure ethanol on the protein extraction was examined. The result obtained is similar to the study done by the Machado group in which they discovered greater extraction yields of blackberry residues attained by applying pressurized liquid extraction when ethanol-water was utilized compared to pure ethanol [18].

Based on Figure 2c, the results show that the protein separation efficiency increased from 80% to 90% of ethanol concentration and reached an optimum level of 82.33% at 90% ethanol concentration. However, the separation efficiency started to show a decreasing trend with ethanol concentration higher than 90%. In the case of the recovery yield, the highest yield obtained was 43.19% in LBF containing 80% ethanol, followed by a 40.2% recovery yield at 90% ethanol. The findings indicate that there was no correlation relationship between the two variables of separation efficiency and recovery yield in the same system. The addition of water to the organic solvent in this case ethanol possibly created a relatively polar medium that facilitated the extraction of protein [18]. In this study, LBF formed using 250 g/L of $(\text{NH}_4)_2\text{SO}_4$ and 90% ethanol was identified as being the most proficient at protein separation and was thus chosen for further optimization.

3.4. Effect of Salt Concentration on Protein Recovery and Separation Efficiency

The concentration of salt in the liquid biphasic system is another important factor to consider because different salts interact differently with the protein, water, and other chemicals. The effects of the concentration of salt on protein recovery and separation are well documented. The presence of salt in the solution will impact the surface tension of water, which will then increase the hydrophobic interaction between the protein and water [19]. Following this change, the targeted protein will shift to or from the aqueous phase depending on the nature of the protein [20]. For this work, the ammonium sulphate salt concentration was varied from 150 g/L to 350 g/L with 50 g/L increments. The alcohol content was set at 90%, while the mass of leaves used was 300 mg. The flotation time for this experiment was set at 10 min.

One important observation for this experiment is that the volume of top and bottom phases changed as the concentration of salt increased. The volume of the two phases reached a plateau when the concentration of salt reached 300 g/L and above. Another important observation to note is that at a low salt concentration (150 g/L), the two phases did not form. This suggests that the lower boundary for salt concentration that allows for two-phase formation is higher than 150 g/L. This is because an increase in salt or alcohol concentration in an alcohol/salt system could increase the tie-line-length (TLL), which could facilitate phase separation [21].

A plot of the effect of salt concentration on the recovery yield and separation efficiency is shown in Figure 2d. From Figure 2d, it is seen that the recovery yield and separation efficiency decreased in tandem with the increase in salt concentration. At a concentration of 200 g/L, the highest recovery yield of 40.69% and separation efficiency of 87.81% was recorded. As the concentration of salt increased, however, the recovery yield and separation efficiency gradually decreased. As the concentration of salt increased, the solubility of protein decreased. This is commonly known as the salting-out effect. Since different proteins salt-out at different salt concentrations, the effect can help us to determine the upper boundary of salt concentration for the LBF system. A higher salt concentration results in a

higher salting-out effect. This could then lead to a higher protein partition coefficient K_e [21]. A high K_e would result in a low yield.

At concentrations of 300 g/L and 350 g/L, however, there was a slight increase in separation efficiency compared to a decrease in recovery yield. There was a sharp decrease in both separation efficiency and recovery yield at a concentration of 300 g/L. This could be the concentration at which the salting-out effect occurred. As the concentration of salt increased, it caused more water to enter the bottom phase. This way, the protein was then impelled to the top phase [21], which caused the slight increase in the recovery yield at a concentration of 350 g/L. Therefore, we can conclude that the optimum salt concentration for the extraction of protein from kesum leaf was between 150 g/L and 300 g/L. The concentration of 200 g/L was then used in the experiments with other parameters in this work.

3.5. Effect of the Kesum Leaf Biomass Amount on the Protein Recovery and Separation Efficiency

The influence of kesum leaf biomass, or the mass of protein source, is another important factor to consider. Generally, increasing the concentration of protein sources can have a profound effect on the performance of phase separation due to the specific partition behavior of the target protein [22]. For this work, the concentration of salt was set to 200 g/L, ethanol concentration was set to 90%, and the flotation time was set to 10 min. The mass of kesum leaves was varied between 100 mg and 400 mg with 100 mg increments.

From Figure 3, it is seen that as the mass of leaves increased, the yield decreased. There was a significant drop in yield (over 40%) when the mass of leaves increased from 100 mg to 200 mg, and the drop continued gradually as the amount of leaves continued to increase. For the separation efficiency, the highest efficiency occurred at 300 mg, which then dropped to its lowest point at 400 mg. It was interesting to see that the yield decreased as the mass of leaves increased. In general, increasing the biomass concentration of the LBF also increases the number of contaminants and impurities in the system, thereby reducing the performance of the LBF separation [23]. In addition, a higher biomass content also increases the precipitation at the interface of the two phases, which could adversely affect the yield [23].

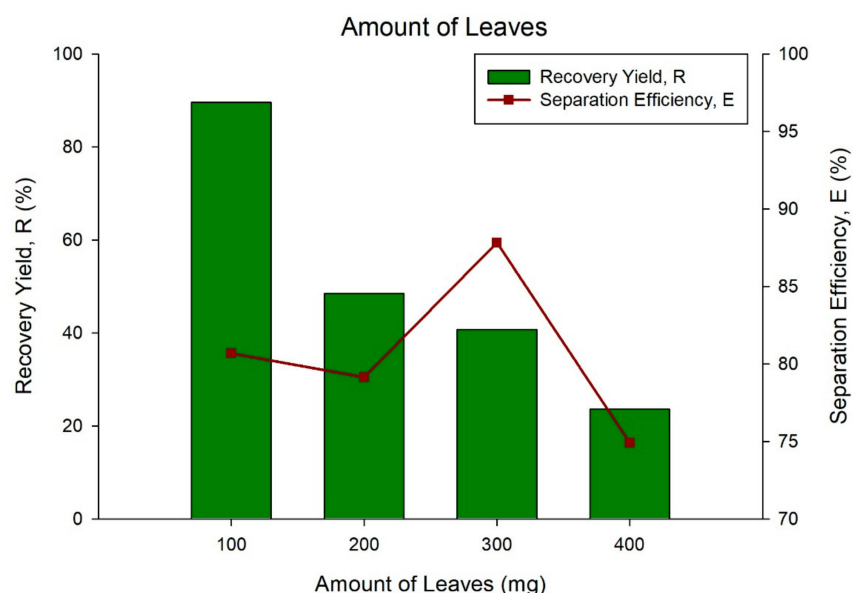


Figure 3. Effect of the kesum biomass amount on the protein recovery yield and separation efficiency.

Based on the definition highlighted in the materials section, separation efficiency is highly dependent on the protein activity in the bottom phase after the LBF process. It determines the concentration of protein extracted and this represents the efficiency of the system in extracting the

protein. As for the recovery yield, it represents the total amount of protein being recovered in the top phase. At 300 mg, the high separation efficiency was obtained with 87.81%; however, the protein recovery yield was low with only 40.69%. The low recovery yield was possibly due to high impurities present in the top phase. Several possibilities contributed to the low recovery yield: (1) protein retrieved in the upper phase was low (CT), or (2) the low phase volume of the top phase (VT) at 300 mg. These could be caused by the decreasing LBF performance as the level of impurities increased due to the increase in kesum leaf mass. Due to the high recovery yield value (89.58%) and separation efficiency of 80.68%, a 100 mg kesum leaves mass was used for the next step of this experiment.

3.6. Influence of pH on the Recovery Yield and Separation Efficiency

The pH value of an LBF system affects the separation outcome by altering the surface properties of the target protein, including the surface net charge, molecular shape, surface hydrophobicity, and the presence of specific binding sites [24]. A simple example is the case of a biomolecule with both polar and non-polar groups that experiences changes in its net charge and surface properties with changing pH values [25]. For this work, the pH of the system was varied from pH 4 to pH 8.

From Figure 4, it is seen that despite the high absorbance at pH 5, the highest recovery yield and separation efficiency occurred at pH 6. The lowest yield and E, on the other hand, occurred at pH 7. This suggests that for the extraction of biomolecules from kesum leaves, the LBF system should be kept in an acidic condition. Both the recovery yield and separation efficiency did not fluctuate much as the pH of the system increased. An interesting observation is that there was no formation of two-phases at pH 8. This experiment shows that as the pH approached basic pH, the hydrophobicity of the system was impacted to the point where it induced a salt-out. In order to prevent a salt-out, the pH of the system should be kept below the neutral level. The optimized pH condition that gave the maximum separation efficiency and recovery yield was at pH 6 with 87.19% and 96.37%, respectively.

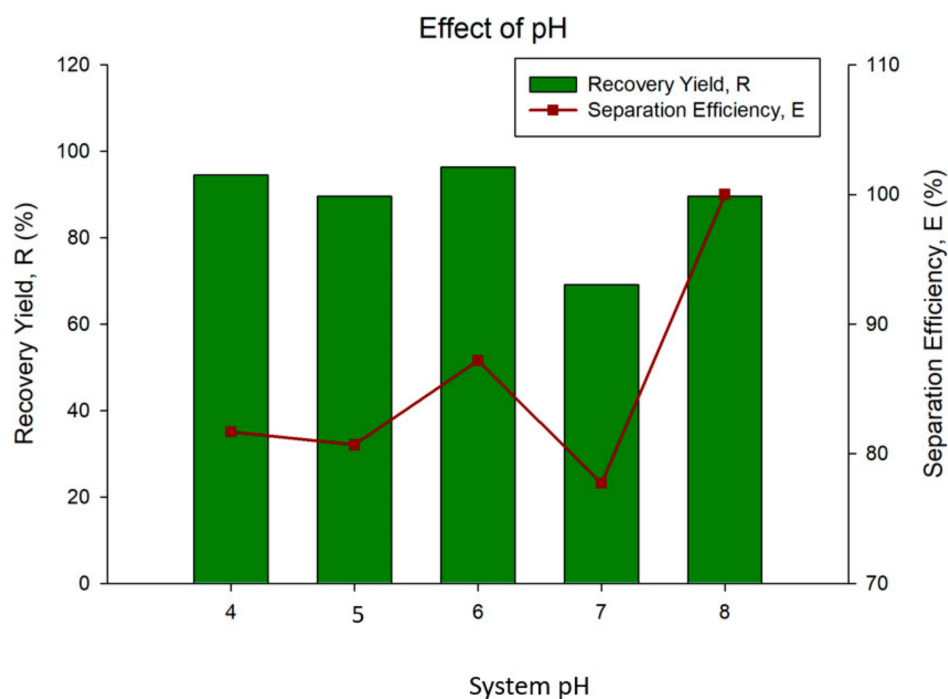


Figure 4. Effect of pH on the protein recovery yield and separation efficiency.

3.7. The Influence of the Flotation Time on the Recovery Yield and Separation Efficiency

The effect of flotation time is one of the most important factors that could affect the LBF recovery yield and separation efficiency. The flotation time affects the outcome of the process by influencing

the area of the air–water interface per unit volume of aqueous solution over time [26]. For this part, the concentration of salt was set to 200 g/L, the pH of the system was maintained at pH 5.0, the ethanol concentration was maintained at 90%, and the mass of kesum leaves consumed was 100 mg. The flotation time was varied from 5 min to 15 min with 2.5 min increments. The results of the experiment are provided in Figure 5.

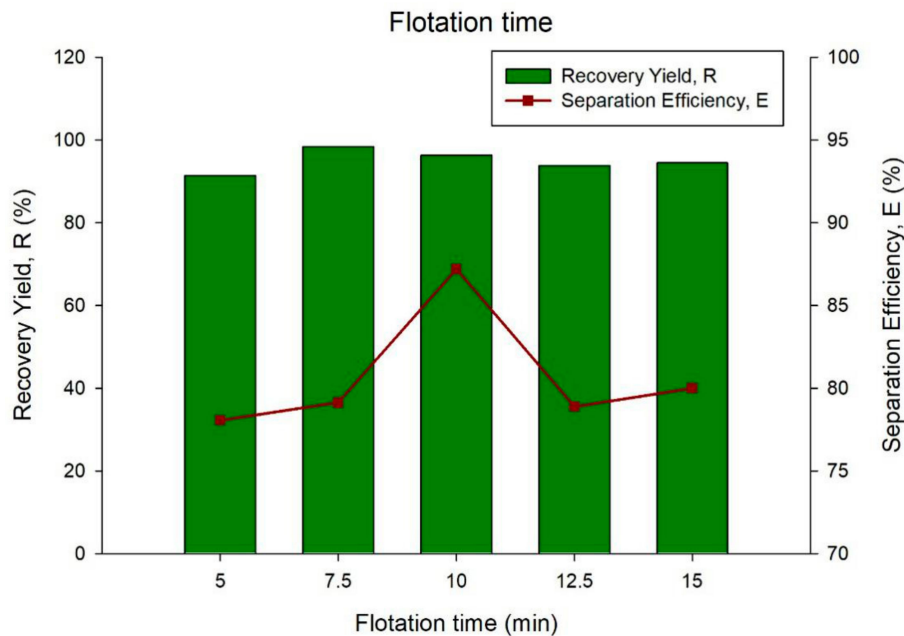


Figure 5. Effect of the the flotation time on the protein recovery yield and separation efficiency.

The resulting yield and separation efficiency, as shown in Figure 5, shows that the flotation time of 7.5 min gave the highest recovery yield with 98.36%, while the flotation time of 5 min gave the lowest recovery yield of 91.42%. For the separation efficiency, the highest occurred at a 10 min flotation time with 87.19% but at 7.5 min, 79.12% was recorded. One possible explanation for this phenomenon is that longer flotation time allowed for the accumulation and build-up of biomolecules in the LBF phases with the movement of gas bubbles [25]. As the flotation time increased, the concentration of targeted biomolecules in the top and bottom phases changed based on the kinetic processes. This explains the general trend of the separation efficiency, which showed a positive gradient, i.e., increasing with increasing flotation time. However, as the flotation time continued to increase, the level of impurity carried upward by the gas bubbles also increased, which then affected the separation performance of the LBF system.

According to Iqbal et al., the flotation force is highly dependent on the flow properties of the phases [27]. As the flotation time increases, the concentration of targeted biomolecules in the top and bottom phases changes based on the kinetic processes [25]. Based on Figure 5, as the flotation time increased, the yield was reduced. From this study, although 10 min gave the highest separation efficiency, 7.5 min was selected as an optimized condition because the focus of this study was to obtain a high protein recovery yield. Additionally, a long flotation time requires high energy consumption, which is costly, non-environmentally friendly, and it is not suitable for large-scale processes.

3.8. Large-Scale Protein Extraction Using the LBF System

In this section, a scale-up study of the protein extraction using the optimized conditions were assessed. By using the operating conditions that were optimized previously, a large-scale study was conducted. This assessment was performed to corroborate the consistency and efficiency of the LBF technique on a large scale. In the large-scale study, it was discovered that the amounts of both top

organic phase and bottom phases increased ten-fold compared with the small-scale experiment. A total of 300 mL of working volume with 150 mL of bottom phase and 150 mL of top phase were employed. Following the results achieved from Table 1, it can be seen that a comparatively higher recovery yield and separation efficiency of 99.44% and 93.28%, respectively, were obtained. Many reported studies have shown that the separation efficiencies of LBF for the recovery of different kind of biomolecules in the comparative study were between 85%–98.5%, which were much higher than SS, which achieved separation efficiencies of 48%–70%. LBF is preferable compared for this case as the separation efficiency achieved was more than 90%.

Table 1. Comparison between small- and large-scale LBF systems for the protein extraction of *P. tenulla* leaf.

| LBF System | Recovery Yield (%) | Separation Efficiency (%) |
|-------------|--------------------|---------------------------|
| Small scale | 98.36 | 79.12 |
| Large scale | 99.44 | 93.28 |

Based on the results achieved, the large-scale version of the LBF system was validated for its reliability, which is beneficial for the extraction of other biomolecules on an industrial scale. Other studies that demonstrated that LBF can be an alternative technology that can be utilized in industries for the extraction of various medicinal components include ortho-phenylphenol [28], puerarin [29], antioxidant peptides from trypsin hydrolysates of whey protein [30], baicalin [31], lipase enzyme [32], C-phycoyanin [33], and betacyanins [34]. All these studies have proven that the separation efficiency and recovery of the biocomponents were enhanced with the utilization of LBF system as their extraction method.

4. Conclusions

The findings from this study revealed that a high protein recovery and separation efficiency can be obtained using this LBF approach. Based on the experiment conducted, the optimized conditions for highest protein recovery and separation efficiency were 90% ethanol, 200g/L of ammonium sulphate, 100 mg of kesum leaf biomass, pH 6, and a flotation time of 7.5 min. The highest protein recovery yield achieved was 98.36% and the separation efficiency was 79.12% for the small-scale system. The study on the large-scale LBF system demonstrated the reliability and consistency of the system in which a recovery yield and separation efficiency of 99.44% and 93.28%, respectively, were attained. The application of the LBF system for protein extraction from herbal leaves involves a simple procedure, short processing time, and cost-effective process with a high recovery yield. This study shows the significance of LBF in downstream processing, especially for the extraction of value-added biomolecules. LBF can be an alternative technology that can be utilized in industries for the extraction of various medicinal leaf extracts. This LBF system is beneficial in the pharmaceutical and nutraceutical industries for the improvement of overall production and biotechnology fields.

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