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EXTRACTION OF LECTIN FROM LITCHI CHINESIS SONN SEEDS VIA LIQUID BIPHASIC FLOATATION

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Abstract

The purification of lectin from *Litchi chinesis Sonn* seed extract was carried out using Liquid Biphasic Floatation, which is an integration of solvent sublation into an aqueous two-phase extraction approach. This study aims to investigate the effects of floatation time and polymer-to-salt ratio on the protein separation efficiency. The results show that that at a moderate PEG: salt ratio of 5:5 at 5-minute flotation time showed the best results at 55% efficiency and a recovery yield of 48%. Using Minitab, it is found that floatation time has the most significant effect on top phase protein concentration, efficiency (%) and recovery yield (%), while the volume of PEG has only significant effect on recovery yield (%). Using a response optimization tool, it was found that the ideal conditions for maximizing the overall system performance is by using a PEG volume of 5.4 mL and a floatation time of 5 minutes.

Keywords: Biphasic system, Extraction, Lectin, *Litchi chinesis Sonn*, Protein.

1. Introduction

Litchi chinesis Sonn belongs to the soapberry family (*Sapindaceae*) and are cultivated in the tropical and sub-tropical regions of the world, where China leads the world in production followed by Thailand, India, and Vietnam [1]. The processing of *Litchi chinesis Sonn* generates 30-40% of byproducts which are usually thrown away as waste by the industry and is estimated that the global production of *Litchi chinesis Sonn* is at ~2.7 million tons which means that ~0.54 million tons of seeds are being produced each year [2]. There are uses of the seed as the nutritional content of the includes phenolic compounds, bioactive and starchy polysaccharides (40.7%), protein (4.93%), oil content (3.2%) and minerals [3]. Despite its low protein content, its specific lectins have been reported for its usage as anti-viral, anti-inflammatory, pro-inflammatory, and anti-tumour agent which makes it a high-value application.

Liquid biphasic flotation (LBF) is an advanced separation technique that integrates aqueous two-phase systems (ATPS) with solvent sublation (SS) to streamline the recovery of biological components, effectively combining concentration, separation, and extraction into a single step. This integration achieves a higher concentration coefficient by focusing surface-active components within a two-phase system [4]. The principle behind this system is based on the adsorption of surface-active components onto the surfaces of air bubbles within a rising gas stream in a salt solution.

The process relies on the adsorption of these components onto air bubbles within a gas stream in a salt solution. As bubbles ascend, they transport surface-active compounds to the hydrophilic, alcohol-rich top phase. This interaction with the salt solution results in the formation of a distinct two-phase system. The adhered surface-active compounds concentrate in the top phase [5].

In this technique, two immiscible aqueous phases form by mixing either an alcohol or polymer with a salt solution, surpassing specific concentration thresholds. LBF offers high biocompatibility, which enhances protein partitioning and separation, ensuring greater selectivity and minimal protein loss. Generally, LBF systems consist of either two polymers or a polymer-salt combination [6]. Figure 1 shows an illustration of the set-up of LBF.

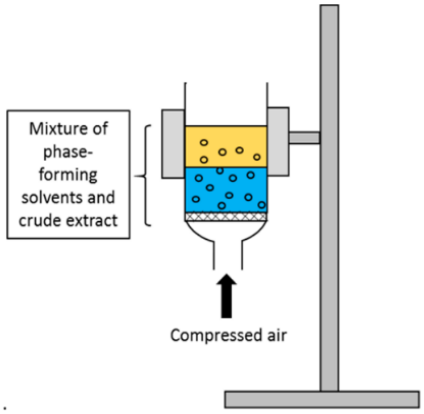

Fig. 1. Setup for the LBF system.

Several factors can influence the effectiveness of LBF, including the polymer-to-salt ratio and the floatation time, which both affect the yield and separation efficiency of the target biomolecule [7]. From a study conducted by Yusree et al., 2022 on recovery of β-amylase from slurry of sweet potato, the biphasic system was made up from polyethene-glycol (PEG) and sodium citrate [8]. Based on the study found, the most optimal values for the highest recovery yield (78.24%) and 3.9608 selectivity, are the factors of 60% citrate concentration with 60% polymer concentration.

Another study in which the LBF was also made up from PEG and citrate salts demonstrated the extraction of α-Lactalbumin from whey, it achieved a separation efficiency of 87.54% and a purification fold of 5.33 [9]. It is proven that the advantages of using innovative bubble-assisted technology over normal liquid-liquid extraction, offering cheap processing costs, fast processing, and high separation yields. Thus, in this study the main objective will be focused on evaluating the efficiency of lectin extraction from *Litchi chinesis Sonn* seeds using the Liquid Biphasic Floatation, LFF system. Specifically, the effects of floatation time, and polymer-to-salt ratios on the lection yield are the parameters that will be studied and optimized.

2. Materials and Methods

2.1. Materials

Polyethylene glycol (PEG) 10000, trisodium citrate (Na3C6H5O7) were found in the Taylor's University labs. Bradford reagent was purchased from Evergreen Engineering and Resources (Selangor, Malaysia). Litchi chinesis Sonn seeds was purchased from Cheng Woh Medical Hall (Penang, Malaysia).

2.2. Preparation of Litchi chinesis Sonn seed extract slurry

The *Litchi chinesis Sonn* seed were first placed in a plastic bag and crushed into smaller pieces before being grounded into fine powder using a mechanical grinder, this would increase the surface area which will maximize its contact and interaction with the solvents during the process of LBF extraction. The grounded *Litchi chinesis Sonn* seed powder then underwent ethanol pretreatment, which would increase the stability or "shelf life" of the extract. Exposure to air can lead to moisture loss and enzymatic degradation, which reduces the nutritional quality of the extract due to nutrient breakdown and the activity of anti-nutritional compounds [10, 11]. Ethanol pretreatment would reduce the presence of any interfering compounds such as lipids, polyphenols and pigments, and reduce the presence of endogenous enzymes.

The pretreatment method was adapted from work previous studies [12]. The seed powder was soaked in 70% (v/v) ethanol at a solid-solvent ratio of 1:10 (w/v), meaning that each gram of the *Litchi chinesis Sonn* seed extract, 10 ml of ethanol solution is used. The soaking is performed under constant agitation using a magnetic stirrer at room temperature for an hour. This would allow for the efficient removal of soluble impurities and the partial defatting of the seed extract.

After the soaking period, the samples are filtered using vacuum filtration which will remove the excess ethanol, followed by oven drying at low temperatures (50°C). Then, the *Litchi chinesis Sonn* seed powder extract was blended with distilled water at a 1:1 (w/w) ratio, and the resulting mixture was allowed to be settled in the chiller at a temperature of 4°C. The supernatant was then collected to obtain a " *Litchi chinesis Sonn* seed slurry" suitable for LBF processing.

2.3. Liquid biphasic floatation method

The LBF apparatus was adapted from the work done from previous studies [13, 14]. The LBF system set up is shown at Fig. 1 and a two-phase separation after 24 hours setting is shown in Fig. 2. The system uses a cylindrical separating column which have a total volume of 80 cm3 and is secured tightly using a retort stand. The system also includes an air pump which consistently pumps out 3 L/mins which will introduce bubbles into the mixture. The usage of air to form bubbles is critical in LBF as it enhances mass transfer and promote effective phase separation. Rising air bubbles help to carry target biomolecules such as lectins, which would either be pushed towards the top polymer phase or away from the interfering compounds. The bubbles flows would create agitation and increased interfacial surface area between the phases, which accelerates the contact between biomolecules and the extraction medium, which would improve the recovery and selectivity. Additionally, air is a cost-effective and easily available gas, which makes it suitable for sustainable and scalable extraction systems. The materials are measured using a precision electronic weighing, to ensure accuracy and consistency.

Fig. 2. A two-phase separation after 24 hours setting.

2.4. Preparation of polymer mixture

To prepare the polymer mixture, it was first decided that 50% w/w of PEG 10000 is used. 25g of PEG 10000 was weighed followed by the addition of 25 ml of distilled water. The mixture is stirred using a hotplate stirrer due to the viscosity of the solution making it difficult to stir, with the settings at 55°C and 550 rpm.

2.5. Preparation of salt mixture

To prepare the polymer mixture, 50% w/w of trisodium citrate was used. 25g of the slat was weighed followed by the addition of 25ml of distilled water. The mixture is stirred using a hotplate stirrer with the settings at 50°C and 500 rpm.

2.6. Analytical procedures

To quantify the protein calibration in the Litchi chinesis Sonn seed extract obtained from the LBF system, a calibration curve was constructed using Bovine Serum

Albumin (BSA) as the standard protein. BSA is chosen as it has a good response in calorimetric protein assays and thus is regularly used as a reference standard [15]. A stock solution (10 mg/ml) was prepared at a concentration at 10 mg/mL by dissolving an appropriate amount of BSA powder in distilled water. Using the stock solution, serial dilutions was done to create concentrations of 2, 4, 6, 8 and 10 mg/ml, with distilled water as the blank (0 mg/mL). Each diluted solution was then mixed with Bradford reagent as the assay protocol and allowed to react under room temperature for 10 minutes. The absorbance of the solutions is then measured at 595 nm using a UV spectrophotometer. The resulting absorbance will be plotted against the known BSA concentrations which generates the calibration curve as shown in Fig. 3. A linear regression was applied into the figure, which would yield the following Eq. (1):

$$y = 0.0879x + 0.0325 \ (R2 = 0.9976) \quad (1)$$

The y represents the absorbance, and the x is the protein concentration (mg/mL). The high coefficient of R2 confirms the linearity and reliability of the standard curve over the tested range. The calibration curve was used to determine the protein concentration on the top and bottom phases of the LBF system. The absorbance of each system was measured, and its corresponding protein concentration was calculated by interpolating the value from the standard curve.

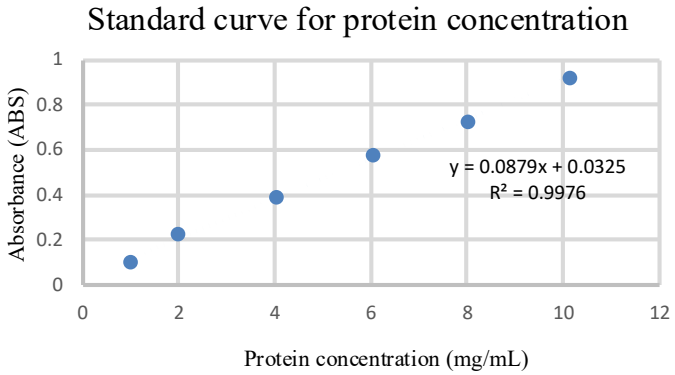

Fig. 3. The standard curve for protein concentration.

The concentration of lectin in the top and bottom phases of the LBF system were determined using the Bradford protein assay. Although Bradford reagent is a general protein assay, it can be used as an approximation method for the total protein quantification, under the assumption that much of the protein recovered was lectin. This assumption is supported by the solubility of lectins is at pH 7.2 which matches the pH of the LBF system used in this study [16]. The absorbance was measured at 595 nm after incubation with Bradford reagent, and the protein concentration were interpolated from the standard BSA calibration curve.

Next, Eqs. (2) to (5) which are used to calculate the separation efficiency and the recovery yield [14, 17].

$$E\% = \frac{Protein\ in\ top\ phase\ \left(\frac{mg}{ml}\right)}{Protein\ in\ feedstock\ \left(\frac{mg}{ml}\right)} \times 100\% \quad (2)$$

The partition coefficient (K) was calculated using Eq. (3), where L_t and L_b are the concentration of lectin (mg/ml) in the top and bottom phases, respectively.

$$K = \frac{L_t}{L_b} \tag{3}$$

The volume ratio (V_r) is the ratio between the volumes of the top and bottom phases which can be calculated using Eq. (4).

$$V_r = \frac{V_t}{V_b} \tag{4}$$

The recovery yield (RC, %) of the lectin from the sample is calculated using Eq. (5).

$$RC = \frac{K \times V_r}{1 + K \times V_r} \tag{5}$$

The statical model was obtained and done through Minitab Statistical Software 22 by Minitab, LLC.

2.7. Spectrophotometer, Bradford reagent, sample preparation and the dilution ratio

As mentioned in Section 2.6, to determine the protein concentration in both the feedstock and the separated phases of the Liquid Biphasic Floatation (LBF) system, the Bradford protein assay was used in combination with a UV-Vis spectrophotometer which is set at 595 nm. The method was selected as it has a rapid response, sensitivity, and compatibility with small volume samples. Bradford reagent relies on the blinding of the Coomasie Brilliant Blue G-250 dye to primarily arginine and other basic amino acids residue of proteins [15]. Upon binding, the dye would undergo a shift in absorbance from 465 mm (reddish form) to 595 nm (blue complex), which would be quantitatively measured.

To ensure the accuracy and comparability across all samples, a consistent dilution protocol was applied to all test solutions, including the feedstock, top phase and bottom phase. Each sample was prepared using 0.05 ml of the sample, 0.2 ml of distilled water, and 2.5 ml of Bradford reagent. This would result in a total dilution factor of 55x, which would be applied in the final concentration calculation by back multiplying the interpolated protein value which is obtained from the protein curve. The method was adopted from previous studies [18].

All absorbance measurements are blanked against a reference solution consisting of distilled water and Bradford reagent. Although PEG and citrate salts in the LBF system could potentially influence the Bradford absorbance, the LBF was pre-diluted with distilled water prior to analysis, which can significantly reduce the concentration of the interfering substances. Therefore, the usage of distilled water and Bradford reagent as the blank can be deemed as acceptable, and unlikely to introduce any bias in the protein quantification.

3. Results and Discussion

3.1. Effect of the ratio of polymer: Salt

Changing the ratio of polymer and salt can easily influence the phase composition and separation efficiency of the biphasic system. These variations will significantly affect the key physiochemical properties such as interfacial tensions, density, and

viscosity. Therefore, this variation will cause the partition behaviour of solutes between the top and bottom phases would be altered. A higher salt proportion relatively to polymers can enhance phase separation through a stronger salting-out effects, which would promote a more distinct boundary. Conversely, a higher polymer concentration can lead to increased viscosity and modify the polarity of the top phase. It is essential that the optimization of the ratio of polymer-to-salt is critical for maximizing extraction efficiency and selectivity in LBF system.

In this case, based on Table 1, a lower PEG: salt ratio (2.5:7.5) would result in increased protein solubility in the bottom phase, which is likely due to the stronger salting-out effect, therefore would also result in a reduction in the recovery. Conversely, a higher PEG: salt ratio (7.5:5.5) would favour protein partitioning into the top PEG-rich phase, as it achieved the highest recovery yield at floatation time of 5 minutes (up to 80.6%), although with slightly lower efficiency than the balanced 5:5 ratio. This suggests that increasing the PEG volume can enhance the protein mitigation to the top phase, which is consistent with reports of PEG known affinity for hydrophilic proteins such as lectins.

Also, in addition to influencing protein partitioning behaviour, the ratio of PEG and salt also affects the physical volume distribution of the two resulting phases after separation. This is important to consider as shown in Eqs. (3) to (5), the recovery yield (RC%) is dependent on both the partition coefficient (K) and the volume ratio (Vr). Therefore, a higher PEG volume would result in a larger top phase and a smaller bottom phase, which would result in an increase of the volume ratio.

Due to this effect, this can boost the recovery yield, even when the partition coefficient remains the same, as the larger top phase would allow for more of the protein mass to be collected in that phase. While, a smaller ratio would result in a smaller top phase and a larger bottom phase, thus decreasing the volume ratio, in turn would limit the maximum recovery, even if the top phase is more concentrated. In this study, due to physical restraints, the Vr was assumed based on the initial mixing volumes of PEG and citrate salt, which was a similar approach taken in previous studies [19].

3.2. Effect of floatation time

Air floatation time plays a crucial role in the efficiency of the LBF system, as it influences the air-water interfacial area per unit volume of the aqueous phase over time [20]. Not only that, but the sizes of bubbles also play in a role where it can directly affect the interfacial area available for gas-liquid mass transfer. Usually, smaller bubbles would merge into larger ones to minimize the interfacial tension [21]. Based on Table 1, based on the trend floatation time from 5 minutes to 10/15 minutes caused a drop from the top phase protein by 50~%, while also the efficiency and recovery yield also decreased significantly.

Therefore, a floatation time of 5 minutes held the greatest yield in the top phase protein concentration and recovery yield, and prolonging the flotation beyond 10 minutes causes a decrease in the efficiency, possibly due to bubble instability, protein redistribution into the bottom phase, or partial protein denaturation [22].

However, there is also the possibility of overly intense bubbling, as high-speed air flow can generate mechanical shear, which unfolds or denatures sensitive proteins, especially lectin which depends on their tertiary structure for solubility and bioactivity, and also cause disruption of stable phase boundaries as excessive

bubbling would disrupt the interface between the top and bottom phases, ultimately causing poor separation, emulsion formation which would lead to proteins becoming trapped between the phases causing lower recovery [23].

Table 1. Full factorial design of ratio of PEG: salt and floatation time with the responses obtained.

Volume of PEG (ml)	Volume of salt (ml)	Floatation time (minutes)	Top phase protein conc. (mg/mL)	Bottom phase protein conc (mg/mL)	Recovery Yield (%)
2.5	7.5	5	164.25	154.24	21.03
2.5	7.5	5	139.22	84.16	29.26
5	5	5	174.89	191.78	32.91
5	5	5	181.77	104.81	48.27
7.5	2.5	5	178.01	42.86	80.6
7.5	2.5	5	128.58	74.15	63.43
2.5	7.5	10	72.27	198.04	8.36
2.5	7.5	10	75.4	119.82	13.59
5	5	10	75.4	121.08	25.1
5	5	10	63.51	122.95	21.75
7.5	2.5	10	81.03	189.28	29.98
7.5	2.5	10	68.52	104.81	39.53
2.5	7.5	15	81.66	217.43	8.58
2.5	7.5	15	66.01	88.54	15.71
5	5	15	126.08	89.79	43.03
5	5	15	87.29	122.33	27.74
7.5	2.5	15	74.77	190.53	28.18
7.5	2.5	15	44.74	120.45	27.08

3.3. Effect of volume of PEG and floatation time on top phase protein concentration

The results suggested in Table 2, the floatation time is the most effective factor with its large negative coefficient (-40.51) and P = 0, which shows that when time increases, the top phase protein concentration significantly drops, as mentioned in Section 3.3, this might be due to the protein settings, bubbling collapse, or redistribution to the bottom phase. While the volume of PEG has no statically significant effect by itself, as suggested at its low coefficient (-1.93) and high P-value (0.735), this means that its impact on protein concentration in the top phase appears inconsistent. As for the quadratic terms, the floatation time2 shows that its significant and positive through its coefficient value of 47.92 and P-value of 0, which could indicate that while longer time would reduce the protein concentration at first, longer duration of floatation time can cause stability and promote recovery on the top phase concentration.

PEG2 shows a negative coefficient of -20.28 and a P-value of 0.057, which suggests that a very low or high PEG volume can cause a reduction in the top phase protein concentration, and that an optimum would be in the middle. The strength of model is based on the R2 of 87.29%, which means that the model explains 87.3% of the variability in the top phase protein concentration, and the adjusted R2 of 82% means that after adjusting for the number of predictors, the model fits the data well, which conclusively highlighted flotation time as a key optimization parameter in the LBF system. The regression analysis shows that the top phase protein concentration, by the liquid biphasic system can be described in Eq. (6).

$$\text{Top phase protein concentration} = 266 + 34.8\,(\text{Vol of PEG}) - 44.87\,(\text{floatation time}) - 3.25\,(\text{Vol of PEG})2 + 1.917\,(\text{floatation time})2 - 0.313\,(\text{Vol of PEG})\,(\text{floatation time}) \quad (6)$$

Table 2. ANOVA for top phase protein concentration.

Term	Coef	SE Coef	T-Value	P-Value
Constant	86.2	10.2	8.48	0.000
Volume of PEG	-1.93	5.57	-0.35	0.735
Floatation time (mins)	-40.51	5.57	-7.27	0.000
Vol of PEG*Vol of PEG	-20.28	9.65	-2.10	0.057
Float time*Float time	47.92	9.65	4.97	0.000
Vol of PEG*Float time	-3.91	6.82	-0.57	0.577

R^2 = 87.29%, R^2 (adjusted) = 82%

3.4. Effect of volume of PEG and floatation time

From Table 3, the ANOVA from the Minitab software shows that floatation time remains the most dominant variable with it having the highest coefficient at -12.46 and a P-Value of 0 which also shows its high significance. This matches with the experimental observation where protein redistribute at longer duration, which means less efficient separation. While the volume of PEG, similar to the observation at Section 3.4, with a coefficient and P-Value of -0.59 and 0.735 respectively, shows that it has a minimal linear effect.

However, the PEG^2 term shows a greater coefficient (14.74) and a lower P-Value (0.057), which suggests non-linear behaviour, which means that too little or too much PEG might both be suboptimal; balanced ratio would work better as explained in Section 3.2. The Time^2 term is significant and positive in terms of the P-Value (0) and the coefficient (14.74), which means that the efficiency would drop at moderate times but might slightly recover or stabilize at very long times (20-30 minutes). The regression analysis can be described in Eq. (7).

$$E\,(\%) = 81.8 + 10.7\,(\text{Vol of PEG} - 13.8\,(\text{floatation time}) - 0.998\,(\text{Vol of PEG}) + 0.59(\text{floatation time})2 - 0.096\,(\text{Vol of PEG})(\text{floatation time}) \quad \text{……...(7)}$$

Table 3. ANOVA for efficiency (%).

Term	Coef	SE Coef	T-Value	P-Value
Constant	26.52	3.13	8.48	0.000
Volume of PEG	-0.59	1.71	-0.35	0.735
Floatation time (minutes)	-12.46	1.71	-7.27	0.000
Vol of PEG*Vol of PEG	-6.24	2.97	-2.10	0.057
Float time*Float time	14.74	2.97	4.97	0.000
Vol of PEG*Float time	-1.20	2.10	-0.57	0.577

R^2 = 87.29%, R^2 (adjusted) = 82%

The contour plot shown in Fig. 4 shows that efficiency (%) is highest (>50%) when floatation time is short (5 minutes) and the PEG volume is at the mid ranges of 4-6 mL. The contour plot supports the ANOVA result which suggests that the floatation time has the strongest influence in efficiency (%). Efficiency declines rapidly when the floatation time increases, as moving upwards the Y-axis, it shows that turn colour turning from green to blue which in the legend shows that the efficiency is decreasing. A low ratio of PEG can cause a less pronounced

separation, which would reduce the partitioning of the desired biomolecules into the desired phase [24]. It can also cause an increase in contamination in the target phase due to less efficient separation and potential carryovers from the other phase. Therefore, the data shows that a balanced ratio is the most optimal for the process.

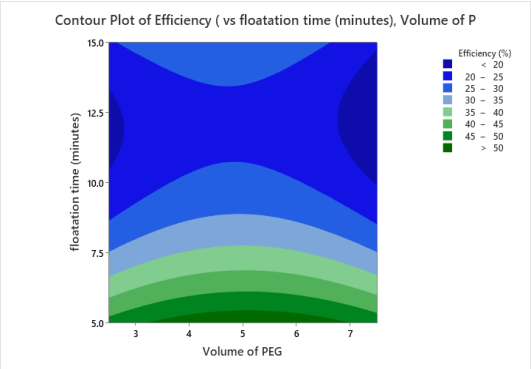

Fig. 4. Contour plot of efficiency (%) vs. floatation time (minutes) and volume of PEG.

3.5. Effect of PEG and floatation time on recovery yield

The most significant positive effect on the recovery yield is the volume of PEG which is at coefficient of 14.36 and P-Value of 0. As PEG volume increases, there is greater protein partitioning into the top phase which causes higher yield. These numbers reflect on the role of PEG in the phase separations and biomolecules affinity. The floatation time shows a negative coefficient of -10.43, and a P-Value of 0.001, which could mean that the longer floatation time reduces recovery, which could be due to settling, denaturation, or redistribution into the bottom phase.

As per the quadratic terms, for Time2, the high coefficient of 12.43 and P-Value of 0.015 shows the yield decreases with time initially but might rebound at a higher time (20-30 minutes), which is possibly due to repartitioning equilibrium [25]. The PEG*time interaction shows significant coefficient of 12.43 and P-Value of 0.026, which means the effect of floatation time depends on the PEG volume, and vice versa. In Tabl4 4, the model's R^2 value of 84.24% shows its suitability for predicting recovery yield within the experimental domain. The regression analysis can be described in Eq. (8).

$$\text{RC (\%)} = 24.6 + 16.32\ (\text{Vol of PEG}) - 8.9\ (\text{floatation time}) - 0.43\ (\text{Vol of PEG})2 + 0.497(\text{floatation time})2 - 0.628\ (\text{Vol of PEG})\ (\text{floatation time}) \quad (8)$$

Table 4. ANOVA for recovery yield (%).

Term	Coef	SE Coef	T-Value	P-Value
Constant	24.84	4.62	5.38	0.000
Volume of PEG	14.36	2.53	5.68	0.000
Floatation time (minutes)	-10.43	2.53	-4.13	0.001
Vol of PEG*Vol of PEG	-2.69	4.38	-0.61	0.551
Float time*Float time	12.43	4.38	2.84	0.015
Vol of PEG*Float time	-7.85	3.10	-2.53	0.026

R^2 = 84.24%, R^2 (adjusted) = 77.67%

3.6. Response optimization

Figure 5 represents a multi-response optimization plot based on the responsive factors like recovery yield (%) and efficiency (%). It helps to visualize the optimal levels of PEG volume and floatation time to simultaneously maximize multiple responses using the desirability functions. From Fig. 5, it shows that the ideal conditions for maximizing the overall system performance is by using a PEG volume of 5.4 mL and a floatation time of 5 minutes. These settings achieved a composite desirability of 0.6459, with high individual desirability scores for top phase protein concentration of 0.95 and efficiency at 0.95. The recovery yield peaked at 51.01% under these conditions, as the medium PEG: salt ratio of (5.4:4.6) would have a smaller volume ratio relative to a large PEG: salt ratio (e.g. 7.5:2.5). The figure also confirms the strong effect of floatation time and the importance of balancing PEG volume to optimize selective protein partitioning.

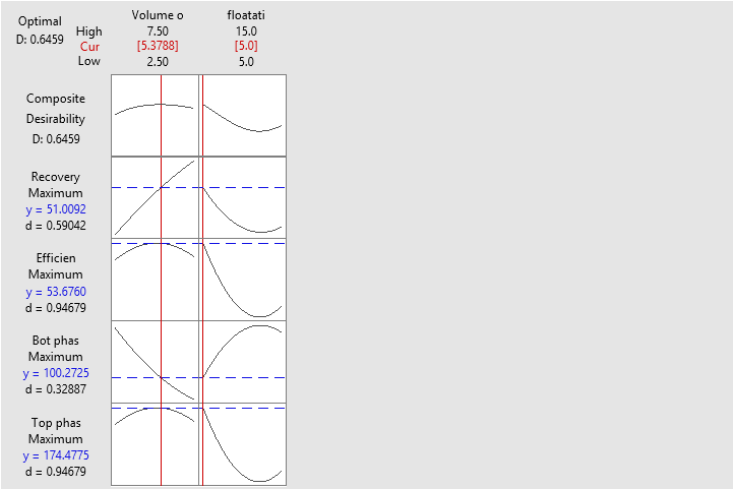

Fig. 5. Response optimization: Recovery Yield (%), Efficiency (%), Bot phase protein concentration, Top phase protein concentration.

3.7. Software limitations

Due to limitation of Minitab, the effect of salt on the flotation time and efficiency could not be studied as the volume of salt and polymer are both codependent of each other (volume of PEG = 10 mL – volume of salt) in this study, and Minitab treats both as independent variables of each other. The DOE method like factorial or response surface designs are not directly applicable in their standard forms. DOE is typically used to optimize a process by identifying the best settings for independent variables (factors) to achieve the desired responses (dependent variables).

3.8. Fourier transformation infrared spectroscopy (FTIR)

A FTIR analysis is conducted to characterize the functional groups which exists in the *Litchi chinesis Sonn* seed extract and to confirm the presence of protein-related bonds. A study by Sandt (2024) shows that FTIR micro spectroscopy can be used to identify pure proteins with high classification accuracy and specificity as well as detecting small differences between different purification

batches [26]. Characteristics absorption bands which are associated with protein identification includes amide I band (~1650 cm-1) which attributes to C=O stretching vibrations of the peptide bond and amide II band (~1540 cm-1) which relates to N-H bending and C-N stretching [27].

Based on Fig. 6, which shows the comparison of 2 different *Litchi chinesis Sonn* seeds sample of the FTIR at a 3-week period. The pretreated *Litchi chinesis Sonn* seed have a much higher peak intensities than the expired one, especially at ~3274 cm-1 which is likely an O-H or N-H stretch, ~1624 cm-1 which is an amide I band which attributes to C=O stretching in proteins, and ~1000-1150 cm-1 which relates to C-O, C-N, or polysaccharide-related peaks. Whereas the expired sample showed a much lower absorbance across the board, which may suggest low concentration of active compounds, degradation or oxidation and water loss/denaturation. However, the expired sample also showed new or shifted peaks at 1290 cm-1 that could indicate breakdown products. There is general signal weakening so there's possible chemical instability or loss of functional components.

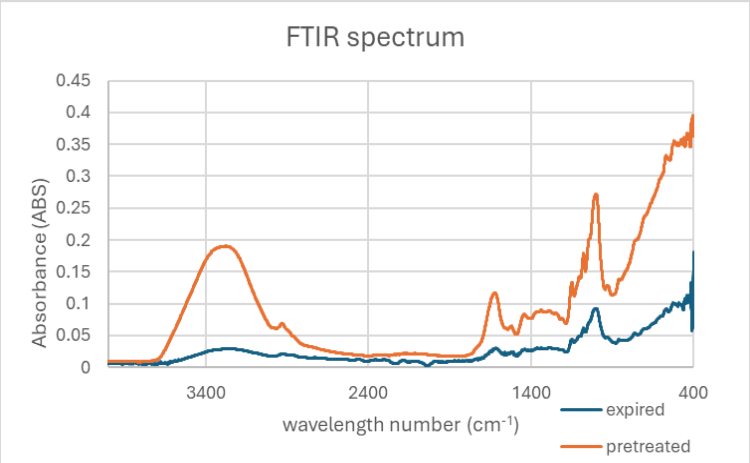

Fig. 6. FTIR spectrum of the expired seed sample and pretreated seed sample.

4. Conclusion

The purification of lectin from *Litchi chinesis Sonn* seeds were performed using LBF, incorporating solvent sublation into an aqueous two-phase system. The results show that that at a moderate PEG: salt ratio of 5:5 at 5-minute flotation time showed the best results at 55% efficiency and a recovery yield of 48%.

Using Minitab, it is found that floatation time has the most significant effect on top phase protein concentration, efficiency (%) and recovery yield (%), while the volume of PEG has only significant effect on recovery yield (%). Using a response optimization tool, it was found that the ideal conditions for maximizing the overall system performance is by using a PEG volume of 5.4 mL and a floatation time of 5 minutes.

For future studies, a consideration should be made to expand the design of the experiment to a full 3-factor level factorial which helps independently evaluate all interactions and quadratic effects, especially for volume of salt.

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