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ORIGINAL RESEARCH

Extraction and Determination of Protein from Edible Oil Using Aqueous Biphasic Systems of Ionic Liquids and Salts

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Abstract

This study aimed to develop the extraction method of protein from edible oil for rapid detection. Firstly, aqueous biphasic systems (ABS) based on six hydrophilic ionic liquids (ILs) and three salts were developed and the phase diagram was drawn by turbidimetric point method. The binodal curves were fitted to the Merchuk equation. On this basis, the ABS composed of IL and salt were applied to extract protein from edible oil. The type of IL or salt, IL concentration, salt concentration, oil mass, extraction pH, and temperature on the extraction efficiency of protein from oil were investigated. The results showed that the optimum conditions for the extraction of protein from edible oil with ABS were as follows: 50% (w/v) K_3PO_4, 20% (w/v) [Bmim]Cl at 35 ℃, and pH 9.0. Under the optimal conditions, the protein extraction efficiency was almost 100%. Also, the extraction mechanism was studied and the main driving factors of protein extraction may be the hydrophobicity, electrostatic interaction, and salting-out between molecules. Finally, the method was used to detect the commercial edible oils from different sources. The results showed that the ABS could also be used to extract protein from other edible oils. In conclusion, the IL-based ABS method is simple and rapid for protein extraction from edible oil, and will highlight novel possibilities in the large-scale separation and purification of protein from oily solution.

Keywords Edible oil · Aqueous biphasic system (ABS) · Ionic liquid (IL) · Protein

Introduction

Edible plant oil is a kind of oil made from the fruits or seeds of plants, such as camellia oil, olive oil, flaxseed oil, peanut oil, and rapeseed oil. Many methods were applied for obtaining edible vegetable oil, including squeezing method (Maugeri, 2009), organic solvent extraction (Li et al., 2009), water-enzyme extraction (Tirgarian et al., 2019), supercritical CO_2 extraction (Bettaieb Rebey et al., 2019; Palsikowski et al., 2019), and subcritical extraction(Gámiz-Gracia & Castro., 2000). No matter what method is used to prepare edible oil, there are residual proteins in the commercial edible oil. Attempts to quantify and characterize the residual proteins of oils indicate that crude oils contain about 100–300 mg/kg, whereas fully refined oils contain at least 100-fold less (Crevel et al., 2000). The presence of trace proteins does not affect the quality of the oil, but may be a risk to individuals who may be allergic to proteins (Wensing et al., 2002). On the other hand, many oil proteins have been extensively utilized as food supplements or emulsifiers owing to their important functional properties and good nutritional quality (Roselló-Soto et al., 2015; Yang et al., 2019). At present, with the in-depth study of extraction technology of edible oil, the protein in oil has also attracted more and more attention by scholars. For example, two protein fractions derived from the emulsion formed during aqueous extraction of camellia oil (Yang et al., 2019). Widyarani et al. (2014) developed biorefinery methods for separation of proteins and oil fractions from rubber seed kernel. Furthermore, some different protocols were reported to extract proteins from the oils, such as low-temperature acetone precipitation (Paschke et al., 2001), phosphate buffered saline (PBS) extraction (Errahali et al., 2002), bicarbonate extraction (Olszewski et al., 1998), borate extraction (Rigby et al., 2011), and cold acetone extraction (Li et al., 2013). However, it is still expected for more and

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more simple, efficient, and green methods using in protein extraction and enrichment from oil, which can provide researchers more new options.

Aqueous biphasic systems (ABS) are a promising new separation and purification technology in recent years (Chong et al., 2020), which consisting of one polymer and another aqueous solution of salts or two mutually insoluble polymers. Since both phases contain water and are incompatible with each other, they are called aqueous biphasic (Gutowski et al., 2003) and particularly suited as medium for the separation and purification of a diverse range of biomolecules (Raghavarao et al., 2003). The extraction mechanism is similar to that of water-organic solvent, which is based on the selective distribution of substances in two phases, but the properties of the extraction system are different (Zhao & Zhan, 2012). In 2002, Dupont et al. found that ionic liquids (ILs) could also form a biphasic system, with the advantages of both IL and the ABS. Compared with the traditional ABS, it has the advantages of low viscosity, short phase separation time, the IL can be recycled, and the extraction system will not emulsify like the polymer ABS (Pereira et al., 2013). At present, the system shows excellent performance in the fields of extraction and separation. For example, He et al. (2018) isolated bioactive ginseng saponins by using IL-aqueous biphasic. Konstantza (2012) extracted and separated polysaccharides by using ILs [C_4min]BF_4 and [C_6min]Cl formed ABS with K_2HPO_4. In addition, He et al. (2005) employed [C_4mim]Cl/K_2HPO_4 ABS to extract testosterone and epitestosterone. The separation and purification of proteins and amino acids by aqueous biphasic extraction have also been widely used. Pei et al. (2009) isolated cytochrome C, bovine serum protein, trypsin, and globulin using [C_4min]Br/K_2HPO_4 ABS. Belchior et al. (2020) studied the optimization of extraction of ovalbumin and lysozyme from tetraalkylammonium based ILs and K_2HPO_4/KH_2PO_4 or K_3PO_4 aqueous systems under different pH conditions. In addition, aromatic and aliphatic amino acids mixtures also can be effective separated by IL-based ABS (Capela et al., 2017). Up to now, lots of diverse proteins, such as BSA, cytochrome C, hemoglobin, lysozyme, myoglobin, pepsin, and trypsin (Dreyer et al., 2009; Lu et al., 2011; Pei et al., 2009), were extracted by IL-based ABS (IL-ABS). However, these extraction and separation methods are based on solids or organisms. Furthermore, in most IL-based ABS studies, hither to the protein in edible oil was not comprehensively investigated. Therefore, the extraction of protein from special raw material of edible oil with IL-based ABS is a new attempt because of its viscosity, insoluble in water, and so on. Also, to the best of our knowledge, there is no study on the effects of IL-ABS on extract of protein from edible oil. Thus, the aim of this study was to develop an IL-ABS method for protein extraction from edible oil and optimize the extraction method to obtain the maximum amount of protein extraction. In addition, the effects of ABS on the different sources of oil were also examined.

In this study, a series of ILs including 1-ethyl-3-methylimidazole bromine salt ([Emim]Br), 1-butyl-3-methylimidazole bromine salt ([Bmim]Br), 1-hexyl-3-methylimidazole bromine salt ([Hmim]Br), 1-ethyl-3-methylimidazole chloride ([Emim]Cl), 1-butyl-3-methylimidazole chloride ([Bmim]Cl), and 1-hexyl-3-methylimidazole chloride ([Hmim]Cl) were studied in this paper. Moreover, K_3PO_4/KH_2PO_4/K_3CO_3 buffer solution, which was relatively mild for protein extraction, was selected to form an ABS to extract the total protein in camellia oil, and its extraction conditions, extraction efficiency, and extraction mechanism were studied in detail. This extraction method can be applied to determine the protein content of edible oil simply and quickly and also provide some preliminary basis for the identification of edible oil on the market.

Experimental

Material and Reagent

All ILs (mass fraction purity ≥ 95%) and salts (K_3PO_4, KH_2PO_4, and K_2CO_3) were purchased from Aladdin Chemical Co. (Shanghai, China). The edible oil used in the preliminary phase diagram and other studies was extracted from camellia seed in our laboratory. Briefly, camellia seeds were dried to 6–8% moisture content, shelled, crushed, and then pressed by the Twin screw press machine. After that, camellia seed oil was obtained by filtered through a 60-mesh filter bag. Other edible oils from different sources are mostly bought at local supermarkets. All reagents and solvents were of analytical purity grade. The freshly ultra-purified water was prepared by a Millipore Direct-Q3 water system (Bedford, MA, USA) and used throughout the whole study.

Determination of the ABS Phase Diagrams

The turbidimetric point method (cloud point method) was used to draw the phase diagram (Pei et al., 2009; Requejo et al., 2020). The 0.5 g IL, 1 mL ultrapure water, and 0.5 g K_3PO_4/KH_2PO_4/K_2CO_3 were placed in a 10-mL graduated centrifuge tube in sequence. Then, the centrifuge tube was placed in a shaker at 200 rpm at 25 ℃ until the solution was stratified. After that, 25 μL of ultrapure water was successively added to the centrifuge tube until the two aqueous phases disappeared. The amount of each substance in the test tube is recorded when the two aqueous phases disappear. An equal amount of K_3PO_4/KH_2PO_4/K_2CO_3 is added to stratify the solution. After repeated, the mass fraction of IL and K_3PO_4/KH_2PO_4/K_2CO_3 in the system with the disappearance of stratification was obtained, namely, the phase

diagram of the IL, and $K_3PO_4/KH_2PO_4/K_2CO_3$ was obtained. The data were fitted according to the empirical nonlinear equation developed by Merchuk et al. (1998):

$$[IL] = a \times \exp[(b \times [salt]^{0.5}) - (b \times [salt]^3)] \quad (1)$$

where [IL] and [salt] are the mass fractions of ILs and salt, respectively; and a, b, and c are fitting parameters.

Tie-Lines

According to the phase diagram determined in the above experiment, a certain amount of IL, saline solution, and pure water were mixed evenly in a plug tube (at this time the system was turbidity), and placed in a 25 ℃ water bath for 12 h until the phase separated (i.e., the upper and lower phases are clarified and have obvious phase interfaces). The upper and lower phases are separately sucked out with a pipettor, and then weighed and recorded by three times, respectively. The phase composition under specific initial concentration of IL and concentration of salting out agent can be calculated by MATLAB software using Eqs. (2)–(4).

$$[IL]_{IL} = a \times \exp[\left(b \times [salt]_{IL}^{0.5}\right) - (c \times [salt]_{IL}^3)] \quad (2)$$

$$[IL]_{salt} = a \times \exp[(b \times [salt]_{salt}^{0.5} - (c \times [salt]_{salt}^3)] \quad (3)$$

$$[IL]_{IL} = \frac{[IL]_M}{\alpha} - \frac{1-\alpha}{\alpha} \times [IL]_{salt} \quad (4)$$

$$[salt]_{IL} = \frac{[salt]_M}{\alpha} - \frac{1-\alpha}{\alpha} \times [salt]_{salt} \quad (5)$$

The subscripts IL, salt, and M refer to the enrichment phase of IL, the enrichment phase of salting-out agent, and the initial mixing system, respectively, and the parameter α is the ratio of the mass of the enrichment phase of IL to the sum of the mass of the enrichment phase of IL and the enrichment phase of salting-out agent.

After above values were determined, the tie line length (TLL) and the slope of the tie line (S) at different compositions and temperatures could also be calculated respectively using Eqs. (6) and (7) as follows:

$$TLL = \sqrt{([IL]_{IL} - [IL]_{salt})^2 + ([salt]_{salt} - [salt]_{IL})^2} \quad (6)$$

$$S = ([IL]_{IL} - [IL]_{salt})/([salt]_{IL} - [salt]_{salt}) \quad (7)$$

Extraction of Proteins Using ABS

The ternary mixture compositions used in the extraction experiments were chosen based on the phase diagrams determined in this work for each IL-salt-water ABS. About 0.5 ml edible oil and a certain amount of IL- and salt-based ABS were shaken 200 rpm at 25 ℃ for 20 min. After completion, the topmost oil layer was removed and the remaining upper and lower phase volumes were recorded. The protein content of upper phase was detected using the method described in the "Determination Protein Content" section. Different type of salts and ILs were investigated to obtain higher partition coefficient (K) and extraction efficiency (E, %), which can be calculated using Eqs. (8) and (9) as follows:

$$E = \frac{C_{IL} \times V_t}{C_{IL} \times V_t + C_{salt} \times V_b} \times 100\% \quad (8)$$

$$K = \frac{C_{IL}}{C_{salt}} \quad (9)$$

where C_{IL} and C_{salt} (mg/mL) represent the concentrations of total protein in the enrichment phases of IL and salting-out agent, respectively. V_t and V_b (mL) represent the volumes of the enrichment phases of IL and salting-out agent, respectively.

Determination Protein Content

The protein content was measured by BCA method (Smith et al., 1985). The determination method followed the instruction in BCA assay kit (Beyotime Institute of Biotechnology, Shanghai, China). The standard curve was made with bovine serum albumin solution as the control. The linear regression equation of the measured standard curve is $y = 0.651x + 0.106$, $R^2 = 0.991$, where y is A_{562} value and x is protein concentration.

Result and Discussion

ABS Phase Diagrams

Unlike the ordinary liquid–liquid extraction system, which can obtain a more suitable two phases by directly mixed with the prebalanced two phases, the ABS needs to be judged according to the phase diagram. Therefore, the system with different initial concentration of IL and salting out agent was configured in this experiment, and the ratio of extraction system was determined according to its phase composition after phase separation. The phase composition was calculated by formulas (2)–(5) according to the fitting curve, and the specific results are summarized in Table 1. According to the obtained R^2 and SD, Eq. (1) has sufficient satisfactory accuracy in fitting binodal data of the investigated system.

Table 1 Values of parameters of Eq. (1) for various ILs + salts + water under 298.15 K at atmosphere pressure ($p = 0.1$ MPa)

ILs	a	b	c	R^2	SD
ILs + K_2HPO_4 + water					
[Bmim]Br	193.68845 ± 14.29444	−0.36784 ± 0.01661	9.79201E-7 ± 5.09676E-7	0.99927	0.04893
[Hmim]Br	259.13519 ± 17.07775	−0.48496 ± 0.01493	8.36571E-6 ± 4.69758E-7	0.99951	0.02176
[Emim]Br	116.72415 ± 11.70767	−0.18655 ± 0.02231	1.16469E-5 ± 6.52854E-7	0.99821	0.20238
[Emim]Cl	92.28672 ± 4.76922	−0.14528 ± 0.01141	1.09974E-5 ± 3.24762E-7	0.99943	0.05471
[Bmim]Cl	169.52675 ± 10.68877	−0.34181 ± 0.01416	9.90745E-6 ± 4.30632E-7	0.99944	0.03596
[Hmim]Cl	191.38995 ± 12.73994	−0.39019 ± 0.015	9.5566E-6 ± 4.6287E-7	0.99943	0.03104
ILs + K_3PO_4 + water					
[Bmim]Br	187.96302 ± 8.50889	−0.31012 ± 0.01056	7.95651E-6 ± 3.57941E-7	0.99938	0.08037
[Hmim]Br	244.16198 ± 2,253,509	−0.38179 ± 0.02258	1.54115E-5 ± 1.054115E-6	0.99864	0.16669
[Emim]Br	167.55173 ± 16.94422	−0.26379 ± 0.02135	4.87503E-6 ± 4.59761E-7	0.9971	0.33615
[Emim]Cl	128.81546 ± 7.82	−0.23561 ± 0.01276	2.70779E-6 ± 2.51402E-7	0.99774	0.20909
[Bmim]Cl	106.79482 ± 4.22955	−0.21052 ± 0.00855	1.64533E-6 ± 1.57485E-7	0.99785	0.21984
[Hmim]Cl	278.44361 ± 27.08398	−0.39448 ± 0.02172	4.13018E-6 ± 6.14473E-7	0.99748	0.32273
ILs + K_2CO_3 + water					
[Bmim]Br	147.56587 ± 21.95375	−0.25191 ± 0.03652	2.91513E-5 ± 1.91771E-6	0.99809	0.15327
[Hmim]Br	373.72224 ± 17.26953	−0.51917 ± 0.01311	3.53443E-5 ± 1.29675E-6	0.99973	0.04224
[Emim]Br	190.19037 ± 32.35178	−0.387 ± 0.0397	1.85288E-6 ± 1.15599E-6	0.98598	1.28791
[Emim]Cl	199.37541 ± 14.2073	−0.28865 ± 0.01603	5.40766E-6 ± 4.29754E-7	0.9983	0.30673
[Bmim]Cl	191.5474 ± 23.30164	−0.32908 ± 0.02897	1.1358E-5 ± 1.14146E-6	0.99689	0.36232
[Hmim]Cl	377.81401 ± 25.94814	−0.46316 ± 0.01716	1.37477E-5 ± 8.3476E-7	0.99927	0.11389

The phase separation mechanism of the ABS consisting of imidazole ILs and salts has been well established: the trend of two-phase separation is consistent with the ability of salt ions to form hydrating complexes, and the stronger the hydrating ability of salt ions, the more likely it is to repel the IL and form the second phase, namely "salting out" effect (Deive et al., 2011; Ventura et al., 2009). According to Fig. 1, the longer the alkyl chain of IL cations, the closer the bimodal curve is to the *x*-axis. The larger area above the bimodal curve (the immiscible region) indicates a higher ability to form two phases; that is, due to the decrease affinity of IL in the water, IL is easier to be salted out by salts. In general, the longer the alkyl carbon chain, the stronger the hydrophobic capacity, the stronger the phase separation trend, and the easier it is to be salinized to form ABS (He et al., 2018; Wei et al., 2013). As can be observed in Fig. 1, the ability of the ILs investigated in their ABS for phase separation follows the following order: $[Hmin]^+ > [Emin]^+ > [Bmin]^+$. For anions, bromide seems to be more likely to form two phases than chlorine. This may be due to the lower proton receptivity of bromide ions, which is consistent with the lower alkalinity of the hydrogen bonds of bromide ions (Claudio et al., 2014).

In previous studies, all kinds of organic salts and inorganic salts have been used in the formation of two-phase system, and potassium is the most frequently used inorganic salt for its high solubility in water (He et al., 2018; Belchior et al., 2020). Furthermore, most reports have focused on phosphate-based salts because these salts easily induce the formation of liquid–liquid biphasic systems with ILs (Yan et al., 2014). In this research, three soluble potassium salts, K_3PO_4, K_2HPO_4, and K_2CO_3 were applied for forming two-phase system with IL. KH_2PO_4 and $C_6H_5Na_3O_7$ were excluded due to their poor solubility in water. According to Gutowski (Gutowski et al., 2003) and Weingärtner (Weingärtner & Schröer, 1995), the kosmotropic ions, e.g., HPO_4^{2-}, SO_4^{2-}, OH^-, CO_3^{2-}, and PO_4^{3-}, are solvophobic one, which exhibit stronger interaction with water molecule than that between water molecules, so they are beneficial to the ABS formation. The relevant parameters of various salts and ILs in Eq. (1) are showed Table 1. Figure 2 shows that the ABS formation capabilities of salts and ILs basically follow the following order: $K_2CO_3 > K_2HPO_4 > K_3PO_4$. According to the data in Table 2, the tie-lines can be compared, and the influence of anions, salts, and alkyl chain length of ILs on the formation of two aqueous phases also be observed. Thus, the formation of the ABS is closely related to the polarity/salting-out ability of IL and salt ions. The length of the tie-lines indicates that the separation degree of the two phases increases. For the same ABS, the longer the line is, the higher the separation degree of two phases is, and the better the extraction effect is. In addition, the obtained data demonstrate that the tie-lines of the aqueous phases conform to the lever rule and the application of the Merchuk

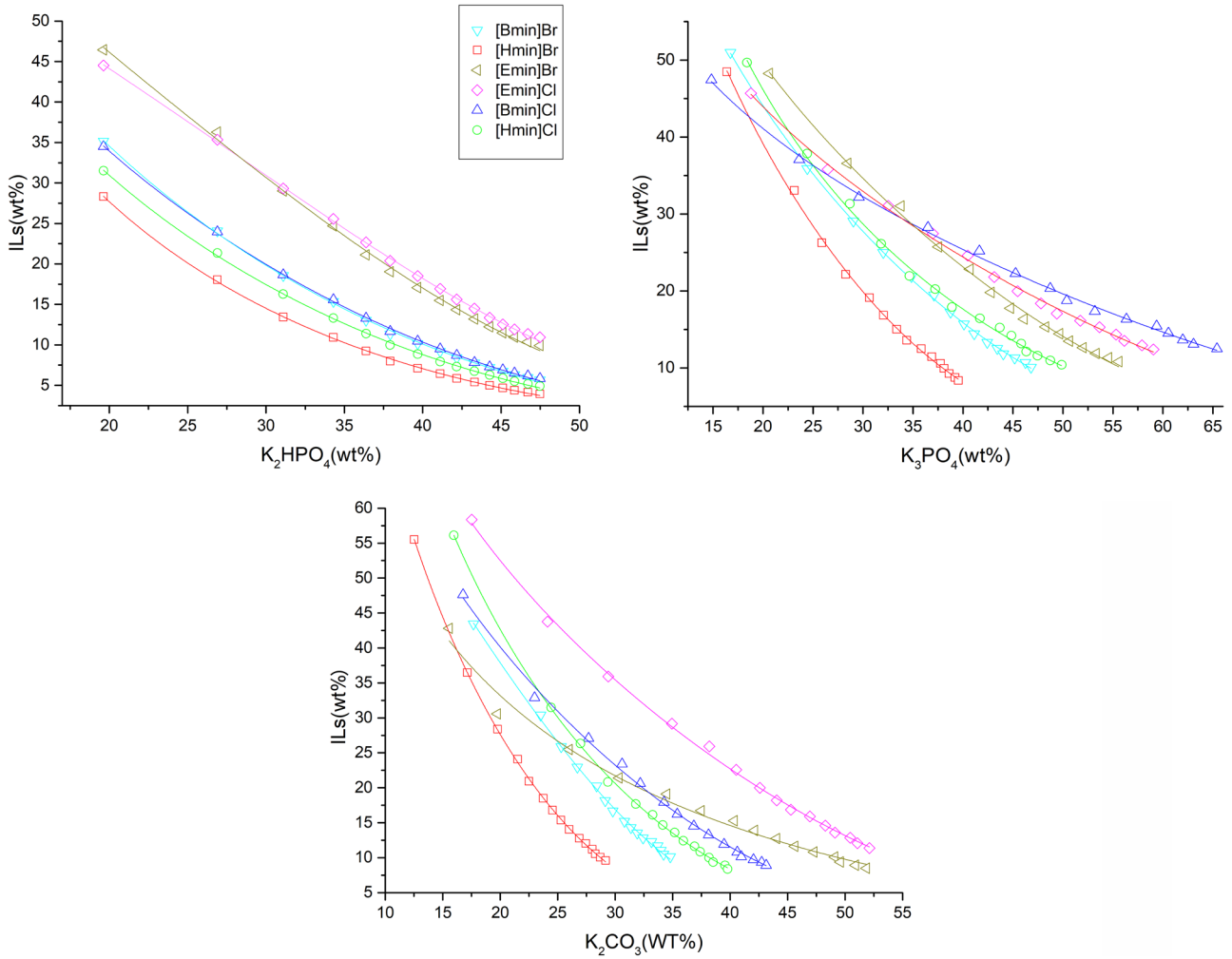


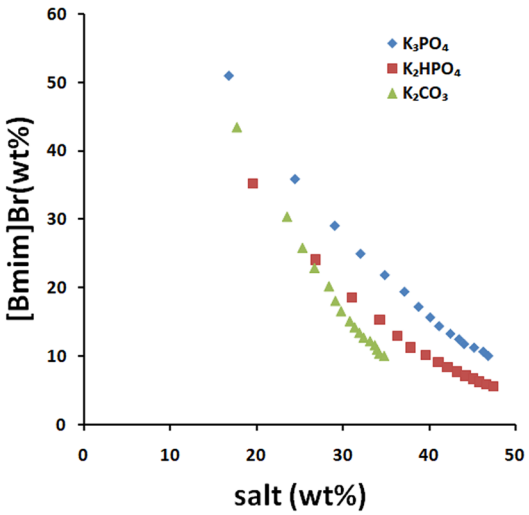
Fig. 1 Phase diagrams in mass fraction units for ABS formed by ILs + K_2HPO_4/K_3PO_4 or K_2CO_3 + H_2O at 25 ℃ and atmospheric pressure. Lines correspond to the fitting by Eq. (1)

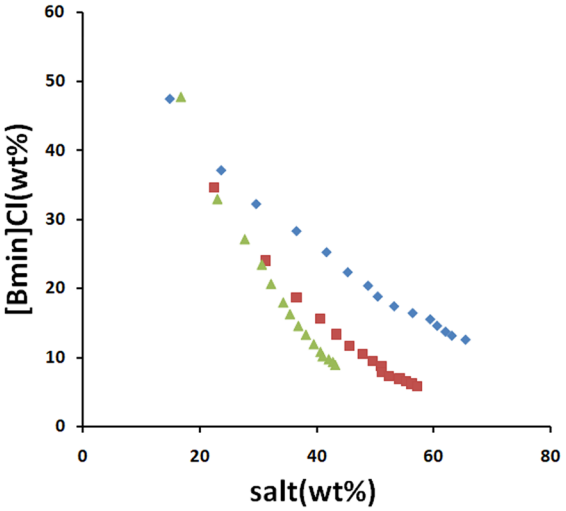
equation to the IL-salt aqueous phases can well replicate our experimental results.

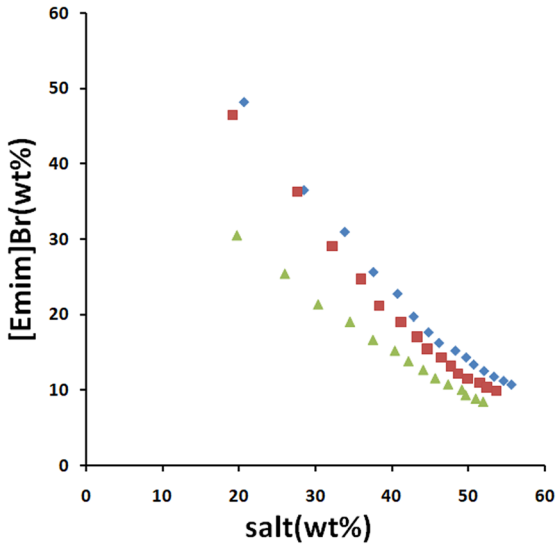
Separation Behavior of Protein from Edible Oil in Under Various IL-ABS Conditions

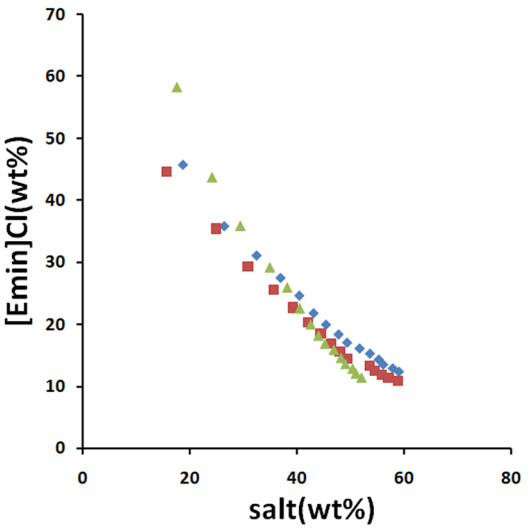
Effect of Salt and IL Types

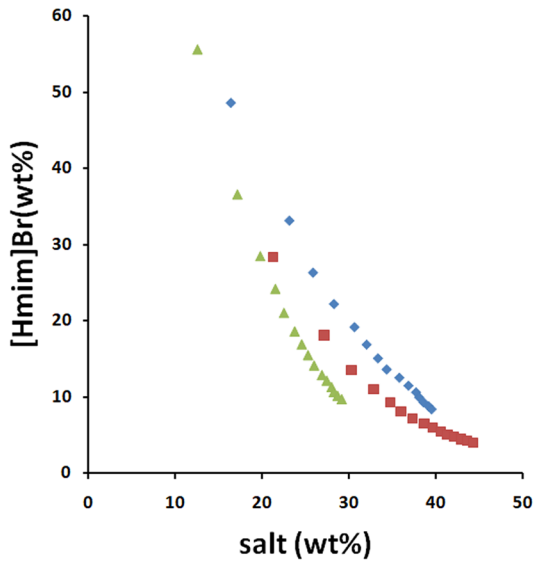
As a stable and non-volatile solvent, ILs have great potential in the application of extraction and separation. The extraction efficiency of ILs with different structures is affected by their physical and chemical properties. IL-ABS are formed by adding appropriate amounts of K_3PO_4, K_2HPO_4, and K_2CO_3 into aqueous ILs, and include [Bmin]Br, [Hmin]Br, [Emin]Br, [Emin]Cl, [Bmin]Cl, and [Hmin]Cl. In the extraction application for target constituents, the requirement for ABS should include not only easy formation, but also ideal extraction efficiency. The extraction efficiency not only depends on the solubility of target compound in the ABS, but also may be affected by the clustering phenomenon of ILs itself or the combination of ILs with the extracted compounds (Lin et al., 2013; Yan et al., 2014). As is known to all, different ILs have different dissolution and clustering behaviors due to their different structures. In order to find out the optimal ILs combined with salt in the ABS for the extraction of proteins, the extraction efficiency (E) and partition coefficients (K) mentioned in the "Extraction of Proteins Using ABS" section were compared in the ABS composition of 25% (w/v) IL + 30% (w/v) salt in a 5 mL system adding 1:1 (w:v) oil. As shown in Fig. 3A, the combination of [Bmim]Cl and K_3PO_4 had the highest extraction efficiency and partition coefficients. With the increase of the carbon chain, ABS are easier to form, but the corresponding solution viscosity will also increase, which is not conducive

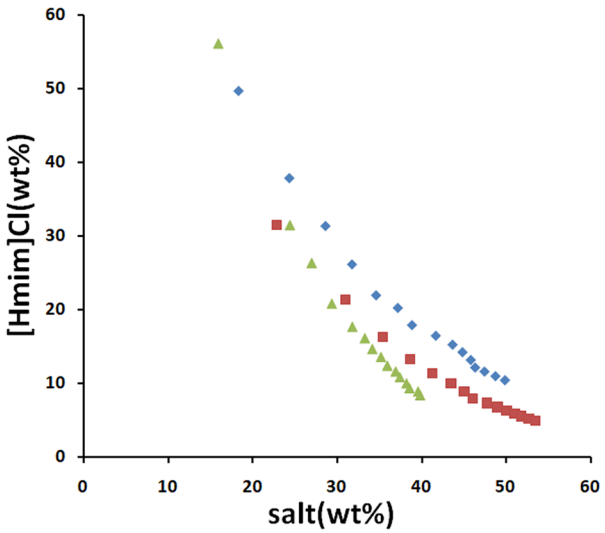

K3PO4
K2HPO4
K2CO3
[Bmim]Br(wt%)
salt (wt%)


[Bmin]Cl(wt%)
salt(wt%)


[Emim]Br(wt%)
salt(wt%)


[Emin]Cl(wt%)
salt(wt%)


[Hmim]Br(wt%)
salt (wt%)


[Hmim]Cl(wt%)
salt(wt%)

◂**Fig. 2** Phase diagrams in mass fraction units for ABS formed by [Bmin]Br/[Emin]Br/[Hmin]Br/[Bmin]Cl/[Emin]Cl/[Hmin]Cl + salts + H_2O at 25 °C and atmospheric pressure

to the dissolution of the target product. Therefore, it is necessary to compare the extraction efficiency through the specific experimental process. In general, proteins can interact with ILs through hydrogen bonding, electrostatic interactions, and dispersion forces, so that proteins are preferred to the top phase (IL-rich phase) rather than the salt-rich phase. Thus, the ILs that established hydrophobic interactions with proteins were those that better induce protein aggregation. In addition, different salts affect the distribution of IL in the top/bottom phase and also change the extraction efficiency (E) of the target component. As mentioned earlier, some salts are not considered due to their poor solubility and difficulty in forming two aqueous phases, e.g., KH_2PO_4 and $C_6H_5Na_3O_7$. According to Fig. 3, the ABS formed by [Bmim]Cl and K_3PO_4 have the highest extraction efficiency (E) (close to 100%) and partition coefficient (K) (close to 90). Therefore, [Bmim]Cl and K_3PO_4 were selected as the extractant in the following experiment.

Effect of Inorganic Salt Concentration

Besides salt type, its concentration also plays an important role in extraction process. With the increase of salts, the hydrophobicity of the base phase increases, and the existence of IL and protein in the bottom phase decreases. This is because salt ions and protein compete with water molecules through intermolecular hydrogen bonds, which leads to less protein dissolved into the salt rich phase; thus, IL-protein complex will be driven from the salt-rich phase to the IL-rich phase. Therefore, it can be observed from Fig. 4A that the initial extraction efficiency (E) increases with the increase of salt concentration. The maximum extraction efficiency (E) was obtained when the concentration of K_3PO_4 was controlled at 50% (w/v). After that, the extraction rate decreased slightly as the salt concentration continued to increase. This result may be attributed to the salt-out effect. High concentrations of salt can destroy the colloidal properties of proteins and reduce their solubility. This is consistent with the conclusion of PEG-inorganic salt ABS (Marchel et al., 2019; Yang et al., 2018). Considering the higher extraction efficiency of protein, 50% (w/v) of K_3PO_4 was selected as the phase-forming salt in [Bmin]Cl-based ABS.

Effect of IL and Sample Mass

The effect of IL concentration on extraction of protein could be observed from the results in Fig. 4B. The extraction efficiency of protein decreased with increasing [Bmim]Cl amount, when the concentration of K_3PO_4 was controlled at 50% (w/v). In the range of concentration of IL that can form

Table 2 Tie line data of ILs + salts + water under 298.15 K at atmosphere pressure ($p = 0.1$ MPa)

ILs	IL (WT%)	Salt (WT%)	$[Salt]_{IL}$ (WT%)	$[Salt]_{salt}$ (WT%)	$[IL]_{IL}$ (WT%)	$[IL]_{Salt}$ (WT%)□	α	TLL	S
ILs + K_2HPO_4 + water									
[Bmim]Br	32.04918	21.90823	19.78441	25.65664	34.69719	27.3756	0.638329	9.38556	−1.24682
[Hmim]Br	37.3672	23.95418	7.86769	40.28669	67.14978	7.128458	0.5038	68.21694	−1.85142
[Emim]Br	50.27278	21.35785	15.25675	38.30604	55.99365	34.37048	0.735343	31.60429	−0.93813
[Emim]Cl	42.86865	24.13376	9.973435	63.20774	57.74709	1.813116	0.733795	77.21723	−1.05071
[Bmim]Cl	43.35749	25.04026	7.83551	66.94272	60.95527	0.497587	0.708924	84.55054	−1.02285
[Hmim]Cl	47.69849	23.8191	6.808387	61.14738	68.97019	1.019907	0.686952	87.00556	−1.25049
ILs + K_3PO_4 + water									
[Bmim]Br	47.33387	23.1776	8.955741	39.99896	73.90789	15.9026	0.541869	65.78978	−1.86853
[Hmim]Br	41.50754	21.76884	10.94844	31.26664	68.05021	18.20922	0.467453	53.82335	−2.45302
[Emim]Br	59.50945	21.87374	6.771043	50.49666	83.71169	13.6409	0.654603	82.59446	−1.60251
[Emim]Cl	52.08291	22.88187	6.066583	54.38387	72.05708	14.663	0.651982	75.02427	−1.18786
[Bmim]Cl	49.84912	22.69161	4.776401	57.34342	67.3996	15.90277	0.659193	73.58815	−0.97964
[Hmim]Cl	42.15157	22.13557	19.71487	26.24883	46.80639	34.2421	0.62952	14.1617	−1.92292
ILs + K_2CO_3 + water									
[Bmim]Br	49.23541	40.34205	98.44983	6.229313	1.01E-11	78.13954	0.369904	120.8735	−0.84731
[Hmim]Br	40.94663	28.80161	5.923495	43.20437	104.8571	0.712219	0.386331	110.6166	−2.79352
[Emim]Br	42.08	32.98	10.06272	48.13117	92.85329	8.512559	0.397998	92.53413	−2.2155
[Emim]Cl	45.74297	24.03614	20.98597	34.36514	50.54636	29.47691	0.772021	24.95845	−1.57479
[Bmim]Cl	49.7681	23.19016	8.262175	51.63592	73.90805	3.768634	0.655829	82.46708	−1.61709
[Hmim]Cl	42.69146	24.85503	13.26696	41.20557	67.71396	7.385192	0.585231	66.48403	−2.15933

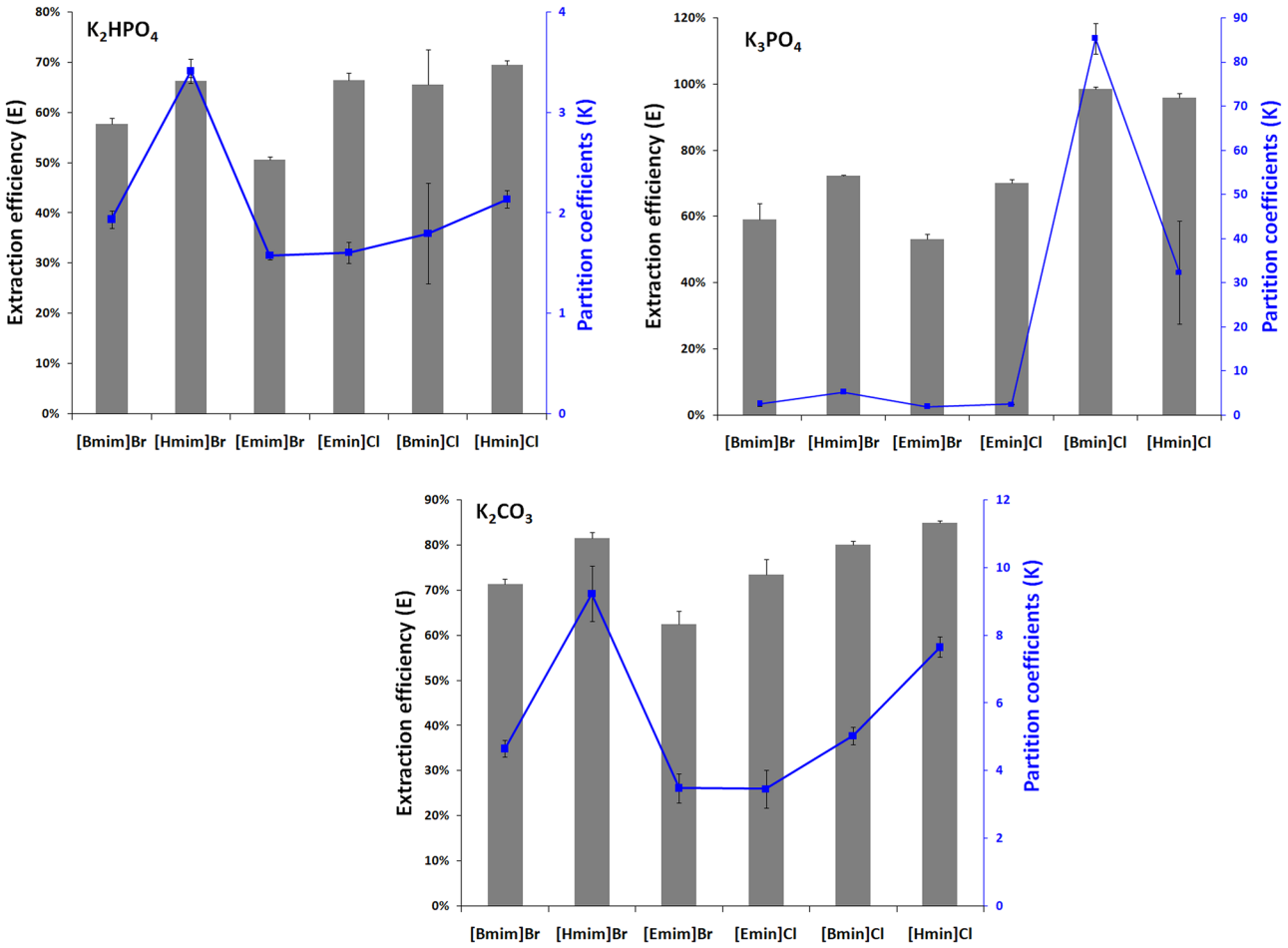


Fig.3 Effects of inorganic salt and IL type on extraction efficiency of proteins. Conditions: 30% (w/v) salt, 25% (w/v) IL, and 1.0 mL oil, the experiments were done at 25 ℃ and atmospheric pressure

two phases, increasing the concentration of IL does not help to improve the extraction efficiency because of the invariance of protein in IL-rich phase when the amount of total protein remains unchanged. In addition, higher [Bmim]Cl content increases solution viscosity and reduces poor mass transfer (Saba et al., 2015). Thus, 20% (w/v) [Bmim]Cl was suitable for the simultaneous separation of proteins with high extraction efficiency. So far, the selection of solvent system was as follows: 20% (w/v) [Bmim]Cl, 50% (w/v) K_3PO_4, and the total solvent system of 5 mL was used in the following experiment.

The extraction efficiencies of 12 different oils from 0.5 to 8 g were studied in the above 5 mL ABS. As shown in Fig. 4C, high extraction efficiency (E) above 99% can be achieved under each sample mass in the range from 0.5 to 8 g. Since the unified system used, we also compared the protein concentrations in the IL enrichment phase (top phase). With the increase of oil content, the protein concentration in the upper phase also increased gradually, and reached the highest at 6 g oil added (Fig. 4C). In the ABS, the IL-rich phase is a non-aqueous phase (He et al., 2018). However, the protein in edible oil is generally liposoluble, so according to the principle of "like dissolves like," the higher initial concentration of the oil promotes more proteins to enter the IL-rich phase, and the IL-rich phase has a strong affinity with the protein. The results indicated that the IL-enriched phase had a higher enrichment capacity to protein.

Effects of pH and Extraction Temperature

The pH value and temperature are crucial to the specific protease activity and the stability of protein extracts (Ochoa-Rivas et al., 2017; Silvestre et al., 2012). When the pH was lower than 5, phosphate solutions and ILs could not form ABS (Ferreira et al., 2017). In addition, the three salt solutions used in the ABS are alkaline. Therefore, we adjusted the pH by adding phosphoric acid between 7.0 and 13.0 to study the extraction efficiency of the system.

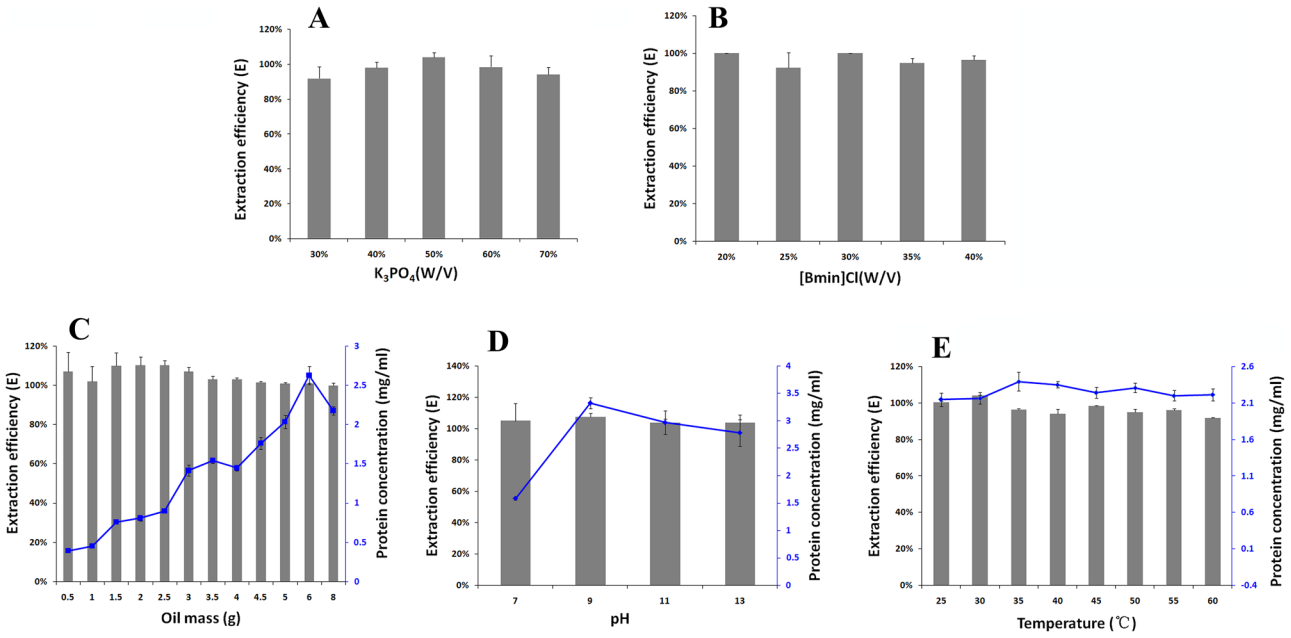


Fig.4 Effects of the (**A**) K_3PO_4 concentration; (**B**) ILs concentration; (**C**) oil mass; (**D**) pH; (**E**) extraction temperature on extraction efficiency of proteins in [Bmim]Cl/K_3PO_4 ABS

As shown in Fig. 4D, although no differences were observed in terms of extraction efficiency, the protein concentration in ILs-enriched phase increased with increasing pH. The maximum protein concentration was obtained at pH 9.0. The increase in the pH leads to an increase in the relative number of negatively charged proteins, which not only possibly established electrostatic interactions with IL cations, and but also resulted in charge–charge repulsion of the proteins and exposure of hydrophobic, SH and SS groups, as described in the literature (Belchior et al., 2020; Negar Gharbi, 2018). The exposure of protein groups can lead to oxidation and ion exchange, which further enhance the hydrophobicity of the protein, which is consistent with our results. In addition, in extreme cases, the stability of the protein will also be affected at pH 13, so the protein concentration will decrease significantly in the same system.

Finally, the extraction temperatures (25, 30, 35, 40, 45, 50, 55, 60 ℃) were studied, and the results are shown in Fig. 4E. With the increase of temperature, the extraction efficiency of protein increased slightly. The increase of temperature can reduce the viscosity of IL and enhance the diffusion and solubility of solvent, so the extraction rate rises. The results indicate that protein extraction is endothermic and higher temperature is beneficial to this process. But when the temperature keeps rising, the extraction rate decreases. For the IL-salt two-phase systems, with the increase of temperature, the two-phase region decreases to some extent (Han et al., 2010), which is related to the salting out agent used (Freire et al., 2012). However, increasing the temperature further reduces the IL concentration at the IL-rich stage (He et al., 2018), thus reducing the efficiency of protein extraction. Furthermore, it also suggests that the protein is more stable at lower temperatures. This may be because the Brownian motion of protein and IL cluster particles is more intense with the increase of temperature; that is, protein and IL tend to be homogeneous, so the extraction rate will decrease. In addition, considering that the sample was easy to degeneration under high temperature, 30–35 ℃ was the ideal temperature as the protein extraction.

Extraction Mechanism

The traditional method of protein extraction usually applied 90% ethanol (Yang et al., 2019), but ethanol is not an ideal solvent for liquid oils because of oil's soluble in ethanol. However, the ABS is feasible for the extraction of protein from oil, because oil is insoluble in water, while protein is soluble in the IL enriched phase. In all investigated systems, protein preferentially partitions to the top (IL-rich) phase in respect to the salt-rich phase, with extraction efficiencies of 100% obtained in a single-step which reflects the preferential partition of a given protein between the coexisting phases. Some scholars believe that π-π interaction and hydrophobicity are the main factors that promote the distribution of protein to the enrichment phase of IL (Zafarani-Moattar & Hamzehzadeh, 2011). In general, imidazole cations in

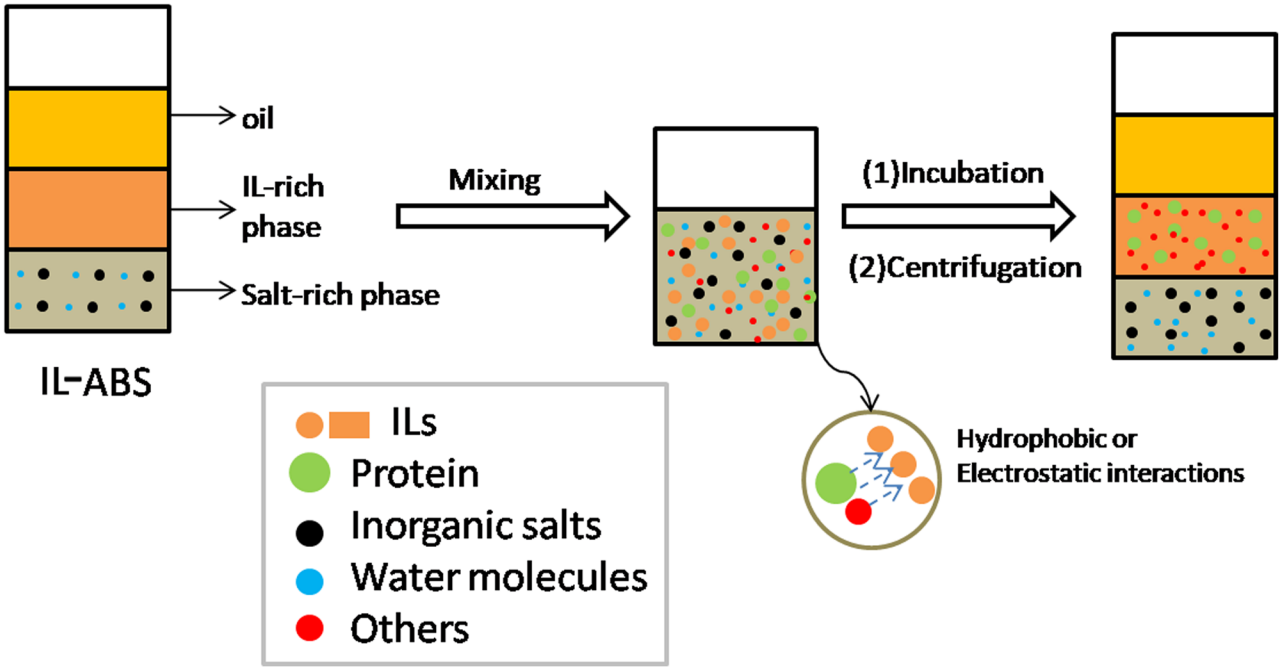


Fig. 5 Extraction process and mechanisms of proteins in IL-ABS

ILs have aromatic π systems. The π-π interaction between imidazole cation and protein aromatic residues may be the driving force of protein extraction (Pei et al., 2009). Figure 5 shows the detailed schematic and mechanism of IL-ABS in extracting proteins. The formation of electrostatic and hydrophobic interactions between protein molecules and ILs will drive the transfer of protein to the top phase. In order to investigate the mechanism of extraction of proteins from edible oil by IL /K_3PO_4 ABS, the structure changes of proteins before and after extraction were studied by UV and fluorescence spectra. The maximum UV absorption peak of protein in water and two phases was basically unchanged (Fig. 6A). This indicates that no new chemical bond is formed between the protein and the IL during the extraction process, and the structure of the protein is also not destroyed. Figure 6B shows the fluorescence emission spectrum of [Bmin]Cl quenching protein at 25 ℃, and the excitation wavelength is 280 nm. In Fig. 6B, the maximum emission wavelength of the samples was all 382 nm. In different extraction systems, the fluorescence intensity of the samples varied. In the ABS, the fluorescence intensity of the sample was quenched, indicating that the IL combined with the luminescent group of the protein, resulting in the quenching or weakening of the fluorophore. In summary, we hypothesized that hydrophobicity, electrostatic interaction, and salting-out between molecules were the main driving factors of protein extraction.

Analysis Different Sources of Oil

According to the results of single factor experiment, the optimal condition was selected to extract the commercial edible oil: [Bmim]Cl 20% (w/v), K_3PO_4 50% (w/v), oil 6:5 (oil:ABS, w/v) at pH 9.0 and 35 ℃. These commercial oils include sunflower oil, corn oil, peanut oil, sunflower seed oil, green tea seed oil, rapeseed oil, soybean oil, blend oil, olive oil, and camellia oil extracted in different ways, such as supercritical extraction, cold pressing, hot pressing, and organic solvent extraction. Through testing, we found that the protein content in peanut oil is generally higher, and the protein content of sunflower seed oil is generally lower. The protein content of refined oil from different sources is as follows: olive oil > peony seed oil > torreya oil > peanut oil > green tea seed oil > flax seed oil > grape seed oil > soybean oil > blend oil > safflower seed oil > corn

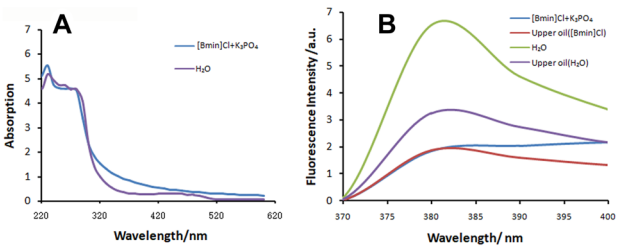


Fig. 6 UV–Vis (**A**) and fluorescence (**B**) spectra

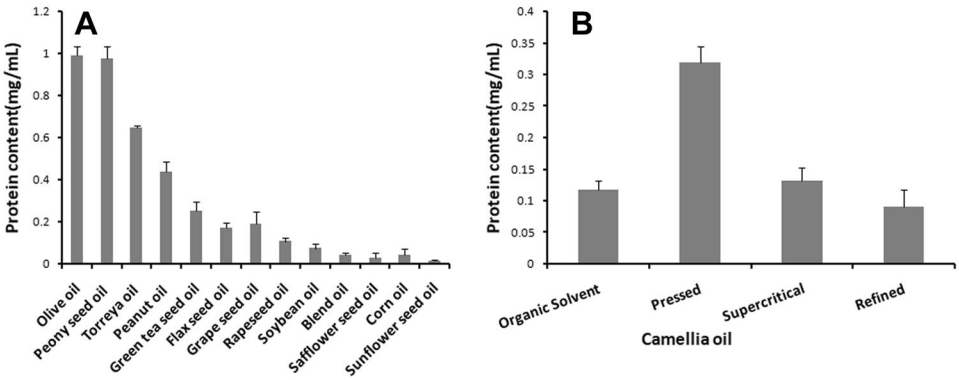


Fig. 7 The protein content of different edible oils. **A** Different sources of oil. **B** Different extraction methods

oil > sunflower seed oil (Fig. 7A). At the same time, it was also found that the protein of refined camellia oil was significantly reduced, indicating that refining process induced the protein loss (Fig. 7B). Secondly, the protein content from pressed camellia oil was generally higher than that from organic solvent and supercritical extraction oil (Fig. 7B). This indicates that different extraction methods also result in significant differences in protein retention. Therefore, it also provides a theoretical basis for using the difference in protein content to identify the sources and processing methods of refined oil.

Conclusion

In this study, ABS extraction ([Bmin]Cl/K_3PO_4) for rapid and efficient separation of proteins from edible oil was established. By optimizing the extraction conditions (salt and IL types and concentration, pH, and temperature), the optimal extraction conditions of [Bmin]Cl-based ABS were obtained. The optimal extraction conditions were as follows: salt concentration was 50% (w/v), IL concentration was 20% (w/v), extraction pH was 9, and extraction temperature was 35 ℃. Under the optimal extraction conditions, the protein extracted from edible oil can reach 2–3 mg/mL. UV and fluorescence spectra were used to characterize the mechanism of the protein extraction under optimized conditions. The results indicated that the main driving forces of protein separation may be hydrophobicity, electrostatic interaction, and salting-out. The protein structure did not change before and after extraction. In addition, [Bmin]Cl/K_3PO_4 ABS was successfully applied to extract and separate the proteins in edible oil under optimized conditions. The results suggested that the protein content in finished edible oil was significantly different, which was mainly caused by the biological source of the oil and the extraction method adopted. The above results indicate that the IL has a good prospect in the application of ABS extraction, and this method is also of great significance in solving the problem of protein separation and enrichment.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

Belchior, D. C. V., Quental, M. V., Pereira, M. M., Mendonça, C. M. N., Duarte, I. F., & Freire, M. G. (2020). Performance of tetraalkylammonium-based ionic liquids as constituents of aqueous biphasic systems in the extraction of ovalbumin and lysozyme. *Separation and Purification Technology, 233*, 116019.

Bettaieb Rebey, I., Bourgou, S., Detry, P., Wannes, W. A., Kenny, T., Ksouri, R., Sellami, I. H., & Fauconnier, M. (2019). Green extraction of fennel and anise edible oils using bio-based solvent and supercritical fluid: Assessment of chemical composition, antioxidant property, and oxidative stability. *Food and Bioprocess Technology, 12*, 1798–1807.

Capela, E. V., Quental, M. V., Domingues, P., Coutinho, J. A. P., & Freire, M. G. (2017). Effective separation of aromatic and aliphatic amino acid mixtures using ionic-liquid-based aqueous biphasic systems. *Green Chemistry, 19*(8), 1850–1854.

Claudio, A. F. M., Swift, L., Hallett, J. P., Welton, T., Coutinho, J. A. P., & Freire, M. G. (2014). Extended scale for the hydrogen-bond basicity of ionic liquids. *Physical Chemistry Chemical Physics, 16*, 6593–6601.

Chong, K. Y., Stefanova, R., Zhang, J., & Brooks, M. S. (2020). Extraction of bioactive compounds from haskap leaves (lonicera caerulea) using salt/ethanol aqueous two-phase flotation. *Food and Bioprocess Technology, 13*, 2131–2144.

Crevel, R. W. R., Kerhoff, M. A. T., & Koning, M. M. G. (2000). Allergenicity of refined vegetable oils. *Food and Chemical Toxicology, 38*, 385–393.

Deive, F. J., Rodríguez, A., Marrucho, I. M., & Rebelo, L. P. (2011). Aqueous biphasic systems involving alkylsulfate-based ionic liquids. *The Journal of Chemical Thermodynamics, 43*, 1565–1572.

Dreyer, S., Salim, P., & Kragl, U. (2009). Driving forces of protein partitioning in an ionic liquid-based aqueous two-phase system. *Biochemical Engineering Journal, 46*(2), 176–185.

Dupont, J., Consorti, C. S., Suarez, P. A. Z., Souza, R. F. D., & Wolff, S. (2002). Preparation of 1-butyl-3-methyl imidazolium-based room temperature ionic liquids. *Organic Syntheses, 79*(6), 236–243.

Errahali, Y., Morisset, M., Moneret-Vautrin, D. A., Kanny, G., Metche, M., Nicolas, J. P., & Frémont, S. (2002). Allergen in soy oils. *Allergy, 59*, 648–649.

Ferreira, A. M., Cláudio, A. F. M., Válega, M., Domingues, F. M. J., Silvestre, A. J. D., Rogers, R. D., Coutinho, J. A. P., & Freire, M. G. (2017). Switchable (pH-driven) aqueous biphasic systems formed by ionic liquids as integrated production–separation platforms. *Green Chemistry, 19*, 2768–2773.

Freire, M. G., Claudio, A. F., Araujo, J. M., Coutinho, J. A., Marrucho, I. M., Canongia, L. J., & Rebelo, L. P. N. (2012). Aqueous biphasic systems: A boost brought about by using ionic liquids. *Chemical Society Reviews, 41*(14), 4966–4995.

Gámiz-Gracia, L., & Castro, M. D. L. D. (2000). Continuous subcritical water extraction of medicinal plant essential oil: Comparison with conventional techniques. *Talanta, 51*(6), 1179–1185.

Gutowski, K. E., Broker, G. A., Willauer, H. D., Huddleston, J. G., Swatloski, R. P., & Holbrey, J. D. (2003). Controlling the aqueous miscibility of ionic liquids: Aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations. *Journal of the American Chemical Society, 125*(22), 6632–6633.

Han, J., Wang, Y., Kang, W., Li, C., Yan, Y., Pan, J., & Xie, X. (2010). Phase equilibrium and macrolide antibiotics partitioning in real water samples using a two-phase system composed of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate and an aqueous solution of an inorganic salt. *Microchimica Acta, 169*(1–2), 15–22.

He, A., Dong, B., Feng, X., & Yao, S. (2018). Extraction of bioactive ginseng saponins using aqueous two-phase systems of ionic liquids and salts. *Separation and Purification Technology, 196*, 270–280.

He, C., Li, S., Liu, H., Li, K., & Liu, F. (2005). Extraction of testosterone and epitestosterone in human urine using aqueous two-phase systems of ionic liquid and salt. *Journal of Chromatography A, 1082*(2), 143–149.

Konstantza, T. (2012). Separation of poly- and disaccharides by biphasic systems based on ionic liquids. *Separation and Purification Technology, 89*, 57–65.

Li, X. M., Tian, S. L., Pang, Z. C., Shi, J. Y., Feng, Z. S., & Zhang, Y. M. (2009). Extraction of cuminum cyminum essential oil by combination technology of organic solvent with low boiling point and steam distillation. *Food Chemistry, 115*(3), 1114–1119.

Li, Y., Yang, W., Chung, S. Y., Chen, H., Ye, M., Teixeira, A. A., Gregory, J. F., Welt, B. A., & Shriver, S. (2013). Effect of pulsed ultraviolet light and high hydrostatic pressure on the antigenicity of almond protein extracts. *Food and Bioprocess Technology, 6*, 431–440.

Lin, X., Wang, Y., Zeng, Q., Ding, X., & Chen, J. (2013). Extraction and separation of proteins by ionic liquid aqueous two-phase system. *The Analyst, 21*(138), 6445–6453.

Lu, Y., Lu, W., Wang, W., Guo, Q., & Yang, Y. (2011). Thermodynamic studies of partitioning behavior of cytochrome c in ionic liquid-based aqueous two-phase system. *Talanta, 85*(3), 1621–1626.

Marchel, M., João, K. G., & Marrucho, I. M. (2019). On the use of ionic liquids as adjuvants in PEG-$(NH_4)_2SO_4$ aqueous biphasic systems: Phase diagrams behavior and the effect of IL concentration on myoglobin partition. *Separation and Purification Technology, 210*, 710–718.

Maugeri, L. (2009). Squeezing more oil from the ground. *Scientific American, 301*(4), 56–63.

Merchuk, J. C., Andrews, B. A., & Asenjo, J. A. (1998). Aqueous two-phase systems for protein separation: Studies on phase inversion. *Journal of Chromatography B, 711*, 285–293.

Negar Gharbi, M. L. (2018). Effect of processing on aggregation mechanism of egg white proteins. *Food Chemistry, 252*, 126–133.

Ochoa-Rivas, A., Nava-Valdez, Y., Serna-Saldívar, S. O., & Chuck-Hernández, C. (2017). Microwave and ultrasound to enhance protein extraction from peanut flour under alkaline conditions: Effects in yield and functional properties of protein isolates. *Food and Bioprocess Technology, 10*, 543–555.

Olszewski, A., Pons, L., Moutete, F., Aimone-Gastin, I., Kanny, G., Moneret-Vautrin, D. A., & Gueant, J. L. (1998). Isolation and characterization of proteic allergens in refined peanut oil. *Clinical and Experimental Allergy, 28*, 850–859.

Palsikowski, P. A., Besen, L. M., Santos, K. A., Silva, C. D., & Silva, E. A. D. (2019). Supercritical CO_2 oil extraction from bauhinia forficata link subsp. pruinosa leaves: Composition, antioxidant activity and mathematical modeling. *Journal of Supercritical Fluids, 153*, 104588.

Paschke, A., Zunker, K., Wigotzki, M., & Steinhart, H. (2001). Determination of the IgE binding activity of soy lecithin and refined and non-refined soybean oils. *Journal of Chromatography b: Biomedical Sciences and Applications, 756*, 249–254.

Pei, Y., Wang, J., Wu, K., Xuan, X., & Lu, X. (2009). Ionic liquid-based aqueous two-phase extraction of selected proteins. *Separation and Purification Technology, 64*(3), 288–295.

Pereira, J. F. B., Rebelo, L. P. N., Rogers, R. D., Coutinho, J. A. P., & Freire, M. G. (2013). Combining ionic liquids and polyethylene glycols to boost the hydrophobic–hydrophilic range of aqueous biphasic systems. *Physical Chemistry Chemical Physics, 15*(45), 19580.

Raghavarao, K. S. M. S., Ranganathan, T. V., Srinivas, N. D., & Barhate, R. S. (2003). Aqueous two phase extraction-an environmentally benign technique. *Clean Technologies & Environmental Policy, 5*(2), 136–141.

Requejo, P. F., Velho, P., Gomez, E., & Macedo, E. A. (2020). Study of liquid-liquid equilibrium of aqueous two-phase systems based on ethyl lactate and partitioning of rutin and quercetin. *Industrial & Engineering Chemistry Research, 59*(48), 21196–21204.

Rigby, N. M., Sancho, A. I., Salt, L. J., Foxall, R., Taylor, S., Raczynski, A., Cochrane, S. A., Crevel, R. W. R., & Mills, E. N. C. (2011). Quantification and partial characterization of the residual protein in fully and partially refined commercial soybean oils. *Journal of Agricultural and Food Chemistry, 59*, 1752–1759.

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Lebovka, N., Grimi, N., & Vorobiev, E. (2015). High voltage electrical discharges, pulsed electric field, and ultrasound assisted extraction of protein and phenolic compounds from olive kernel. *Food and Bioprocess Technology, 8*, 885–894.

Saba, H., Zhu, X., Chen, Y., & Zhang, Y. (2015). Determination of physical properties for the mixtures of [BMIM]Cl with different organic solvents. *Chinese Journal of Chemical Engineering, 23*(5), 804–811.

Silvestre, M. P. C., Carreira, R. L., Silva, M. R., Corgosinho, F. C., Monteiro, M. R. P., & Morais, H. A. (2012). Effect of pH and temperature on the activity of enzymatic extracts from pineapple peel. *Food and Bioprocess Technology, 5*, 1824–1831.

Smith, P. K., Krohn, R. I., & Hermanson, G. T. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry, 150*(1), 76–85.

Tirgarian, B., Farmani, J. & Milani, J.M. (2019). Enzyme-assisted aqueous extraction of oil and protein hydrolysate from sesame seed. *Food Measure, 13*, 2118–2129.

Ventura, S. P., Neves, C. M., Freire, M. G., Marrucho, I. M., Oliveira, J., & Coutinho, J. A. (2009). Evaluation of anion influence on the formation and extraction capacity of ionic-liquid-based aqueous biphasic systems. *Journal of Physical Chemistry B, 113*, 9304–9310.

Wei, X. L., Wang, X. H., Ping, A. L., Du, P. P., Sun, D. Z., Zhang, Q. F., & Liu, J. (2013). Formation and characteristics of aqueous

two-phase systems formed by a cationic surfactant and a series of ionic liquids. *Journal of Chromatography B, 939*, 1–9.

Weingärtner, H., & Schröer, W. (1995). Liquid-liquid phase separations and critical behavior of electrolyte solutions driven by long-range and short-range interactions. *Studies in Physical and Theoretical Chemistry, 83*, 107–114.

Wensing, M., Penninks, A. H., Hefle, S. L., Koppelman, S. J., Bruijnzeel-Koomen, C. A., & Knulst, A. C. (2002). The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *Journal of Allergy & Clinical Immunology, 110*(9), 915–920.

Widyarani, R., & E., Sandersa, J. P. M., & Bruinsa, M. E. (2014). Biorefinery methods for separation of protein and oil fractions from rubber seed kernel. *Industrial Crops and Products, 62*, 323–332.

Yan, J., Ma, H., Pei, J., Wang, Z., & Wu, J. (2014). Facile and effective separation of polysaccharides and proteins from Cordyceps sinensis mycelia by ionic liquid aqueous two-phase system. *Separation and Purification Technology, 135*, 278–284.

Yang, H., Chen, L., Zhou, C., Yu, X., Yagoub, E. G. A., & Ma, H. (2018). Improving the extraction of l-phenylalanine by the use of ionic liquids as adjuvants in aqueous biphasic systems. *Food Chemistry, 245*(apr.15), 346–352.

Yang, J. Y. J., Peng, B. P. B., Wang, M. W. M., Zou, X. Z. X., Yin, Y. Y. Y., & Deng, Z. D. Z. (2019). Characteristics and emulsifying properties of two protein fractions derived from the emulsion formed during aqueous extraction of camellia oil. *Food Hydrocolloids, 87*, 644–652.

Zafarani-Moattar, M. T., & Hamzehzadeh, S. (2011). Partitioning of amino acids in the aqueous biphasic system containing the water-miscible ionic liquid 1-butyl-3-methylimidazolium bromide and the water-structuring salt potassium citrate. *Biotechnology Progress, 27*(4), 986–997.

Zhao, S., & Zhang, L. (2012). Application of ionic liquids in extraction technology. *Chemistry, 75*(11), 1001–1008.