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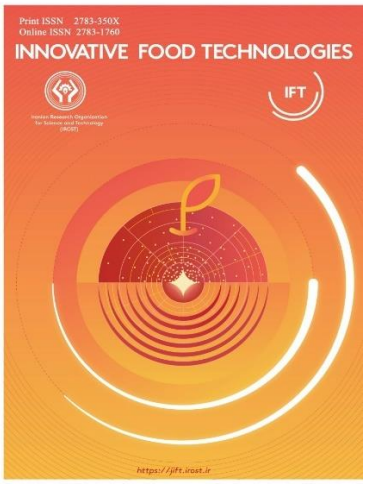

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Extraction of Protein from Sesame By-Product Using Alkaline, Salt, and Choline Chloride-Acetic Acid Deep Eutectic Solvent Methods: Extraction Yield, Functional and Structural Properties

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Abstract

In this study, sesame protein was extracted from by-product of oil industry through different methods including alkaline extraction, salt extraction and choline chloride-acetic acid deep eutectic solvent (ChCl-AA DES) extraction method. The yield and protein recovery were calculated; and the functional and structural properties of extracted protein such as solubility, water and oil holding capacity, emulsifying and foaming properties, FTIR and Differential scanning calorimetry were investigated. The results showed lower ($P < 0.05$) yield and protein recovery (17.74% and 24.94%, respectively) of the ChCl-AA DES than the other methods. The solubility, water holding capacity, emulsifying properties and foam capacity of alkaline extracted protein (AEP) and salt extracted protein (SEP) were significantly higher ($P < 0.05$) than ChCl-AA DES-assisted extraction protein (DEP). However, the oil holding capacity of the DEP was not significantly different from the SEP. In addition, the secondary structure of the protein in DEP was changed according to FTIR spectra. In addition, the lower denaturation enthalpy of DEP than other samples, supports this finding. The extracted proteins from sesame seed by-product can be used in food formulations duo to their acceptable functional and structural properties.

Keywords: Extraction methods; Functional and structural properties; Sesame by-product; Sesame protein concentrate; Extraction yield.

1. Introduction

Nowadays, the significant increase in the world's population (10 billion in 2050) has threatened food security (Sá, da Silva et al. 2021). Particularly, protein-energy malnutrition is a prevalent health issue in developing nations, leads to significant increase of morbidity and mortality rates, especially among newborns and children in low-income households (Khalid, Elhardallou and Elkhalifa 2012). From one hand, animal proteins are not readily available or affordable for many people due to population expansion and economic concerns. On the other hand, plant protein, especially proteins obtained from the by-products of food industry, is a good substitute for animal protein and provides protein deficiencies. In addition, plant proteins have health benefits, including low saturated fatty acid (Sá, Pacheco et al. 2022).

Oilseed meal, which is considered as a by-product of the edible oil industry after oil extraction were usually discarded or were used as animal feed or fertilizer (Sá, da Silva et al. 2021).The recovery of protein from this agricultural food by-product, can overcome the limitation of proteins and bioactive compounds duo to high protein content (15 - 50 %) of it (Sá, da Silva et al. 2021). Sesame seed is one of the oldest oilseeds used in the food industry; sesame seeds and their derived products are widely used in the production of various foods, including sesame oil, sesame paste, and food additives (Hou, Wang et al. 2025). Sesame seeds (Sesamum indicum L.) have been widely grown and recognized as "queen of oilseeds" due to their superior quality and quantity of sesame oil (Gandhi and Srivastava 2007, Onsaard and Singharaj 2015). The sesame seed is composed of approximately 50% oil and 25% protein (Onsaard, Pomsamud and Audtum 2010, Saini, Sharma and Sharma 2018). Also, it is a unique plant protein source in terms of its nutritive value, particularly sulfur-containing amino acids, which are often deficient in most plant proteins. Furthermore, other essential amino acids make up about 30% of total amino acids in sesame. High protein nutritive value, together with ease of digestion and absorption in the body, makes it an excellent plant protein source (Achouri, Nail and Boye 2012, Di, Li et al. 2022). Sesame meal, as a byproduct of sesame oil extraction, can be used as a protein source for human consumption due to its high protein content (approximately 50%) (Onsaard, Pomsamud and Audtum 2010, Onsaard and Singharaj 2015).

According to our literature review, conventional protein extraction techniques include alkaline extraction, salt extraction, and a new method such as extraction suing deep eutectic solvents (DES) (Cao, Zhang et al. 2023). The DES method are mixtures of a hydrogen bond acceptor (HBA) and one or more hydrogen bond donors (HBD) (Bai, Wei and Ren 2017, Jiang, Yuan et al. 2018). HBAs are frequently quaternary ammonium salts, such as choline chloride, while HBDs are often amines, carboxylic acids, alcohols, polyols, acid amides, and carbohydrates (Benvenutti, Zielinski and Ferreira 2019). The technique used to extract protein can have a notable impact on the functional characteristics of the protein obtained (Onsaard, Pomsamud and Audtum 2010). Although alkaline and salt extraction methods have been extensively applied to plant proteins, DES-assisted food-protein extraction remains comparatively underexplored. DESs are highly tunable systems, and the identity of the HBA and HBD, their molar ratio, and the water content can substantially influence solvent–protein interactions, extraction efficiency, protein conformation, and techno-functional properties. Consequently, the results obtained for a particular DES cannot be directly generalized to other formulations (Yue et al., 2021; Zhou et al., 2022).

However, the choline chloride–acetic acid system and its direct comparison with both alkaline and salt extraction have not been adequately investigated. Therefore, the aim of this study is to comprehensively compare the conventional (alkaline and salt extraction) and unconventional

(deep eutectic solvents) methods of plant protein extraction with respect to protein recovery, yield extraction and functional and structural properties of the extracted protein. The findings of this study can aid in the creation of a sustainable and effective protein extraction procedure from sesame meal that can be implemented for a range of purposes within the food industry, ultimately improving food security.

2. Materials and Methods

2.1. Materials

Sesame meal was provided from Dordaneh Mashregh Zamin Co. (Tehran, Iran). Analytical grade chemicals were used in this research, including NaOH and HCl purchased from Merck (Darmstadt, Germany), as well as choline chloride and acetic acid procured from EXIR GmbH (Wien, Austria).

2.2. Methods

2.2.1. Sesame meal flour preparation

Sesame meal obtained from cold press was dried for 24 hours at 40 °C in a hot air oven. Subsequently, the dried meal was ground using a high-speed multi-functional grinder (BEST Mill Model 3000A, China) and sifted through a 50-mesh screen. The resulting meal flour was packed into polyethylene bags and stored in a cool and dry location away from light.

2.2.2. Proximate composition of sesame meal flour

The moisture, ash, lipid and protein of meal flour were determined according to AOAC (2005) (Horwitz and Latimer 2005). Carbohydrate percentage was calculated based on the weight difference of other components out of 100.

2.2.3. Sesame protein extraction procedures

Techniques that were used in this study for protein extraction from sesame meal including alkaline extraction, salt extraction and choline chloride-acetic acid deep eutectic solvent extraction (ChCl-AA DES). The extraction method of each technique is described below.

In the alkaline extraction, 10 g of sesame meal flour was mixed with 200 mL of distilled water. The pH of the suspension was adjusted to 11 using 1M NaOH, and the extraction process was carried out for 1 hour using a magnetic stirrer at a temperature of 47 °C. Following the extraction process, centrifugation at 4000g for 15 minutes was performed to separate the supernatant from the sediment. The pH of the supernatant was then adjusted to the isoelectric point of sesame protein (pH 4.5) by using 1M HCl, resulting in the precipitation of the soluble protein. The precipitated protein was subsequently isolated by centrifugation at 8000g for 15 minutes, washed with distilled water, and neutralized to pH 7. Finally, the alkaline extracted protein (AEP) was freeze-dried and kept in a freezer at -10 °C for further evaluations (Onsaard, Pomsamud and Audtum 2010).

In the salt extraction, a total of 10 g of sesame meal flour was combined with 200 mL of 1M NaCl. The mixture was stirred for 1 hour at 47 ºC. After the extraction was completed, the solution of protein was precipitated by shifting the pH to the isoelectric point. The precipitated protein was extract by centrifugation at 8000g for 15 minutes, washed with distilled water, and neutralized to pH 7. Salt extracted protein (SEP) was freeze-dried and stored at -10 °C for further evaluations (Kanu, Kerui et al. 2007).

Protein extraction using DES was performed according to the method of (Rui-Lin 2017, Hernández-Corroto, Plaza et al. 2020) with minor modifications. The DES ingredients (HBA and HBD) and their levels were selected based on previous studies and pre-tests. Choline chloride (ChCl) and acetic acid (AA) were prepared, as HBA and HBD respectively, in the selected ratio of dry matter to solvent of 1:30. HBA and HBD solutions were mixed in the molar ratio of 1:1 at

80 °C for 45 minutes to 3 hours until a clear and stable liquid was formed. In the extraction process, 10 g of sesame meal flour was mixed with 300 mL of ChCl-AA DES, and the mixture was processed for 2 hours at 47 °C. After centrifugation at 4000g for 15 minutes, the supernatant was separated from the insoluble phase. The sesame protein was precipitated by adjusting the pH of the supernatant to 4.5 and then centrifuging at 8000g for 15 minutes. The protein was washed with distilled water, and the pH was adjusted to 7. Finally, the ChCl-AA DES-assisted extraction protein (DEP) was freeze-dried and preserved in a freezer at -10 °C for further evaluations.

2.2.4. Determination of sesame protein yield, purity and recovery

The protein content was measured based on kjeldahl nitrogen. Extraction yield and protein recovery were calculated according to formulas (Yue, Zhu et al. 2021):

Extraction yield (%) = $W_1/W_2 \times 100$

Protein recovery (%) = $(W_1 \times P_1)/ (W_2 \times P_2) \times 100$

Where W_1 is the weight of extracted protein, W_2 is the weight of sesame meal flour; P_1 and P_2 is protein content in the extracted protein and sesame meal flour, respectively.

2.2.5. Functional properties of sesame protein concentrate

2.2.5.1. Solubility

The pH of a protein concentrate dispersion in distilled water (1% w/w) was adjusted to 7 for determine protein solubility according to the method of (Britten, Giroux and Gaudin 1994). Protein dispersion was centrifuged at 20,000g for 15 minutes at 25 °C. The dispersion was diluted with buffer (50 mM EDTA, 8 M urea at pH 10) in the 1:10 (v/v) ratio. Subsequently, the absorbance of samples was recorded at 280 nm, and the solubility was determined according to Eq:

Solubility (%) = (Absorbance of the supernatant/ Absorbance of the dispersion before centrifugation) ×100

2.2.5.2. Water and oil holding capacity

One g protein concentrate was added in 10 mL distilled water or vegetable oil and vortexed for 1 min and then held for 30 min. The samples were centrifuged at 3000g for 10 min, the pellet was separated and weighed. Water (WHC) and oil (OHC) holding capacities were reported as the weight of water or oil absorbed to protein per initial weight of protein (Sá, Pacheco et al. 2022).

2.2.5.3. Emulsion activity index (EAI) and emulsion stability (ES)

The emulsifying properties of sesame extracted protein were determined according to the method of (Mastani, Bahmanyar et al. 2024) with slight modifications. Ten mL of vegetable oil were homogenized with 10 mL of protein dispersion (1% w/w) in a rotor–stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at speed 5 for 2.5 min. After centrifugation at 1000g for 5 min, the height of the emulsified layer was measured, and the EAI was calculated using the following Eq:

EAI (%) = (Height of the emulsified layer in the tube/ Height of the total content in the tube) ×100

To determination of ES, emulsions were subjected to heating at 80 °C for 30 min before centrifugation and calculated according to Eq:

ES (%) = (Height of the emulsified layer in the tube after heating / Height of the total content in the tube) ×100

2.2.5.4. Foaming capacity (FC) and foam stability (FS)

The FC and FS of protein concentrate were measured according to the method of (Mirmoghtadaie, Kadivar and Shahedi 2009) with slight modifications. 33.3 mL protein dispersion (3% w/v) was stirred for 45 min with a magnetic stirrer, followed by exactly 6

minutes at speed 4 in a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany). The mixture was promptly transferred to a 500 mL measuring cylinder, and the quantity of foam was noted. The FS was gauged by reading the foam volume after 10, 30, 60, and 120 minutes.

2.2.6. Physical and structural properties of sesame protein concentrate

2.2.6.1. Fourier-transform infrared spectra (FTIR)

FT-IR spectra were obtained utilizing a FT-IR spectrophotometer (Perkin Elmer Spectrum RX I, USA) that covers the wavenumber range from 400 to 4000 cm^{-1}. To prepare the pellet, dried samples were mixed with KBr and compressed. Each spectrum was generated by collecting 16 scans at a resolution of 4 cm^{-1} (Mastani, Bahmanyar et al. 2024).

2.2.6.2. Differential scanning calorimetry (DSC)

To obtain the DSC thermograms of the samples, a Shimadzu DSC-60 differential scanning calorimeter (Shimadzu, Kyoto, Japan) was employed. A total of 2.88 mg of each sample was sealed hermetically in aluminum pans, with the empty aluminum pan serving as a reference. The samples were heated from 25 to 135 °C at a scanning rate of 10 °C/min.

2.3. Statistical analysis

The measurements were performed in triplicate, and statistical analysis was conducted using SPSS vs.21 software. To evaluate the data, one-way analysis of variance (ANOVA) was utilized and the significance of the results was determined by Duncan's multiple range test with a 95% confidence interval. The results were presented mean values ± standard deviation.

3. Results and discussion

3.1. Yield, purity and recovery of the sesame extracted proteins

Table 2 shows the yield and purity of extracted products, and protein recovery. The alkaline extraction demonstrated a greater ($P < 0.05$) yield while the salt extraction resulted in a purer protein powder. When protein recovery was taken into account, the results showed that the alkaline and saline extractions were not significantly different, but were significantly higher ($P < 0.05$) than ChCl-AA DES-assisted extraction. The yield, protein content, and protein recovery rate of the ChCl-AA DES-assisted extraction were 17.94%, 59.4%, and 24.94%, respectively, and were significantly lower than those of the salt and alkaline methods in all parameters. Previous studies (Grudniewska, de Melo et al. 2018, Yue, Zhu et al. 2021); also achieved similar results for DES-assisted extraction. In line with these results, (Cao, Zhang et al. 2023) reported that the alkali-soluble acid precipitation extraction has a higher extraction rate compared to the DES-assisted extraction from sesame meal. These authors noted that the variation in extraction rate may be due to differences in extraction principles (Cao, Zhang et al. 2023). DES-assisted extraction depends on the hydrogen bond between HBA and HBD, and then forms hydrogen bonds with the protein. So, different types of DES-assisted extraction exhibit varying physical and chemical properties, resulting in distinct protein extraction yield (Cao, Zhang et al. 2023).

3.2. Functional properties of protein concentrates extracted through different methods

3.2.1. Solubility

Protein functional properties play a crucial role in food processing and formulation, and among them, solubility is an important factor that can impact other protein functional properties (Koysuren, Oztop and Mazi 2021). Protein solubility is promoted by electrostatic repulsion, ionic hydration, and the net charge of the protein at pH higher and lower than the isoelectric pH (Achouri, Nail and Boye 2012). Fig.1. shows that the solubility of AEP and SEP was not significantly different from each other, but these are significantly higher than DEP. Protein solubility depends on the secondary structure of the protein (Yue, Zhu et al. 2021).

In the ChCl-AA DES-assisted extraction, choline chloride and acetic acid establish extensive hydrogen-bonding interactions with the protein backbone, disrupting the native hydrogen-bonding network and promoting conformational rearrangements. These structural changes may facilitate protein aggregation, reducing protein–water interactions and consequently decreasing protein solubility, which is consistent with the FTIR and DSC results. Additionally, the lower amount of protein in the ChCl-AA DES-assisted extraction and the presence of more impurities may contribute to the low protein solubility. Also, higher solubility in alkaline and salt extraction may be due to the electrostatic repulsion of proteins, which prevents protein aggregation and increases protein-solvent interaction (Sharma, Singh and Sharma 2016). Under alkaline extraction, increases the negative charge of protein molecules leading to electrostatic repulsion and partial unfolding of the protein structure. Also, salt extraction mainly modifies electrostatic interactions and hydration of protein molecules.

3.2.2. Water and oil holding capacity

WHC refers to the protein's capacity to hold water, which may be indicative of its texture and viscosity, while OHC indicates a protein's ability to hold lipids and reflects its thickening and emulsifying properties (Ma, Ren et al. 2018, Gundogan and Karaca 2020). The characteristics of WHC and OHC are linked to the polarity of the protein side chains as well as the hydrophobicity, level of denaturation, size, and flexibility of protein (Achouri, Nail and Boye 2012). The results of WHC and OHC of protein under different extraction methods are presented in Table 3. There were no significant differences in WHC between SEP (1.83g/g) and AEP (1.79g/g) but, they were significantly more than DEP (1.15g/g). Protein extraction using DES can alter protein secondary structure (Yue, Zhu et al. 2021). According to our observations, these changes led to a decrease in polarity, resulting in reduced protein solubility and WBC. These findings confirm the results of solubility in previous step.

The OHC (1.91 g/g) of AEP increased significantly and no significant difference was observed between SEP and DEP. Several previous studies have indicated a direct relationship between OHC and surface hydrophobicity. The increase in the OHC of AEP in this study may be to the extraction condition of AEP which resulted in the unfolding of protein structures, exposing hydrophobic group on the surface of protein molecules (Olasunkanmi, Omolayo and Olusegun 2017).

In summary, the characteristics of WHC and OHC are linked to the polarity of the protein side chains as well as the hydrophobicity, level of denaturation, size, and flexibility of protein (Achouri, Nail and Boye 2012).

3.2.3. Emulsifying properties

Proteins possess emulsifying activity due to the simultaneous presence of polar and nonpolar/charged and uncharged amino acids (Kanu, Kerui et al. 2007). The EAI of proteins is related to their ability to disperse oil droplets in the liquid phase by adequately covering the oil droplets, which prevents oil phase coalescence (Onsaard, Pomsamud and Audtum 2010). Several factors, including pH, droplet size, net charge, surface tension, viscosity, and protein configuration, can affect emulsion stability (Onsaard, Pomsamud and Audtum 2010). The EAI showed (Table 3) that there was no significant difference between AEP and SEP, but they were significantly higher than DEP. Also, SEP and AEP showed the highest ES, respectively. Several studies have reported a positive relationship between solubility and hydrophobicity, and emulsification (Mirmoghtadaie, Kadivar and Shahedi 2009, Cano-Medina, Jiménez-Islas et al. 2011, Nazari, Mohammadifar et al. 2018, Di, Li et al. 2022). Higher hydrophobicity allows protein molecules to reach the water-fat interface more quickly (Di, Li et al. 2022). So, the use of

NaOH in alkaline extraction can cause protein denaturation and exposure the hydrophobic groups on the surface of unfolded protein which leads to improved emulsification properties of AEP (Kanu, Kerui et al. 2007). Also, in salt extraction, proteins absorb a lot of chloride ions, which can increase the non-polar parts and surface hydrophobicity of protein thereby improving its emulsifying properties of SEP (Achouri, Nail and Boye 2012).

The emulsifying properties of the DEP was significantly lower compared to AEP and SEP. The presence of more protein molecules with ability to reach to the oil-water interface leads to more coverage of oil droplets and more emulsifying properties (Mirmoghtadaie, Kadivar and Shahedi 2009). Therefore, the lowest emulsifying properties of ChCl-AA DES can be due to its more impurities compared to the other two samples. Additionally, the alterations in the conformation of ChCl-AA DES, as shown by FTIR, may also affect its emulsifying properties (Zhao, Liu et al. 2012).

3.2.4. Foaming properties

Foaming properties of sesame protein through three different extraction methods are shown in Table 4. Numerous factors can impact the foaming properties of proteins including protein solubility, hydrophilic-lipophilic balance of amino acids, protein conformation, the composition of the protein fractions, the extraction method, pH and temperature (Onsaard, Pomsamud and Audtum 2010, Achouri, Nail and Boye 2012).

The results showed that SEP had the highest ($P < 0.05$) FC. The higher FC of SEP compared to AEP can be attributed to the higher protein content in SEP (88.64% vs. 73.24%). In contrast, the ChCl-AA DES-assisted extraction showed lower FC than the salt and alkaline extraction due to their lower protein content and solubility. These findings confirm results of previous steps.

Based on the results of FS, the SEP showed the highest FS in the first 10 min. After 10 min, there was no significant difference between AEP and SEP, and both showed more stability than DEP. The low FS of all three samples in long term can be attributed to the low intermolecular interactions between the proteins, resulting in a lack of formation of a mechanically stable and cohesive protein multilayer film between liquid and air phase, which can to prevent protein coalescence (Mirmoghtadaie, Kadivar and Shahedi 2009).

3.3. Structural and thermal properties of protein concentrates extracted through different methods

3.3.1. Fourier-transform infrared spectra

FTIR spectroscopy is a widely used and effective method to confirm protein conformational changes during extraction. The characteristic absorption bands of protein IR spectrum usually include nine bands (Surewicz, Mantsch and Chapman 1993). Among them, two notable absorption bands are related to amid I (1700–1600 cm^{-1}) and amide II (1575–1480 cm^{-1}), which are the main vibrational bands of the polypeptide backbone and are referred to the C=O stretching vibrations and C—N stretching vibrations of peptide bonds, respectively (Bahmanyar, Pashaei et al. 2025). The amide I region is recognized as the most sensitive part of the spectrum to protein secondary structure alterations (Xu, Wang et al. 2015).

As shown in Fig. 3, two main bands of amide I and II are present in AEP and SEP spectra, at almost similar wavelengths of 1659 and 1657 cm^{-1} for amide I and 1530 and 1535 cm^{-1} for amid II, respectively, and they are far from the DEP sample. In general, Certain structural (α-helix, β sheets, etc.) constituents have formed the protein's secondary structure (Zhao, Ma et al. 2004). When the amide I peak is located around 1650-1660 cm^{-1}, such as for AEP and SEP, the protein has high α-helical contents.

In the DEP sample, the absorption bands were shifted or disappeared and its absorption spectrum pattern was different from the other two samples. This might be implied that the functional

groups of peptide bonds have possibly been broken or altered. As affected by Choline Chloride-Acetic acid, the secondary structure of sesame protein has probably been altered (Bai, Wei and Ren 2017).

3.3.2. Differential scanning calorimetry

The thermal characteristics of sesame proteins were explored using DSC. Table 5 represents T_o (onset denaturation temperature), T_p (peak denaturation temperature), T_e (temperature of denaturation end set), and ΔH (enthalpy of denaturation) of all three protein concentrates. T_p is the temperature at which protein denaturation takes place, and ΔH is the energy needed for protein denaturation (Kaushik, Dowling et al. 2016). These are two considerable indicators of protein thermal properties (Di, Li et al. 2022).

As seen in Table 5, the T_p and ΔH for AEP are lower than those for SEP, which may be due to the relative structural unfolding for AEP during the extraction process. (Kanu, Kerui et al. 2007, Koysuren, Oztop and Mazi 2021) observed similar results and reported that unfolding of the protein conformation through salt extraction is unlikely. As shown in Table 5, the enthalpy of the DEP sample is much lower than that of the AEP and SEP, which may indicate less energy required to unfold the DEP protein structure. This issue, as mentioned in the FTIR section, can be related to alterations in the protein structure as well as changes in the intra and intermolecular bonds of the sesame protein as a result of extraction using ChCl-AA DES.

4. Conclusions

The present study aimed to extraction the protein from sesame meal as a byproduct of the oil factory. In this respect, alkaline and salt extraction as conventional methods, and ChCl-AA DES-assisted extraction as novel method were selected. The protein recovery of alkaline and salt extraction was not significantly different from each other and was almost twice the ChCl-AA DES-assisted extraction. The solubility, WHC, EAI, ES, FC and FS 30, of AEP and SEP were significantly higher than DEP. But there were no significant differences in solubility, WHC and EAI between AEP and SEP. Moreover, the OHC of the DEP was not statistically different from the SEP ($P < 0.05$). The FTIR spectrum showed that the secondary structure of DEP was different from the other two samples. The results obtained by DSC also show a much lower enthalpy for the DEP samples compared to other samples. This means that the structural order of this sample is reduced compared to other samples, and less energy is required for denaturation. Overall, based on the findings, the protein extracted through conventional methods showed better functional properties, while the recovery of protein extraction was higher compared to the ChCl-AA DES-assisted extraction. Since the extraction performance of DESs is highly dependent on HBA/HBD composition and the subsequent protein-recovery procedure, it is recommended to study other solvents as hydrogen bond donors in order to remove the limitation of the ChCl-AA DES-assisted extraction. Moreover, the findings of this study introduce a sustainable and effective protein extraction procedure from sesame meal that can be implemented for a range of purposes in the food industry, including salad dressings, sauces, meat products, bread, and cakes.

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Statements and Declarations

The authors declare that there is no conflict of interest.

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Table 1. Proximate composition (%) of cold-pressed sesame meal.

Sample	Protein	Fat	Moisture	Carbohydrate	Ash
CSM	42.72 ± 0.74	12.2 ± 0.64	3.4 ± 0.18	31.18 ± 0.94	10.5 ± 0.46

Table 2. Protein yield, purity and efficiency of different methods extractions.

Extraction methods	Extraction yield (%)	Purity of Protein (%)	Protein recovery (%)
Alkaline extraction	30.43 ± 1.92^{a}	73.24 ± 0.02^{b}	52.16 ± 3.29^{a}
Salt extraction	25.23 ± 1.76^{b}	88.64 ± 0.04^{a}	52.34 ± 3.67^{a}
DES extraction	17.94 ± 1.21^{c}	59.40 ± 0.03^{c}	24.94 ± 1.69^{b}

Different letters show significant differences in each column by Duncan test ($P < 0.05$).

Table 3. Functional properties of protein comcentrate.

Treatments	WHC (g/g)	OHC (g/g)	EAI (%)	ES (%)
AEP	1.79 ± 0.06^{a}	1.91 ± 0.04^{a}	33.95 ± 0.94^{a}	30.18 ± 1.63^{b}
SEP	1.83 ± 0.05^{a}	1.42 ± 0.03^{b}	35.29 ± 1.69^{a}	33.33 ± 0.98^{a}
DEP	1.15 ± 0.03^{b}	1.46± 0.04^{b}	28.18 ± 1.57^{b}	23.63 ± 0.91^{c}

Different letters show significant differences in each column by Duncan test ($P < 0.05$).

Table 4. Foaming properties (mL) of protein comcentrate.

Treatments	FC	FS 10	FS 30	FS 60	FS 120
AEP	53.33 ± 1.52^{b}	51.66 ± 1.52^{b}	47.33 ± 2.51^{a}	36.66 ± 1.52^{a}	33.66 ± 0.57^{a}
SEP	67.33 ± 1.52^{a}	61.00 ± 1.00^{a}	46.33 ± 1.15^{a}	35.66 ± 0.57^{a}	34.00 ± 0.00^{a}
DEP	42.66 ± 0.57^{c}	41.00 ± 1.00^{c}	37.33 ± 0.57^{b}	35.66 ± 0.57^{a}	33.66 ± 0.57^{a}

Different letters show significant differences in each column by Duncan test ($P < 0.05$).

Table 5. Thermal properties of protein concentrate.

Treatments	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)
AEP	48.4	80.3	105.2	84.17
SEP	67.8	93.7	96.5	126.5
DEP	66.4	92.8	94.7	17.06

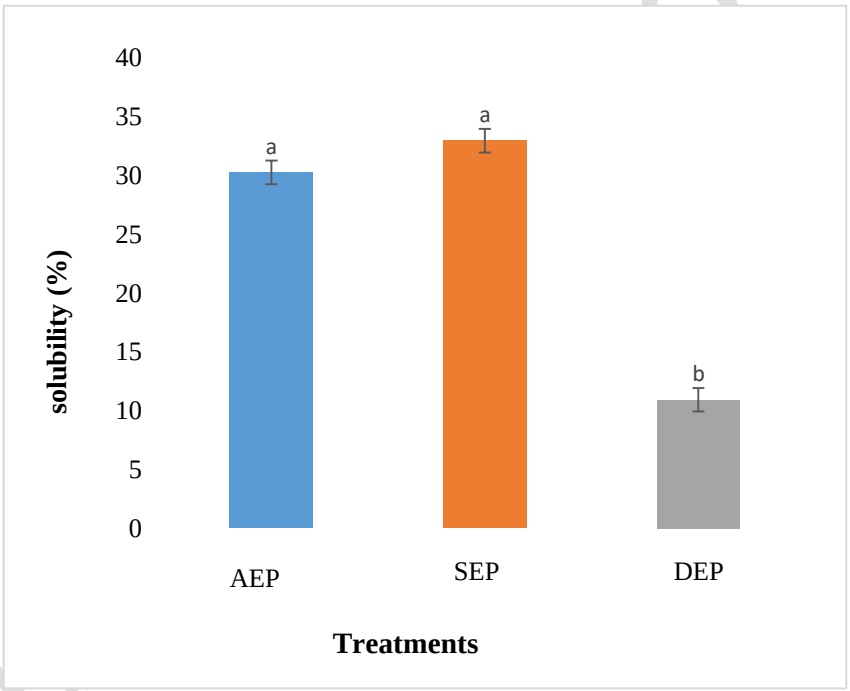

Fig.1

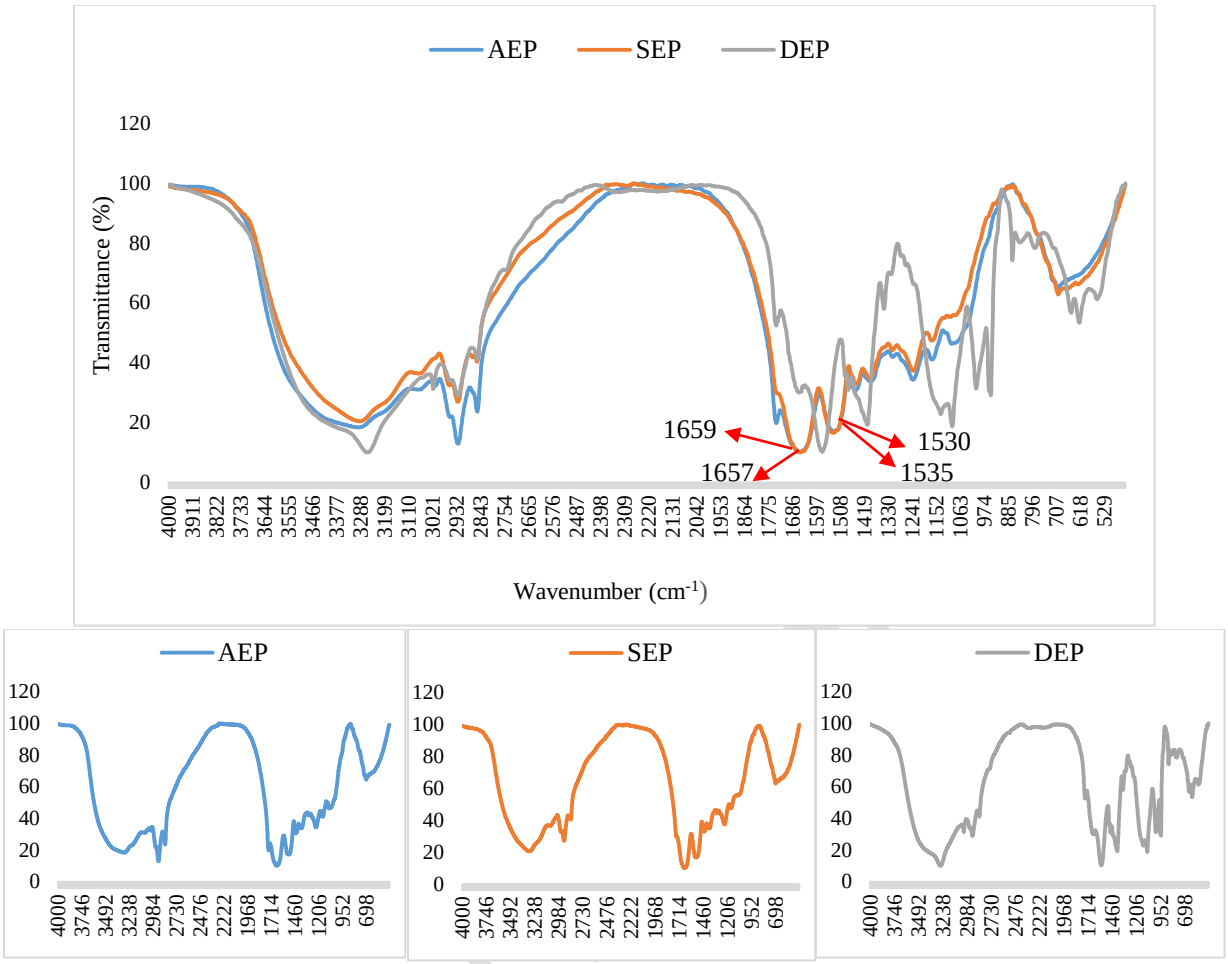

Fig.2

Figure Caption:
Fig.1. Solubility of extracted protein comcentrate through different methods.
Fig.2. FTIR spectra of extracted protein comcentrate through different methods.