Sorghum Oleoresins: Effect of Extraction on Compositional and Structural Characteristics

Eda Kaya[1], Umut Yucel[1], Shanta Peiris[2], and Fadi Aramouni[2]

[1]Kansas State University
[2]USDA-ARS

June 19, 2023

Abstract

Oleoresins are resin-like viscous materials obtained from plants, oilseed, or spices with functional properties. The extraction process determines their stability, composition, and physicochemical properties. Oleoresins were obtained from ground waxy burgundy whole grain sorghum with and without ball milling by using the following solvents: two types of novel ionic liquids (IL1: 1-n-Hexyl-3-methylimidazoliumchloride, IL2: 1-Ethyl-3-methylimidazoliumchloride), ethanol and dichloromethane. The effects of processing were evaluated for the extraction yield, protein, fat and total phenolic content, fatty acid composition, particle size and zeta potential, and FTIR spectra. The use of ILs and ball mill process significantly ($P < 0.05$) affected the extraction yield and physicochemical properties. The highest extraction yields increased (31.35% $\pm$ 0.58) when ball milling used with IL2 in comparison to the lowest (18.37% $\pm$ 0.77) obtained by traditional ethanol extraction. In a similar way, protein concentration and phenolic content were the highest (1.37% $\pm$ 0.05 and 0.57% $\pm$ 0.01, respectively) with ball milling extraction and IL1. The FTIR spectra indicated higher phospholipids (at 1200 cm-1) and protein-phospholipid bonding (at 1700 cm-1) by ILs, and ball milling as compared to traditional extraction. Overall, wet milling-assisted extraction by using ball mill and ILs can provide control for the composition of the oleoresins important for their functional properties with higher extraction efficiencies as compared to traditional techniques.

Introduction

Oleoresins are phytocomplexes extracted from plant sources, herbs and oilseeds as oil-rich viscous materials. These resinous extracts include terpenoids, fatty acids, phospholipids, essential oils, proteins, and phenolic compounds (Napoli et al., 2019). Oleoresins offer great potential for value-added food applications (e.g., beverages, sauces and meat products, baked products, and confectionaries) related to their chemical stability, uniformity, complex composition of biopolymers, lipids, polar lipids, and health beneficial phytochemicals. Oleoresins carry surface active molecules, such as phospholipids and proteins. The interactions between these proteins, triacylglycerols and phospholipids are responsible for the integrity and stability of their dispersions and emulsions.

The extraction method plays an important role in the stability, composition, yield and structural characteristics of oleoresins. Conventional solvent extraction involves the use of organic solvents, such as ethanol, hexane or ethyl acetate, to simultaneously obtain volatile and non-volatile phytochemicals with functional properties (Khasanah et al., 2017). Ultrasound- and microwave-assisted extraction systems were used to increase the extraction yield (Ayub et al., 2023). Wet milling strategies, such as ball milling, can be used to improve the extraction efficiency by breaking solid enclosures where the oleoresins are trapped. For example, ball milling was used to extract sesame paste from seeds with improved viscosity and reduced particle size (Jin et al., 2022). A similar strategy was used for the improving the functional properties of the proteins, such as surface activity, solubility, and hydrophobicity of proteins extracted from hempseed (Julakanti et al., 2023).

In addition to the extraction method, the type of solvent determines the extraction efficiency and physicochemical properties of the oleoresins. The poly-ionic liquids (ILs) are molten organic salts below 100°C, which combine the characteristics of different ionic chemicals, received considerable attention as substitutes of volatile organic solvents due to their versatility, less toxicity, remarkable solubilization for organic and inorganic compounds (Tolesa et al., 2017). ILs offer great potential to enhance the extraction efficiencies for complex material, such as oleoresins containing biopolymers, surface active molecules and small phytochemicals and to provide control over their composition. Among the four general groups (imidazolium, phosphonium, pyridinium, and ammonium), imidazolium based ILs were widely used for their low viscosity and stability in oxidative and reductive conditions. In addition, they can also provide high affinity for lipids and phenolic molecules as compared to conventional organic solvents, such as ethyl acetate, methanol, and hexane. The objectives of this study are to investigate the combined effects of wet milling (i.e., ball milling) and novel ILs (1-n-Hexyl-3-methylimidazolium chloride, 98% and 1-Ethyl-3-methylimidazolium chloride, 97%) on the on the extraction efficiency of oleoresins from waxy sorghum rich in phenolics and evaluate their yield, composition (protein, lipid, fatty acid, phenolic contents) and structural properties of their dispersions (particle size, zeta potential and FT-IR spectrum) in comparison to conventional solvent extraction (i.e., ethanol and dichloromethane).

2. Experimental Procedures

2.1. Materials

The extraction solvents ethanol (99.5%, ACS reagent), dichloromethane (99.5%, ACS reagent), and ionic liquids (ILs) (IL_1: 1-n-Hexyl-3-methylimidazolium chloride, 98% and IL_2: 1-Ethyl-3-methylimidazolium chloride, 97%) were purchased from Fisher Scientific (Fisher Scientific, Waltham, MA, USA). The selection of ionic liquids was based on previous literature for higher affinity for polar lipids and phenolic molecules. The solutions of ILs (40 mL) were prepared by mixing two ionic liquids with ethanol (99%) as co-solvent at 1:2 (w/v) ratio. The waxy burgundy whole grain sorghum was kindly donated by Nu Life Market LLC (Nu Life Market LLC, Scott City, KS, USA).

2.2. Preparation of Oleoresin

Oleoresin were extracted from ground waxy burgundy whole sorghum grain with or without wet milling using traditional (ethanol, dichloromethane) and novel solvents (IL_1 and IL_2). The traditional extraction without ball milling was done following the method of Khasanah et. al (2017) with some modifications. A batch of whole grain sorghum (50 g) was soaked in $NaHCO_3$ (0.1 M) at 1:1 (w/v) ratio 12 h at 21 ± 2 °C. The soaked sorghum grains were ground using a high-shear blender (Waring Commercial, Model 7011BU, McConnellsburg, PA, USA) at the highest speed (22000 rpm) with three-time intervals of 5 minutes. The ground sorghum (10 g) and the solvent (40 mL) were mixed using a magnetic stirrer for 30 minutes. The slurry was filtered through a cheese cloth to remove the undissolved solids. The filtrate was centrifuged at 3000 x g for 15 min at 20 ± 2 °C to separate oleoresin-rich organic top layer, which was collected in hermetically sealed glass tubes. The extraction from the filtrate repeated three times until no sediment was visible. The weight of sediment and organic phase was recorded in each dilution step for calculation of the extraction yield. The oleoresins were concentrated by removing the solvent using a refrigerated vapor trap (Savant SpeedVac, RVT5105, Model SPD300DDA, Thermo Fisher Scientific, Marietta, OH, USA). The wet milling extraction was done using the same sorghum and solvent mixture by using a ball mill (Retsch, PM 100 type, Retsch GmbH, Germany) equipped with a milling bowl (250 mL) and ceramic balls (diameter:10 mm; quantity: 100 pieces) and operated at 500 rpm with 2 cycles of 15 minutes. The filtrate containing the oleoresin was separated and concentrated similar to traditional extraction.

2.4. Oleoresin Characterization

2.4.1. Extraction Percentage Yield

The extraction yield was calculated following the Equation 1:

Extraction yield (%) $= \frac{wt[?]}{\mathrm{wt}[?]} \quad x\ 100$ (1)

where wt_e is the total weight of the extracted oleoresin and wt_a is the weight of the whole grain sorghum.

2.4.2. Total Protein Content

The total protein content was analyzed with the Dumas technique at the Soil Testing Laboratory, Kansas State University. The oleoresins (0.15 +-0.01 g) were combusted, and the nitrogen content was measured on weight percent basis by using a LECO TruSpec CN Combustion Analyzer (TrueSpec Micro, LECO, St. Joseph, MI, USA). EDTA was used as the calibration protein, and the total protein content of samples was calculated by multiplying the total combustion nitrogen with a factor of 6.25.

2.4.3. Total Lipid Analysis

Total lipid content was determined following a gravimetric method. An aliquot of oleoresins (1 g) added in a dry glass tube (15 mL) with chloroform (2 mL) and methanol (4 mL) and sealed with Teflon-lined screw caps. The tube was shaken well for 10 minutes at 325 rpm. Then, additional chloroform (2 mL) and water (2 mL) were added, and the tube was shaken for another 10 minutes. Then, the tube was centrifuged at 2300 rpm for 10 min to separate the organic phase containing the lipid from the aqueous phase. The organic top layer was collected using a Fisherbrand borosilicate glass Pasteur pipet (Fisher Scientific, Waltham, MA, USA). Additional chloroform (4 mL) was added to the tube, centrifuged at 2300 rpm, and the top layer with lipid collected. The solvent was evaporated at 60 degC, and the total lipid in oleoresin sample was calculated using Equation 2:

$$\text{Total lipid content (wt\%)} = \frac{(W2-W1)x\ V[?]\ x\ 100}{(V[?]\ x\ S[?])} \quad (2)$$

where W_2 is the total weight of the glass tube and the extract (g), W_1 total weight of empty dried glass tube (g), V_l is the total volume of chloroform layer (mL), V_a is the volume of the extract (mL) and S_x is the weight of the oleoresin (g).

2.4.4. Fatty Acid Composition

The fatty acid composition was measured using the fatty acid methyl esters (FAME) method described by Taghvaei et al. (2021). In brief, an aliquot of oleoresin (1 mL) was transferred into glass test tube (15 mL) with boron trifluoride (14%) in methanol (3 mL) and heated at 100°C for 40 minutes. Saturated NaCl solution (15 mL) and hexane (3 mL) was added, and the tube was inverted 3 times. The mixture was allowed to settle for a few minutes for the separation of organic and aqueous phases. The organic top layer was collected using a Pasteur pipette into a clean test tube, where a small amount (1 g) of anhydrous sodium sulfate was added to remove any residual moisture. The methylated organic layer (1 µL) was injected into a GC-MS system (HP 5890–5973 MS, Agilent) equipped with an HP-23 FAME capillary column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA). Helium (1 mL/min) was used as the carrier gas. The initial oven temperature was 60 °C ld for 0.5 min), then brought to 150 °C at a rate of 5°C / min, followed by an increase of 15°C / min to a final temperature of 230°C. The program was finalized with a hold at 230°C for 4 min. In addition to the National Institute of Standards and Technology (NIST) 2014 database, a FAME standard (> 95 %, FAME Standard Mixture, St, Louis, MO, USA) over a concentration range of 1.25 -10 mg/mL was used for identification and quantification. The quantification was performed by using the peak areas.

2.4.5. Phenolic Content and Composition

The total phenolic content was determined using Folin Ciocalteu method (Lamuela-Raventós, 2017). The calibration curve was plotted by mixing aliquots (1 mL) of Gallic acid solutions over a range of concentrations (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) with Folin Ciocalteu reagent (5 mL) and anhydrous sodium carbonate solution (4 mL). The phenolic content of the oleoresin was measured by mixing the sample (2.5 mL) and Folin Ciocalteu- anhydrous sodium carbonate reagents at the 1:1 (v/v) ratio. The solution was kept for 1 h at 25°C. The absorbance was measured at 725 nm using a spectrophotometer. Total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of oleoresin (mg GAE/g oleoresin) using Equation 3:

$C = C_1 \text{ x } \frac{V}{m}$ (3)

where C is the total phenolic content (mg/g) in GAE, $C_1$is the concentration of gallic acid established from the calibration curve in mg/mL, V and m are the volume and weight of oleoresin, respectively.

The phenolic compounds were determined using the protocol described by (Tohma et al., 2017). The samples were prepared by mixing oleoresin (20 mg) with a mixture (2mL) of methanol:ethanol (90:10 v/v) in a glass tube with screw top (15 mL) and shaken in a wrist shaker for 1 h. Then, the solution was filtered with a PTFE filter (0.45 µm) and transferred to a 1 mL polypropylene vial (12 mm x 32 mm). The analysis was conducted using an HPLC system (Agilent Technologies, 1100 series, Santa Clara, CA, USA) equipped with a C18 column (TOSOH Bioscience LC, TSKgel ods-80, 4.6 mm ID×25 cm, 5µm Millipore Sigma, MA, USA) and a diode-array detector (DAD) (Hewlett-Packard, Palo Alto, CA, USA) operated at 260-430 nm. The mobile phase (elution rate of 1.0 ml/min) was composed of acetonitrile (solvent A), methanol (solvent B) and water with acetic acid (solvent C) with a gradient system as follows: 0-5 min 5-8% A, 5-8% B and 90-84% C; 5-15 min 8-10% A, 8-10% B and 84-80% C; 15-25 min 10-25% A, 10-0% B and 80-75% C; 25-35 min 25-30% A, 75-70% C; 35-45 min 30-60% A and 70-40% C. The injection volume was 20 µL and the column temperature was 30°C. Each phenolic compound was identified by their retention time in comparison with standard compounds. The peak areas were quantified using Chem Station software as areas under the curve and calibration curve prepared by their analytical standards. The concentration of each phenolic compound in oleoresin samples was determined using Equation 4:

$\text{Concentration of compound} = \frac{\text{Area of sample}}{\text{Area of standard}} \text{ x Concentration of standard}$ (4)

2.4.6. Fourier Transform Infrared (FTIR) Analysis

The FTIR analysis was conducted at the Center for Grain and Animal Health Research (USDA-ARS, Manhattan, KS, USA). Attenuated total reflectance (ATR)-FTIR spectra collected at 400–4000 cm^{-1} range with a resolution of 4 cm^{-1}, data spacing of 0.482 cm^{-1}, and 32 scans using a Thermo Nicolet iS50 FTIR (Thermo Fisher Scientific Co., Waltham, MA) spectrometer equipped with a single-bounce diamond crystal and a deuterated triglycine sulfate detector. Measurements were repeated twice for each sample at ambient temperature. The quantitative analysis was done by "peak fitting" procedure and individual component bands according to a Gaussian curve fit (GRAMS/AI Spectroscopy Software Suite, Thermo Scientific, Waltham, MA, USA).

2.4.7. Particle Size and Zeta Potential of Oleoresin

Particle size and zeta potential were analyzed using a dynamic light scattering (DLS) particle size analyzer (Delsa Max Pro, Beckman Coulter, Kraemer Blvd. Brea, CA, USA) at 25 °C. Prior to analysis, oleoresins were diluted in ultra-pure water at the 1:100 (w/v) ratio and dispersed using an ultrasound bath for 10 minutes and high-shear homogenizer (Omni THModel TH-115, Omni International, Kennesaw GA, USA) for 10 min with inner and outer probe diameters of 7 mm x 125 mm (de Aguiar et al., 2021; Nikiforidis & Kiosseoglou, 2009). The measurements were repeated twice for each replication and their average was used.

2.5. Statistical Analysis

All samples were prepared in triplicate. The experimental data were analyzed using Minitab (version 17.1, LLC, State College, PA, USA) and the values were expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to analyze significant differences among samples at $\alpha = 0.05$.

3. Results and Discussion

3.1. Effect of extraction conditions on yield

The solvent type had a significant ($P < 0.05$) effect on the extraction yield of oleoresins (Fig. 1). Overall, ILs increased ($P < 0.05$) the extraction yield (25.11 ± 0.84 wt% for IL_1 as compared to 18.37 ± 1.34 wt% for ethanol) via traditional extraction (i.e., without ball milling) (Fig.1). The higher extraction efficiencies

with the imidazolium based ILs were as expected due to the combined extractive power of the composite systems. In a similar way, other researchers showed that imidazolium based ILs can increase the extraction yield of bioactive phytochemicals, such as flavonoids, alkaloids, caffein, etc., from plant tissue (Bogdanov & Svinyarov, 2013; Claudio et. al, 2013). The increase in the extraction efficiencies can be explained by the combination of dipole, ionic, and hydrophobic interactions of the IL components. In parallel to the extraction efficiency, lipid, protein, and phenolic compositions were also significantly ($P < 0.05$) affected by the extraction conditions as discussed in the following sections (Table 1). The efficiency of traditional solvent extraction with ethanol and dichloromethane was the same ($P > 0.05$); however, the compositions of their oleoresin extracts were different. There was no significant difference ($P > 0.05$) in the extraction yield of two ILs as well (Fig. 1). Thus, the difference between the type of charged groups in ILs was not responsible for breaking the solid enclosure required for extraction. In a recent study, Cheng et. al (2020) used different ILs with 11 anions and 8 imidazolium-based cations including the ILs in our study for extraction of isoflavone compounds from *Puerariae lobatae.* They showed that the extraction efficiencies of the ethyl and hexyl substituted imidazolium ILs were the same, yet changed with ion concentration in their solution. Therefore, we can conclude that imidazolium ILs regardless of the cation and anion composition were more effective than traditional solvents due to their ability to disrupt cellular structure that determine extraction capacity. On the other hand, the chemical difference between the two ILs affected the oleoresin composition (Table 1) as discussed later.

The wet milling process with a ball mill significantly ($P < 0.05$) increased the extraction yield as compared to traditional solvent extraction. For example, the extraction yield increased more than 50% when IL_2 was used with ball mill (31.36 ± 1.01 wt%) as compared to traditional batch extraction (18.92 ± 0.68 wt%) (Fig. 1). This can be explained by the ability of ball mill to break down the integrity of the hard enclosure trapping the oleoresins via omnidirectional impact of the beads and associated high shear.

3.2. Oleoresin Composition

In parallel with the extraction efficiency results, it was not surprising to observe that the solvent type had significant ($P< 0.05$) effect on the total lipid, protein and phenolic content of oleoresins. Following the traditional solvent extraction approach, the highest protein (0.67 ± 0.03 wt%), lipid (69.22 ± 0.04 wt%) and phenolic contents (48 ± 0.01 mg GAE/g) were obtained with IL_1 (Table 1). The lowest extraction for protein (0.36 ± 0.02 wt%) was obtained using dichloromethane, while the lowest lipid (53.12 ± 0.08 wt%) and phenolic contents (21 ± 0.02 mg GAE/g) were observed using ethanol. Wet milling extraction with a ball mill increased ($P < 0.05$) the total lipid (82.19±0.07 wt%), protein (1.37 ± 0.05 wt%), and phenolic content (57 ± 0.01 mg GAE/g) of oleoresin when IL_1 used as compared to total lipid (69.22 ±0.04 wt%), protein 0.67 ± 0.03 wt%), and phenolic content (48 ± 0.01 mg GAE/g) with traditional extraction (Table 1). In a similar way to traditional extraction, IL_1 and IL_2 were same ($P> 0.05$) for lipid, protein, phenolic content. Jin et. al (2022) also found that using ball mill increased the overall content of protein, fat, and volatile compounds in the sesame seed extracts as compared to colloid and stone mills. The increase was higher for protein (51.1%) than lipid (15.8 %) at the conditions for the highest extraction yield when IL is used (Table 1).

The fatty acid composition of the oleoresins was analyzed by using gas chromatography and shown in Table 2. Oleic acid, palmitic acid, palmitoleic acid and myristic acid were the most abundant fatty acids in all the oleoresins. Octadecanoic acid was present only in trace amounts for all extracts with a concentration range between 0.292 to 1.792%. This aligned with the fatty acid content of raw sorghum varieties of reported in previous studies (Espitia-Hernández et al., 2022). The solvent type had a significant ($P < 0.05$) effect on the fatty acid composition. The unsaturated fatty acids (oleic and palmitoleic acids) increased more than saturated ones (myristic and palmitic acids) when ILs were used instead of dichloromethane and ethanol. This was probably related to the double bonds of unsaturated fatty acids that provided lower free energy for temporal ionization and to interact with cations and anions of ionic liquids. In another study, Li et al. (2009) showed that higher polyunsaturated fatty acid extracts from cod liver oil were obtained with ILs in comparison to organic solvents, such as acetone, methanol, acetonitrile, and chloroform. The percentages of

oleic, palmitic and myristic acids were not significantly ($P > 0.05$) different when IL_1 and IL_2 were used with traditional extraction process while it was significantly ($P < 0.05$) different for palmitoleic acid. In contrast, the percentage of these fatty acids were significantly ($P < 0.05$) different when dichloromethane or ethanol was used. The concentrations of both oleic and palmitic acids were significantly ($P < 0.05$) lower with both traditional solvents than ILs when traditional extraction was conducted.

The extraction method also had a significant ($P < 0.05$) effect on the fatty acid composition (Table 2). The percentage of oleic and palmitoleic acids significantly ($P < 0.05$) increased while myristic and palmitic acids significantly ($P > 0.05$) decreased when ball milling process was conducted instead of traditional process. The highest concentration of oleic acid, the most abundant unsaturated fatty acid in the oleoresins was 23.663% ± 0.003 with the combined action of ball milling and IL_1. In addition to this, the increase in oleic acid was 45.89% and 29.15% when ball milling was used with $IL_2$and ethanol respectively, as compared to traditional solvent extraction with the same solvents. On the other hand, the decrease in myristic acid was 24.57% and 34.44% when ball milling was used with IL_2 and ethanol respectively, as compared to traditional extraction using IL_2 and ethanol. Similar changes in unsaturated (palmitoleic and oleic acids) and saturated (myristic and palmitic acids) fatty acid concentrations were observed by Korber et. al (2022) where ball mill extraction was applied for lettuce plants (*Lactuca sativa L.)* to extract galactolipids and sulfolipids. Furthermore, the percentages of oleic, palmitoleic, myristic and palmitic acids were not significantly ($P > 0.05$) different when IL_1 and IL_2 were used with ball milling extraction. Similar to the traditional extraction, the percentage of these fatty acids changed when dichloromethane and ethanol were selected. The use of ILs increased ($P < 0.05$) the total phenolic content of the oleoresins. Oleoresins were rich in eight phenolic compounds: caffeic acids, coumaric acid, ferulic acids, apigenidin, luteolidin, 7-methoxy apigeninidin, eriodictyol and narencenin (Table 3). The HPLC chromatogram was given in Supplementary Materials (Fig. S1). Caffeic acid, coumaric acid and luteolidin were the major phenolic compounds present in all oleosome samples. Among these, caffeic acid followed by coumaric acid were the most abundant and they remained so with all types of solvents. The solvent type significantly ($P < 0.05$) affected the total phenolic content and composition (Table 3). The percent increase in caffeic acid was 74.84% when IL_1 was used instead of dichloromethane with the traditional extraction process. In addition to this, there was no significant ($P > 0.05$) difference between IL_1 and IL_2 while ethanol and dichloromethane showed significant ($P < 0.05$) difference using the same extraction method.

The extraction method also had a significant ($P < 0.05$) effect on the phenolic composition (Table 3). The increase in caffeic acid was 28.93% and 50.16% when ball milling was combined with IL_1 and dichloromethane respectively, as compared with the traditional extraction process using the same solvents. Similar effects were observed in luteolinidin when ball milling and traditional extraction processes were compared: the increase in luteolinidin was 21.78% and 57.68% when ball milling extraction was performed with IL_2 and ethanol respectively, as compared with traditional extraction. Furthermore, the percentages of caffeic acid were not significantly ($P > 0.05$) different when IL_1 and IL_2 with the combined action of ball milling process, but there was a slight significant difference when ethanol and dichloromethane were used. Similarly, in another study, Talekar et al. (2019) showed that total phenolics and antioxidant "punicalagin" increased when ball-mill pre-treatment was used for pomegranate peel waste, and it was more efficient than ultrasound-assisted and Soxhlet extraction.

3.3. Oleosome Structure

The organic moieties containing O-H and bonds, such as phospholipid bilayer, glycosidic bond and protein-phospholipid complexes, were analyzed by FTIR spectroscopy (Fig. 3). The stretching band of C=O at 1100 cm^{-1} and 1600 cm^{-1} was assigned to the polysaccharides and phospholipid bilayers, respectively (Li et al., 2022). The H-OH stretching around 2800-2900 cm^{-1} was assigned to glycosidic bond between glycoprotein units, and the O-H stretching between 3000-3500 cm^{-1} to the presence of protein-phospholipid complexes (Qi et al., 2017). The FTIR spectra was not affected ($P > 0.05$) by the solvent type but affected ($P < 0.05$) by the extraction process. The intensity of the peak at around 1600 cm^{-1} was lower with ball milling as compared to traditional extraction. This was probably related to the amount of phospholipid compounds

extracted and potentially improve the stability of oil bodies. The intensity of the peak around 2800-2900 cm^{-1} decreased with ball mill treatment. This can be explained by protein-polysaccharide interaction. Ball mill treatment increased the amount of released compounds into the solvent matrix. Among these, soluble polysaccharides became more available for intermolecular interactions with proteins. These interactions were seen as reduced intensity of spectra around 2800-2900 cm^{-1}representing glycosidic bond between amino group of protein and carboxylic group of polysaccharides. This may prove the formation of protein-polysaccharide complex (Guerrero et al., 2014).

Particle size and zeta potential are important factors that relate to their dispersibility and stability. When oleoresins dispersed in an aqueous environment without additional surfactants, they showed remarkable homogeneity and stability. The particle size of oleoresins ranged between 323-1760 nm (Figure 4A). Both solvent type and ball mill treatment significantly ($P < 0.05$) affected the particle size. With traditional extraction, the particle size decreased from a max of 1760 nm to 575 nm when dichloromethane and IL_2 used, respectively (Fig. 4). This showed that charged groups in ionic liquids were anchored on the interface of oil bodies that resulted in increased electrostatic repulsion and reduced the average particle size (Liu et al., 2020). The ball mill process reduced the average particle size of oleoresins. For instance, the average particle size was reduced by nearly 50% when ball mill treatment was applied using ethanol compared to conventional method. It was observed that the friction and shear forces during ball milling treatment reduced the particle size. This can be explained in consideration of the FTIR analysis. For oleoresin dispersions of lower particle size, the intensity of the FTIR band at 1100 cm^{-1}, 1600 cm^{-1} and 2800-2900 cm^{-1} increased. In addition, the zeta potential was measured as an indicator of the stability and extent of charged polymers, mainly protein fraction of oleosome structure. The results ranged from -8 mV to -35 mV (Fig 4B). In parallel to the particle size data, small particles from ball mill extracts showed higher zeta potential values, which demonstrate greater physical stability. The low molecular weight oleosins as major protein fraction plays critical role in the stability of the oleoresin dispersions. due to their hydrophobic nature, oleosins can penetrate deep into the triacylglycerol core of their emulsions with large detachment energies that reinforce their integrity and stability (Wijesundera & Shen, 2014). Another protein fraction, caleosins have shorter hydrophobic sequence and longer hydrophilic unit as compared to oleosin with special N-terminal segments bearing single Ca^{2+} ions responsible for the change in the polarity (Hanano et al., 2023). The difference in the smaller caleosin content of the oleoresins were probably responsible for the formation of smaller particle size as expected with higher diffusion rate of smaller polymers, and the higher zeta potential observed for them.

Conclusions

Our results are original to demonstrate the potential of oleoresins obtained from sorghum grain for value-added food applications. The oleoresins are rich in polyphenols with strong antioxidant potential as expected related to characteristics of the sorghum grain. The composition and structural properties of the oleoresins can be modulated by using novel ILs with composite anions and cations. Therefore, ILs can offer great potential for clean and controlled extraction of oleoresins as compared to traditional solvent extraction. Moreover, the wet milling process via the use of ball mill can help to break solid enclosure to improve extraction yield, and at the same time improve protein functionality for higher emulsification power.

Acknowledgements

This research was partially supported by the Kansas Agricultural Experiment Station (KAES) and by the U.S. Department of Agriculture (award number 2020-67013-30897).

References

Ayub, M. A., Choobkar, N., Hanif, M. A., Abbas, M., Ain, Q. U., Riaz, M., & Garmakhani, A. D. (2023). Chemical composition, antioxidant, and antimicrobial activities of P. roxburghii oleoresin essential oils extracted by steam distillation, superheated steam, and supercritical fluid CO_2 extraction. *Journal of Food Science ,December 2022* , 1–14.

Bogdanov, M. G., & Svinyarov, I. (2013). Ionic liquid-supported solid-liquid extraction of bioactive alkaloids.

II. Kinetics, modeling and mechanism of glaucine extraction from Glaucium flavum Cr. (Papaveraceae). *Separation and Purification Technology* ,*103* , 279–288. https://doi.org/10.1016/j.seppur.2012.10.035

Cheng, Y., Yu, S., & Xue, F. (2020). Comparison of the Extraction Efficiency of Isoflavone Compounds from Puerariae lobatae by Ionic Liquids with 11 Anions and 8 Imidazolium-Based Cations. *ACS Omega* , *5* (15), 8962–8971. https://doi.org/10.1021/acsomega.0c00724

Cláudio, A. F. M., Ferreira, A. M., Freire, M. G., & Coutinho, J. A. P. (2013). Enhanced extraction of caffeine from guaraná seeds using aqueous solutions of ionic liquids. *Green Chemistry* , *15* (7), 2002–2010. https://doi.org/10.1039/c3gc40437d

De Aguiar, A. C., de Paula, J. T., Mundo, J. L. M., Martínez, J., & McClements, D. J. (2021). Influence of type of natural emulsifier and microfluidization conditions on Capsicum oleoresin nanoemulsions properties and stability. *Journal of Food Process Engineering* ,*44* (4), 1–13. https://doi.org/10.1111/jfpe.13660

Espitia-Hernández, P., Chávez González, M. L., Ascacio-Valdés, J. A., Dávila-Medina, D., Flores-Naveda, A., Silva, T., Ruelas Chacón, X., & Sepúlveda, L. (2022). Sorghum (Sorghum bicolor L.) as a potential source of bioactive substances and their biological properties. *Critical Reviews in Food Science and Nutrition* , *62* (8), 2269–2280. https://doi.org/10.1080/10408398.2020.1852389

Guerrero, P., Kerry, J. P., & De La Caba, K. (2014). FTIR characterization of protein-polysaccharide interactions in extruded blends. *Carbohydrate Polymers* , *111* , 598–605. https://doi.org/10.1016/j.carbpol.2014.05.005

Hanano, A., Blee, E., & Murphy, D. J. (2023). Caleosin/Peroxygenases: multifunctional proteins in plants. *Annals of Botany* , 387–409. https://doi.org/10.1093/aob/mcad001

Jin, L., Zhao, X. J., Zhao, Y., Gao, S. A., Liu, H. M., Ma, Y. X., Wang, X. De, & Hou, L. X. (2022). Physicochemical properties of the sesame paste produced by a novel process technology—ball milling.*International Journal of Food Science and Technology* ,*57* (11), 7254–7266. https://doi.org/10.1111/ijfs.16074

Julakanti, S., Charles, A. P. R., Zhao, J., Bullock, F., Syed, R., Myles, Y., & Wu, Y. (2023). Hempseed protein (Cannabis sativa L.): Influence of extraction pH and ball milling on physicochemical and functional properties. *Food Hydrocolloids* , *143* (May), 108835. https://doi.org/10.1016/j.foodhyd.2023.108835

Khasanah, L. U., Kawiji, Prasetyawan, P., Utami, R., Atmaka, W., Manuhara, G. J., & Sanjaya, A. P. (2017). Optimization and Characterization of Cinnamon Leaves (Cinnamomum burmannii) Oleoresin.*IOP Conference Series: Materials Science and Engineering* ,*193* (1). https://doi.org/10.1088/1757-899X/193/1/012021

Lamuela-Raventós, R. M. (2017). Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity.*Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications* , 107–115. https://doi.org/10.1002/9781119135388.ch6

Li, M., Pittman, C. U., & Li, T. (2009). Extraction of polyunsaturated fatty acid methyl esters by imidazolium-based ionic liquids containing silver tetrafluoroborate-Extraction equilibrium studies. *Talanta* ,*78* (4–5), 1364–1370. https://doi.org/10.1016/j.talanta.2009.02.011

Li, R., Pu, C., Sun, Y., Sun, Q., & Tang, W. (2022). Interaction between soybean oleosome-associated proteins and phospholipid bilayer and its influence on environmental stability of luteolin-loaded liposomes. *Food Hydrocolloids* , *130* (January), 107721. https://doi.org/10.1016/j.foodhyd.2022.107721

Napoli, E., Gentile, D., & Ruberto, G. (2019). GC–MS analysis of terpenes from Sicilian Pistacia vera L. oleoresin. A source of biologically active compounds. *Biomedical Chromatography* ,*33* (2), 1–9. https://doi.org/10.1002/bmc.4381

Nikiforidis, C. V., & Kiosseoglou, V. (2009). Aqueous extraction of oil bodies from maize germ (Zea mays) and characterization of the resulting natural oil-in-water emulsion. *Journal of Agricultural and Food Chemistry* , *57* (12), 5591–5596. https://doi.org/10.1021/jf900771v

Qi, B., Ding, J., Wang, Z., Li, Y., Ma, C., Chen, F., Sui, X., & Jiang, L. (2017). Deciphering the characteristics of soybean oleosome-associated protein in maintaining the stability of oleosomes as affected by pH. *Food Research International* , *100* (July), 551–557. https://doi.org/10.1016/j.foodres.2017.07.053

Taghvaei, M., Tonyali, B., Sommers, C., Ceric, O., Linghu, Z., Smith, J. S., & Yucel, U. (2021). Formation kinetics of radiolytic lipid products in model food–lipid systems with gamma irradiation. *JAOCS, Journal of the American Oil Chemists' Society* , *98* (7), 737–746. https://doi.org/10.1002/aocs.12513

Talekar, S., Patti, A. F., Vijayraghavan, R., & Arora, A. (2019). Rapid, enhanced and eco-friendly recovery of punicalagin from fresh waste pomegranate peels via aqueous ball milling. *Journal of Cleaner Production* , *228* , 1238–1247. https://doi.org/10.1016/j.jclepro.2019.04.392

Tohma, H., Gülçin, İ., Bursal, E., Gören, A. C., Alwasel, S. H., & Köksal, E. (2017). Antioxidant activity and phenolic compounds of ginger (Zingiber officinale Rosc.) determined by HPLC-MS/MS. *Journal of Food Measurement and Characterization* , *11* (2), 556–566. https://doi.org/10.1007/s11694-016-9423-z

Tolesa, L. D., Gupta, B. S., & Lee, M. J. (2017). The chemistry of ammonium-based ionic liquids in depolymerization process of lignin.*Journal of Molecular Liquids* , *248* , 227–234. https://doi.org/10.1016/j.molliq.2017.10.054

Wijesundera, C., & Shen, Z. (2014). Mimicking natural oil bodies for stabilising oil-in-water food emulsions. *Lipid Technology* ,*26* (7), 151–153. https://doi.org/10.1002/lite.201400036

Tables

Table 1. Total protein (wt%), total lipid (wt%) and phenolic (mg GAE/g) contents of oleoresins extracted from sorghum grain using different methods. The results are expressed as the mean ± standard deviation. The lower and upper superscripts indicate the significant differences between the solvent type for conventional solvent or ball mill-assisted extraction processes, respectively. The Roman numerals show the differences between the type of extraction for each solvent.

							Conventional extraction	**Conventional extraction**	**Conventional extraction**	**Conventional extraction**	**Conventional extraction**	**Ball milling**	**Ball milling**	**Ball milling**	**Ba**
Solvent type	Total protein (wt %)	Total protein (wt %)	Total protein (wt %)	Total lipid content (wt %)	Total lipid content (wt %)	Total lipid content (wt %)	Total lipid content (wt %)	Total phenolic content (mg GAE/g)	Total phenolic content (mg GAE/g)	Total protein (wt%)	Total protein (wt%)	Total protein (wt%)	Total lipid content (wt%)	Total lipid content (wt%)	To lip co ter (w
IL_1	IL_1	0.67 ± 0.03^{a,I}	0.67 ± 0.03^{a,I}	0.67 ± 0.03^{a,I}	69.22 ± 0.04^{a,I}	69.22 ± 0.04^{a,I}	69.22 ± 0.04^{a,I}	69.22 ± 0.04^{a,I}	48 ± 0.01^{a,I}	48 ± 0.01^{a,I}	1.37 ±0.05^{A,II}	1.37 ±0.05^{A,II}	1.37 ±0.05^{A,II}	82.19±0.5	± 0.0
IL_2	IL_2	0.58 ± 0.01^{a,I}	0.58 ± 0.01^{a,I}	0.58 ± 0.01^{a,I}	66.33± 0.06^{a,I}	66.33± 0.06^{a,I}	66.33± 0.06^{a,I}	66.33± 0.06^{a,I}	42 ± 0.01^{b,I}	42 ± 0.01^{b,I}	1.32 ±0.06^{A,II}	1.32 ±0.06^{A,II}	1.32 ±0.06^{A,II}	80.26±0.5	± 0.0
dichloromethane (GC grade, 99%)	dichloromethane (GC grade, 99%)	0.36 ± 0.02^{c,I}	0.36 ± 0.02^{c,I}	0.36 ± 0.02^{c,I}	57.26 ± 0.02^{b,I}	57.26 ± 0.02^{b,I}	57.26 ± 0.02^{b,I}	57.26 ± 0.02^{b,I}	29 ± 0.02^{c,I}	29 ± 0.02^{c,I}	1.06 ±0.01^{C,II}	1.06 ±0.01^{C,II}	1.06 ±0.01^{C,II}	76.32 ±0.04^{B,II}	76 ±0

							Conventional extraction	**Conventional extraction**	**Conventional extraction**	**Conventional extraction**	**Conventional extraction**				
												Ball milling	**Ball milling**	**Ball milling**	**Ba**
ethanol (GC grade, 99%)	ethanol (GC grade, 99%)	0.43 ± 0.01^{b,I}	0.43 ± 0.01^{b,I}	0.43 ± 0.01^{b,I}	53.12 ± 0.08^{c,I}	53.12 ± 0.08^{c,I}	53.12 ± 0.08^{c,I}	53.12 ± 0.08^{c,I}	21 ± 0.02^{d,I}	21 ± 0.02^{d,I}	1.15 ± 0.11^{B,II}	1.15 ± 0.11^{B,II}	1.15 ± 0.11^{B,II}	70.41 ± 0.09^{C,II}	70 ± 0.0

Table 2. Fatty acid composition (wt%) in oleoresins extracted from sorghum grain using different methods. The results are expressed as the mean ± standard deviation. The lower and upper superscripts indicate the significant differences between the solvent type for conventional solvent or ball mill-assisted extraction processes, respectively. The Roman numerals show the differences between the type of extraction for each solvent.

	Solvent type	Myristic acid (C14)	Myristic acid (C14)	Myristic acid (C14)	Myristic acid (C14)	Palmitic acid (C16)	Palmitic acid (C16)	Palmitoleic acid (C16:1 ([?]9))	Palmitoleic acid (C16:1 ([?]9))	Octadecanoic acid (C18:0)	Octadecanoic acid (C18:0)	Octadecanoic acid (C18:0)	Oleic acid (C18 ω9-cis)
Conventional extraction	IL_1	32.946 ±0.003^{a,I}	32.946 ±0.003^{a,I}	16.819±0.[illegible]^{a,I}	16.819±0.[illegible]^{a,I}	16.819±0.[illegible]^{a,I}	16.819±0.[illegible]^{a,I}	16.819±0.[illegible]^{a,I}	16.819±0.[illegible]^{a,I}	16.188 ± 0.292^{a,I}	16.188 ± 0.292^{a,I}	1.646 ±0.172^{c,I}	1.646 ±0.172^{c,I}	17.24 ± 0.329
	IL_2	32.888 ±0.006^{a,I}	32.888 ±0.006^{a,I}	17.240 ±0.723^{a,I}	17.240 ±0.723^{a,I}	17.240 ±0.723^{a,I}	17.240 ±0.723^{a,I}	17.240 ±0.723^{a,I}	12.855 ±0.265^{b,I}	12.855 ±0.265^{b,I}	1.577 ± 0.126^{c,I}	1.577 ± 0.126^{c,I}	15.61 ± 0.215
	dichloromethane (99%)	30.959 ±0.018^{c,I}	30.959 ±0.018^{c,I}	15.758±0.[illegible]^{b,I}	15.758±0.[illegible]^{b,I}	15.758±0.[illegible]^{b,I}	15.758±0.[illegible]^{b,I}	15.758±0.[illegible]^{b,I}	12.411 ±0.261^{ab,I}	10.411 ±0.261^{ab,I}	7.482 ± 0.212^{b,I}	7.482 ± 0.212^{b,I}	13.14 ± 0.243
	ethanol (99%)	31.582±0.[illegible]^{b,I}	31.582±0.[illegible]^{b,I}	13.983 ±0.277^{c,I}	13.983 ±0.277^{c,I}	13.983 ±0.277^{c,I}	13.983 ±0.277^{c,I}	13.983 ±0.277^{c,I}	8.130 ± 0.266^{c,I}	8.130 ± 0.266^{c,I}	8.130 ± 0.266^{c,I}	9.993 ±0.173^{a,I}	14.70 ± 0.211
Ball mill	IL_1	26.064±0.[illegible]^{A,II}	26.064±0.[illegible]^{A,II}	12.758±0.[illegible]^{A,II}	12.758±0.[illegible]^{A,II}	12.758±0.[illegible]^{A,II}	12.758±0.[illegible]^{A,II}	12.758±0.[illegible]^{A,II}	19.275 ± 0.477^{A,II}	19.275 ± 0.477^{A,II}	19.275 ± 0.477^{A,II}	1.792±0.[illegible]^{C,II}	22.26 ± 0.003
	IL_2	26.402±0.[illegible]^{A,II}	26.402±0.[illegible]^{A,II}	26.402±0.[illegible]^{A,II}	11.478 ± 0.69^{A,II}	11.478 ± 0.69^{A,II}	11.478 ± 0.69^{A,II}	11.478 ± 0.69^{A,II}	19.717 ± 0.291^{A,II}	19.717 ± 0.291^{A,II}	19.717 ± 0.291^{A,II}	1.723±0.[illegible]^{C,II}	22.78 ± 0.891
	dichloromethane (99%)												

	Solvent type	Myristic acid (C14)	Myristic acid (C14)	Myristic acid (C14)	Myristic acid (C14)	Palmitic acid (C16)	Palmitic acid (C16)	Palmitoleic acid (C16:1 ([?]9))	Palmitoleic acid (C16:1 ([?]9))	Octadecanoic acid (C18:0)	Octadecanoic acid (C18:0)	Octadecanoic acid (C18:0)	Oleic acid (C18 ω9-cis)
		21.868±0	21.868±0	21.868±0	9.436±0.	67.456±0.	67.456±0.	67.456±0.	17.290 ± 0.281^{B,II}	18.290 ± 0.281^{B,II}	18.290 ± 0.281^{B,II}	8.637±0.3	20.74 ± 0.87
	ethanol (99%)	23.491±0	23.491±0	23.491±0	8.263±0.	80.703±0.	80.703±0.	80.703±0.	17.633 ± 0.456^{C,II}	17.633 ± 0.456^{C,II}	17.633 ± 0.456^{C,II}	10.638±0	18.89

Table 3. Concentration of phenolic compounds as gallic acid equivalents (mg GAE/g oleoresin) in oleoresins extracted from sorghum grain using different methods. The results are expressed as the mean ± standard deviation. The lower and upper superscripts indicate the significant differences between the solvent type for conventional solvent or ball mill-assisted extraction processes, respectively. The Roman numerals show the differences between the type of extraction for each solvent.

	Solvent type	Caffeic acid	Coumaric acid	Ferulic acid	Ferulic acid	Apigenidin	Apigenidin	Luteolinidin	Luteolinidin	7-methoxy apicidinin	7-methoxy apicidinin	7-methoxy apicidinin	Erio
Conventional extraction	IL_1	20.280 ± 0.007^{a,I}	18.409 ± 0.003^{a,I}	10.456 ± 0.058^{a,I}	10.456 ± 0.058^{a,I}	9.556 ± 0.003^{a,I}	9.556 ± 0.003^{a,I}	12.673 ± 0.017^{a,I}	12.673 ± 0.017^{a,I}	12.673 ± 0.017^{a,I}	8.360 ± 0.08^{a,I}	8.360 ± 0.08^{a,I}	3.032 ± 0.00
	IL_2	19.208 ± 0.051^{a,I}	13.602 ± 0.321^{b,I}	10.446 ± 0.291^{a,I}	10.446 ± 0.291^{a,I}	6.319 ± 1.112^{a,I}	6.319 ± 1.112^{a,I}	10.380 ± 2.67^{a,I}	10.380 ± 2.67^{a,I}	10.380 ± 2.67^{a,I}	6.470 ± 1.342^{a,I}	6.470 ± 1.342^{a,I}	3.158 ± 0.37
	dichloromethane	11.599 ± 0.053^{c,I}	10.651 ± 0.331^{d,I}	3.113 ± 0.281^{c,I}	3.113 ± 0.281^{c,I}	3.096 ± 1.171^{c,I}	3.096 ± 1.171^{c,I}	4.251 ± 0.015^{c,I}	4.251 ± 0.015^{c,I}	4.251 ± 0.015^{c,I}	4.405 ± 0.371^{b,I}	4.405 ± 0.371^{b,I}	3.085 ± 0.24
	ethanol (99%)	14.296 ± 0.007^{b,I}	11.385 ± 0.038^{c,I}	3.315 ± 0.456^{b,I}	3.315 ± 0.456^{b,I}	4.374 ± 0.562^{b,I}	4.374 ± 0.562^{b,I}	6.023 ±2.131^{b,I}	6.023 ±2.131^{b,I}	6.023 ±2.131^{b,I}	4.486 ± 0.461^{b,I}	4.486 ± 0.461^{b,I}	3.177 ±0.5
Ball mill	IL_1	26.147 ± 0.005^{A,II}	22.965 ± 0.075^{A,II}	6.082 ± 0.292^{A,II}	6.082 ± 0.292^{A,II}	12.360 ± 0.081^{A,II}	12.360 ± 0.081^{A,II}	13.111 ± 1.332^{A,II}	13.111 ± 1.332^{A,II}	13.111 ± 1.332^{A,II}	4.486 ±0.713^{A,II}	4.486 ±0.713^{A,II}	3.575 ±0.2
	IL_2	26.075 ± 0.005^{A,II}	20.159 ± 0.492^{A,II}	6.072 ± 0.265^{A,II}	6.072 ± 0.265^{A,II}	11.927 ± 0.064^{B,II}	11.927 ± 0.064^{B,II}	13.271 ± 2.671^{A,II}	13.271 ± 2.671^{A,II}	13.271 ± 2.671^{A,II}	4.606 ±0.136^{A,II}	4.606 ±0.136^{A,II}	3.312 ±0.4

Solvent type	Caffeic acid	Coumaric acid	Ferulic acid	Ferulic acid	Apigenidin	Apigenidin	Luteolinidin	Luteolinidin	7-methoxy apicidinin	7-methoxy apicidinin	7-methoxy apicidinin	Erio
dichloromethane												
	17.417 $\pm 0.004^{C,II}$	13.135 $\pm 0.881^{C,II}$	2.175 $\pm 0.261^{B,II}$	2.175 $\pm 0.261^{B,II}$	9.278 $\pm 1.112^{C,II}$	9.278 $\pm 1.112^{C,II}$	4.907 $\pm 0.148^{C,II}$	4.907 $\pm 0.148^{C,II}$	4.907 $\pm 0.148^{C,II}$	2.458 $\pm 0.722^{B,II}$	2.458 $\pm 0.722^{B,II}$	3.145 ± 0.2
ethanol (99%)												
	19.720 $\pm 0.002^{B,II}$	18.869 $\pm 0.320^{B,II}$	3.376 $\pm 0.266^{B,II}$	3.376 $\pm 0.266^{B,II}$	11.321 $\pm 0.562^{B,II}$	11.321 $\pm 0.562^{B,II}$	11.321 $\pm 0.562^{B,II}$	9.497 $\pm 0.156^{B,II}$	9.497 $\pm 0.156^{B,II}$	2.540 $\pm 0.211^{B,II}$	2.540 $\pm 0.211^{B,II}$	3.237

Legends to the Figures

Figure 1. Oleoresin extraction yield (%) from sorghum grain as a function of extraction condition. The results are expressed as the mean of 3 replicates and standard deviation (bars). The lower and upper superscripts indicate the significant differences between the solvent type for conventional solvent or ball mill-assisted extraction processes, respectively. The Roman numerals show the differences between the type of extraction for each solvent.

Figure 2. FTIR spectra of oleoresins obtained from sorghum grain with different extraction conditions. The arrows indicate bond stretching of interest.

Figure 3. Particle size (A) and zeta potential (B) of oleoresin dispersions from sorghum grain as a function of extraction condition. The results are expressed as the mean of 3 replicates and standard deviation (bars). The lower and upper superscripts indicate the significant differences between the solvent type for conventional solvent or ball mill-assisted extraction processes, respectively. The Roman numerals show the differences between the type of extraction for each solvent.

Figure 1

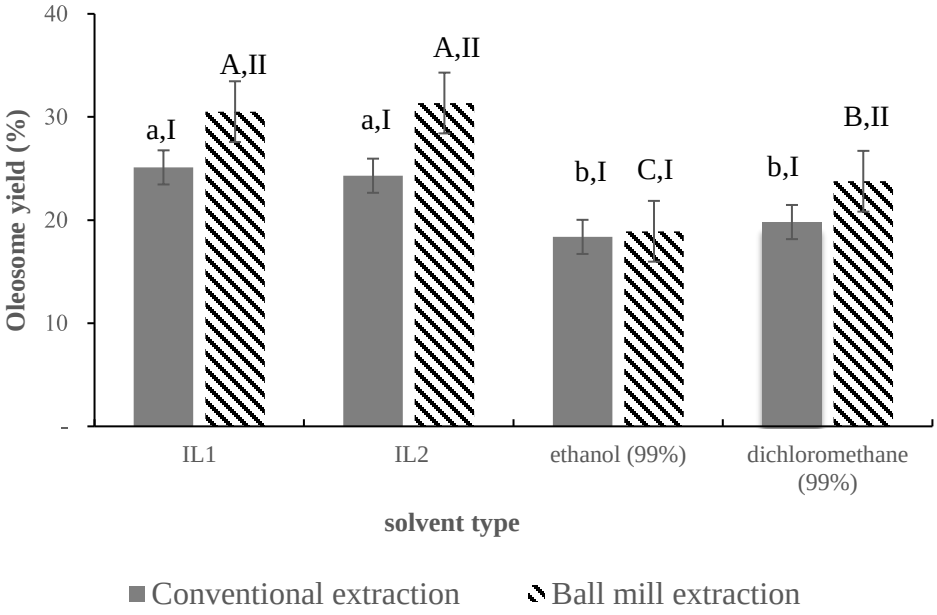

40
30
20
10
-
Oleosome yield (%)
a,I
A,II
a,I
A,II
b,I
C,I
b,I
B,II
IL1
IL2
ethanol (99%)
dichloromethane (99%)
solvent type
Conventional extraction
Ball mill extraction

Figure 2.

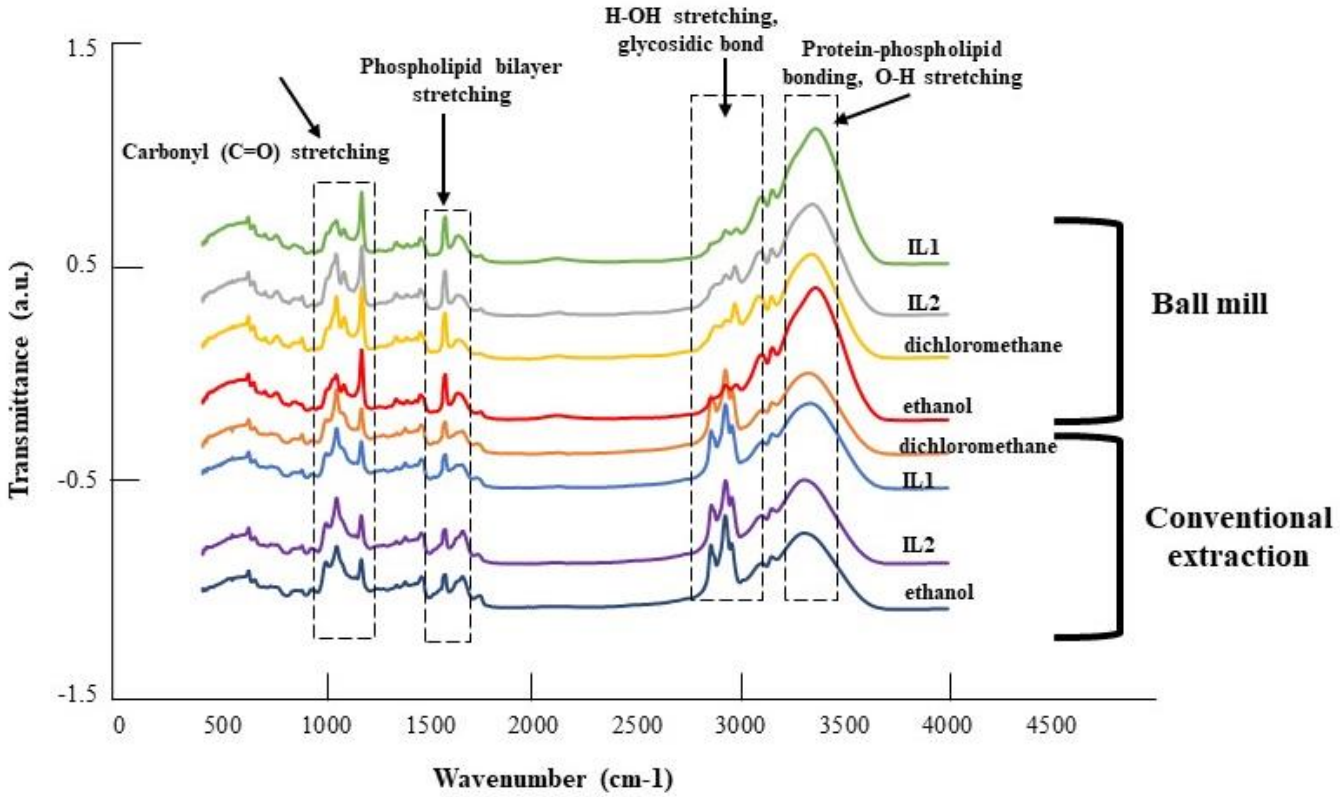

H-OH stretching, glycosidic bond
Protein-phospholipid bonding, O-H stretching
Phospholipid bilayer stretching
Carbonyl (C=O) stretching
IL1
IL2
dichloromethane
ethanol
dichloromethane
IL1
IL2
ethanol
Ball mill
Conventional extraction
Transmittance (a.u.)
1.5
0.5
-0.5
-1.5
0
500
1000
1500
2000
2500
3000
3500
4000
4500
Wavenumber (cm-1)

Figure 3A

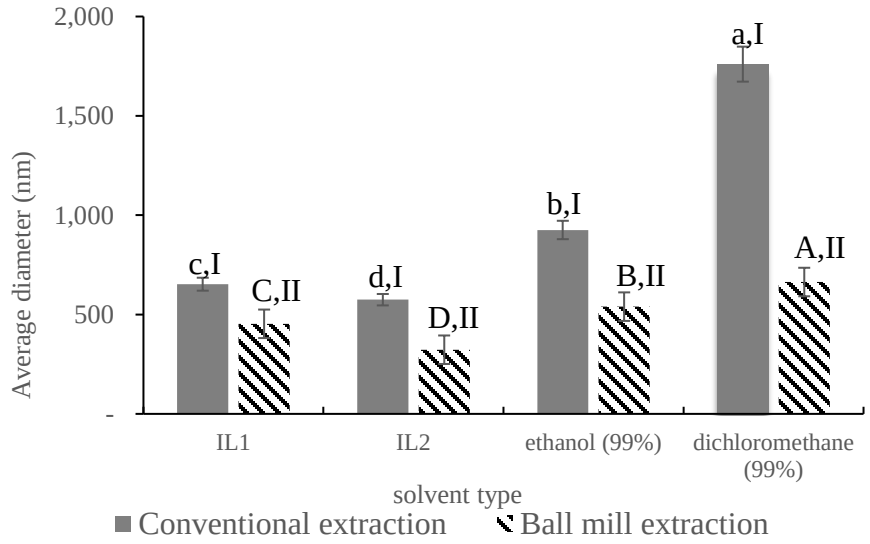
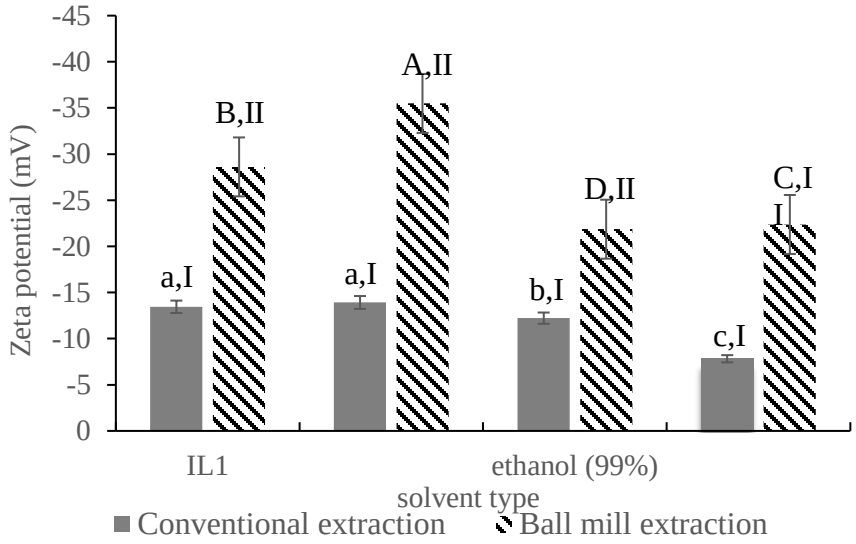

2,000
1,500
1,000
500
-
Average diameter (nm)
c,I
C,II
d,I
D,II
b,I
B,II
a,I
A,II
IL1
IL2
ethanol (99%)
dichloromethane (99%)
solvent type
Conventional extraction
Ball mill extraction

Figure 3B


-45
-40
-35
-30
-25
-20
-15
-10
-5
0
Zeta potential (mV)
a,I
B,II
a,I
A,II
b,I
D,II
c,I
C,I
I
IL1
ethanol (99%)
solvent type
Conventional extraction
Ball mill extraction