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Impact of ultrasound on nutrition recovery and physicochemical properties of oat beverage

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ABSTRACT

Oat beverage has become increasingly popular, yet its processing removes valuable nutritional components and results in waste stream. This study evaluated the use of ultrasound as a pre-treatment technology to improve nutrient recovery in oat beverage preparation. Various ultrasound power levels (60, 240, and 960 W) and treatment times (1, 5, and 9 min) were investigated for their effects on physical and chemical changes during oat beverage preparation. Ultrasound effectively reduced the size of larger oat grain fibrous particles while largely leaving smaller size compounds unaffected. Partial disintegration of cell wall structures was also observed under confocal laser scanning microscopy. This resulted in a significant increase (up to 39%) in β-glucan recovery with minor alteration to its molecular weight in oat beverage, preserving its inherent health benefits. Protein recovery was not improved, but ultrasound led to partial unfolding of tertiary structure, improving protein solubility and foaming properties of oat beverage, which could be beneficial in developing coffee-based beverages and desserts. The shear-thinning behavior of oat beverage was maintained after ultrasound treatment, benefiting processing efficiency and mouthfeel. Overall, these findings highlight the potential of ultrasound as a sustainable pre-treatment technology to enhance the nutritional quality and physicochemical properties of oat beverage.

1. Introduction

The global growth of the plant-based beverage sector is propelled by environmental sustainability consideration, their potential health benefits (including cholesterol lowering effect, and antioxidative and anti-inflammatory properties) and dairy allergies (Siddiqui et al., 2023). Besides soy and tree nuts-based beverages, oat beverage has emerged as one of the most popular plant-based beverages in North American markets. Oats are an excellent source of β-glucan, a water soluble dietary fiber, which is well known for its health beneficial effects including blood-glucose and blood-cholesterol lowering ability, and satiety effect (Cui, Jia, Zhao, Hou, & Zhou, 2023). The Food and Drug Administration (FDA) and Health Canada both have claimed that consume a 3 g per day of oat β-glucan would reduce the risk of coronary heart dieases by lowering blood-cholesterol (Hughes & Grafenauer, 2021). Additionally, oats contain good amount of protein with a higher lysine content compared to other cereals, along with a well-balanced amino acid essential profile (Cui et al., 2023).

The production of oat beverage involves several processing steps, including wet or dry grinding, enzymatic hydrolysis with α-amylase and separation (Yu et al., 2023). According to Triantafyllou (2014), wet grinding yielded a 24% higher protein content than dry grinding. This method is preferable for industry as it is more effective in releasing nutritional components, particularly proteins and β-glucan, from the oat endosperm. Subsequently, α-amylase is introduced to hydrolyze oat starch, limiting starch gelatinization that tends to induce high viscosity and enhances the fluidity of oat beverage (Yu et al., 2023). Afterward, the solid residues are separated from the oat beverage by centrifugation or descanting. However, during this step, a significant amount of valuable compounds, such as dietary fiber and protein, is lost in the residues due to inefficient grinding and extraction. Similar inefficiencies are observed in other cereal grain processing, such as sorghum (Amoura, Mokrane, & Nadjemi, 2020) and corn (Ozturk, Kaasgaard, Palmén, Vidal, & Hamaker, 2021). Therefore, developing a more efficient processing technology to enhance nutrients recovery in oat beverage is important.

Ultrasound, an environmentally friendly technology, has garnered increased attention in food processing for its ability to enhance

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extraction yields and modify physicochemical properties of plant-based materials. The cavitation effect of ultrasound ruptures plant cell walls, enhancing the extractability of intercellular materials through high shear rates and the collapse of microbubbles in a liquid medium (Yusoff, Mat Taher, Rahmat, & Chua, 2022). Several studies had revealed that ultrasound improved the extractability of intracellular materials from oat including β-glucan (Chen et al., 2018) and protein (Perez-Vega et al., 2023) compared to conventional methods. Hematian Sourki, Koocheki & Elahi (2017) observed ultrasound disintegrated barley β-glucan structure, and Falsafi et al. (2019) found similar effects on oat starch, improving paste fluidity. Li and Xiong (2021) utilized ultrasound to modify oat protein isolates, observing a reduction in particle size and an increase in protein solubility and functional properties. Other studies have demonstrated ultrasound as a post-preparation process improved the physical stability of plant-based beverages, preventing creaming and component phase-separation (Lu et al., 2019; Vallath & Shanmugam, 2022). However, the use of ultrasound as a pre-treatment technology to improve β-glucan and protein content, and its impact of physicochemical properties of oat beverage, remains unclear.

This work aimed to evaluate ultrasound as a pre-treatment technique for oat beverage processing. The impact of ultrasound power levels (60, 240, and 960 W) and treatment durations (1, 5, and 9 min) on a series of component changes during oat beverage preparation was studied including the extraction content of protein and β-glucan. Additionally, the oat grain size reduction and cell wall disintegration were studied by particle size measurement and confocal laser scanning microscopy (CLSM) observation. Moreover, the protein and β-glucan structure changes in the ultrasound treated oat beverage were characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), size exclusive-high-performance liquid chromatography (SE-HPLC), Fourier-transform infrared spectroscopy (FT-IR) and intrinsic fluorescence analysis. The generated knowledge will provide scientific insight into the oat protein and β-glucan extraction and modification by ultrasound as a pre-treatment technology during oat beverage preparation. Increasing nutrients recoveries through ultrasound will provide a more sustainable method to help oat beverage industry in improving the product nutritive quality and enhancing competitiveness in the global market.

2. Materials and methods

2.1. Materials

Dehulled oat grains (*Avena sativa)* were purchased from a Wedge Farms Ltd., Manitoba, Canada. The α-amylase from *Bacillus* spp. (EC 3.2.1.1., 500 U/mL), acid fusion and calcofluor white used for confocal laser microscopic analysis were purchased from Sigma-Aldrich Co. (Oakville, Ontario, Canada). The β-glucan assay kit used for β-glucan content determination was purchased from Megazyme International Ltd (Bray, Co. Wicklow, Ireland). Distilled water was used for the oat beverage preparation, and Milli-Q water (Millipore, Billerica, Ma, USA) was used for analysis. All chemicals were reagent graded.

2.2. Preparation of oat slurry and ultrasound treatment

The 0.2 g/mL oat slurry was prepared with oat grains (stored at 4 °C) mixed with distilled water and grounded with a laboratory blender (Blender 7012 S, Warning Commercial Blender, USA) for 2 min. The oat slurry then was treated by an ultrasonic processor (Model JY98 – IIIDN, Ningbo Scientz Biotechnology Co., Ltd., China) equipped with a titanium probe (diameter 3/4 inch). The samples were treated at different power output levels (60, 240, and 960 W) for 1, 5, and 9 min (pulse duration: on-time, 5 s; off-time, 5 s). The temperature was controlled by using an ice bath as not exceeding 45 °C.

2.3. Enzymatic treatment of the oat slurry

The oat slurries (100 mL) were adjusted to pH 7 using 1 mol/L NaOH, then hydrolyzed with 1 mL α-amylase at 60 °C for 30 min, followed by inactivation in a 90 °C water bath for 10 min. After cooling, the oat slurries were tested for particle size analysis and observed by an optic microscopy with a AxioCam ERc 5s digital camera (Zeiss Pimostar 1, Jena, Germany) with a 10 × magnification. Afterward, the slurries were centrifugated at 2000 rpm for 2 min to collect the oat beverage (supernatant) were stored were stored at 4 °C or lyophilized for subsequent analysis. The oat beverage samples had a final pH range of 6.26–6.40. The residues were also collected, which were then stored at 4 °C for CLSM analysis. Sodium azide (0.02 mg/mL) was added in all oat beverage samples and residues as an antimicrobial reagent. The oat beverage sample without ultrasound treatment was also prepared as control.

2.4. Particle size distribution

The particle size distributions of oat slurries and oat beverages were determined by a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Worchester, UK) with Hydro LV extension at 25 °C. The samples were added dropwise to the instrument with distilled water as a dispersion medium until the obscuration rate of 10–12% was obtained. The refractive index of particle and water set as was 1.47 and 1.33, respectively. The volume surface (D_{43}) was recorded.

2.5. Rheological analysis

The flow behavior of the oat beverage samples was studied using a DHR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40 mm diameter parallel plate and the gap size was set at 1000 μm. The viscosity was measured over the shear rate range of 0.1–1000 1/s at 25 °C.

Flow behavior index (n) and consistency coefficient (k) values were calculated by fitting the power-law model (Eq. (1)) (Hematian Sourki, Koocheki, & Elahi, 2017):

$$\tau = k\dot{\gamma}^n \tag{1}$$

where τ is the shear stress (Pa), k is the consistency coefficient ($Pa.s^n$), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow behavior index (dimensionless).

2.6. Protein characterization

2.6.1. Protein content and solubility

The protein content in the oat beverages was determined by using a combustion nitrogen analyzer (CN-628, Leco Corporation, St Joseph MI, USA) with a factor of 5.83 used for protein conversion. The total solid content of oat beverage samples was determined by oven-drying method. The protein content (mg/mL) in oat beverages was calculated from the following equation (Eq. (2)):

$$\textit{Protein content}\left(\frac{mg}{mL}\right) = \frac{\textit{Protein content of oat beverage (\% db)}}{100} \times \textit{total solid content of oat beverage}\left(\frac{mg}{mL}\right) \tag{2}$$

The protein solubility of the oat beverages was measured by Eckert et al. (2019) with modifications. Aqueous oat beverage was mixed in equal volume of Milli-Q water for 30 min, and then centrifuged at 3500 rpm for 30 min to collect the precipitate for lyophilization and weighting. The protein solubility (%) was calculated from the following equation (Eq. (3)):

$$\textit{Protein solubility}\ (\%) = \left(1 - \frac{\textit{mass of insoluble protein}}{\textit{mass of protein in oat beverage}}\right) \times 100\% \tag{3}$$

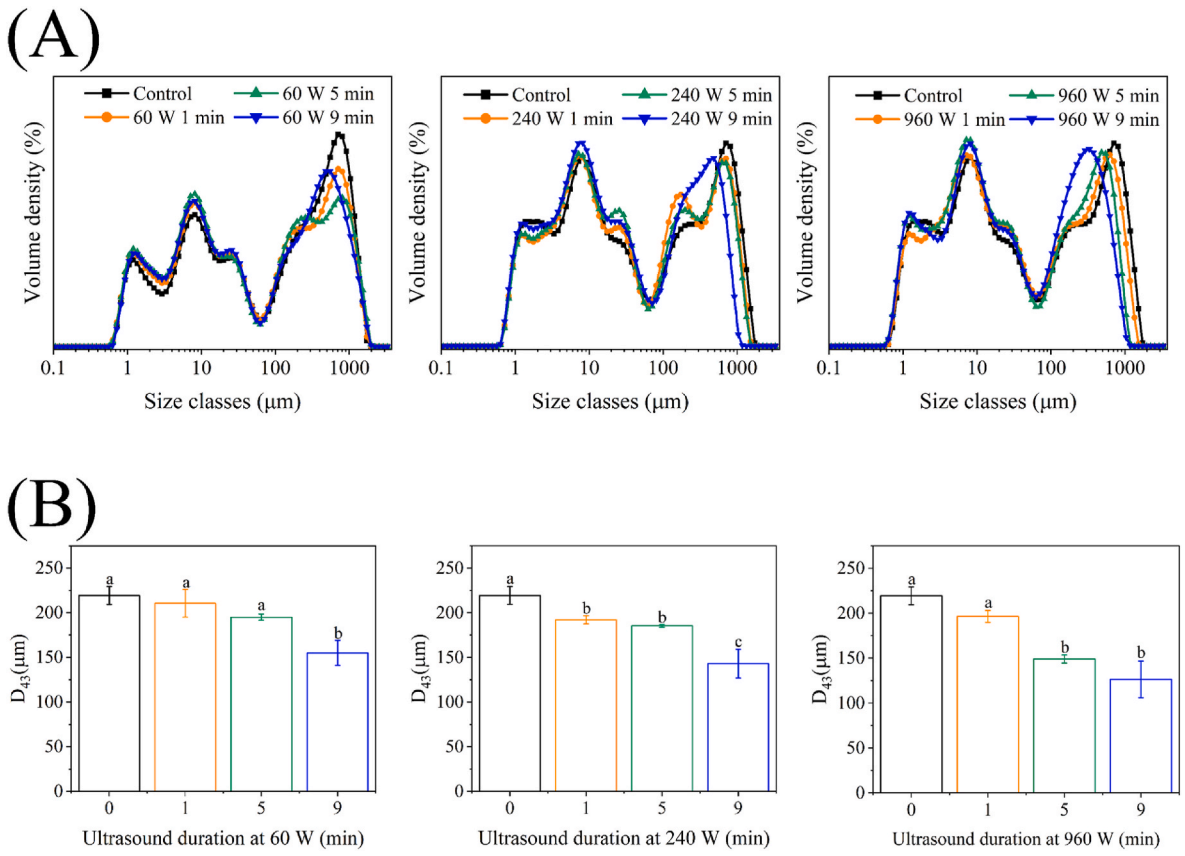

Fig. 1. Effect of ultrasound (60, 240, and 960 W) for 1, 5, and 9 min on the (A) particles size distribution of oat slurries (before centrifugation), and (B) D_{43}. Control (0 min) was the sample without ultrasound treatment. Different letters above column indicate significant differences ($p < 0.05$).

2.6.2. SDS-PAGE

SDS-PAGE was performed under reducing and non-reducing conditions (Zhang, Huang, Roopesh, & Chen, 2022). The samples (3 mg protein/mL) were mixed with 2 × sample buffers (Bio-Rad Laboratories Inc.) with or without 2-mercaptoethanol and boiled for 5 min. After centrifugation (9703 rpm for 5 min), 12 μL of supernatants were loaded on each lane of 4% stacking gel and 12% separating gel. The electrophoresis was run at a constant voltage of 80 V. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 and destained in water: methanol: and acetic acid solution was at the ratio of 8:1:1 (v/v/v).

2.6.3. Fourier-transform infrared spectroscopy (FT-IR)

The conformation of protein in the oat beverages was characterized by FT-IR. Lyophilized oat beverages were mixed and pressed with potassium bromide in 1:100 ratio. The spectra were recorded by a Nicolet 6700 spectrophotometer (Thermo Fisher scientific Inc., MA, US) at 400 to 4000 cm^{-1} with 128 scans at a 4 cm^{-1} resolution. Fourier self-deconvolution for amide I region (1700-1600 cm^{-1}) was analyzed at a bandwidth of 24 cm^{-1} and an enhancement factor of 2.5 using Omnic 8.1 software (Byler & Susi, 1986).

2.6.4. Intrinsic fluorescence

The intrinsic fluorescence spectra of the oat beverages were measured using a spectrophotometer (SpectraMax M3, Molecular Devices, Sunnyvale, US). Lyophilized samples were prepared in 1 mg protein/mL with distilled water before testing. The excitation wavelength was set at 295 nm and emission wavelength was in the range of 315–500 nm (slit = 2 nm).

2.7. β-glucan characterization

2.7.1. β-glucan quantification

β-glucan content in lyophilized oat beverages was determined using the Megazyme β-glucan (AOAC 992.28) method. The absorption readings were measured at 510 nm using the same Spectra Max spectrophotometer.

2.7.2. Determination of β-glucan molecular weight (M_w)

The M_w of β-glucan in the ultrasound treated and untreated oat beverage samples was determined by SE-HPLC using an Agilent 1100 series HPLC system equipped an Ultrahydrogel™ Linear (7.8 × 300 mm, Waters Corp., Mass., USA) according to Åman, Rimsten, & Andersson, R. (2004) with modifications. The lyophilized oat beverages (200 mg) were mixed with 10 mL Milli-Q water and 70 μL 100% ethanol, and then hydrolyzed with 50 μL thermostable α-amylase from *Bacillus* spp. (EC 3.2.1.1., 3000 U/mL) (Megazyme International Ireland Ltd) at 90 °C for 2 h to remove starch and polysaccharides. Then, the mixtures were centrifugated at 2500 rpm for 15 min. The supernatants filtered with 0.45 μm membrane prior to SE-HPLC analysis. The column was kept at 40 ± 0.5 °C, and the flow rate of mobile phase was at 0.5 mL/min. Signals were detected by a refractive index detector (RID). Pullulans of different M_w (10,000, 21,700, 48,800, 113,000, 200,000, and 348,000 Da) were used as standards to estimate the β-glucan M_w.

2.8. Confocal laser scanning microscopy (CLSM)

The ultrasound treated and untreated residual samples were observed by CLSM. Acid Fuchsin and Calcofluor White were used to highlight the endosperm protein and plant cell wall, respectively (Dornez et al., 2011). Specifically, the residual samples were stained with

Acid Fuchsin (0.1 mg/mL) for 2 h, followed by Calcofluor White (0.01 mg/mL) for 2 h. After each staining, the samples were washed with Milli-Q water twice through centrifugation for 30 s at 500 rpm to remove excess stain. When the stained samples were imaged with a confocal laser scanning microscope (Zeiss LSM 710, Zeiss AxioObserver, Jena, Germany) with 10 × and 20 × objectives. The images were analyzed at the wavelength of 405 and 561 nm for Calcofluor White and Acid Fuchsin, respectively. The ZEN 2011 software (Carl Zeiss AG, Oberkochen, Germany) was used to process the images.

2.9. Foaming properties of oat beverage

The foaming properties of the oat beverages were measured according to Xiong et al. (2018) with modifications. 5 mL of oat beverage samples (stored at 4 °C) were whipped at a high speed (24,000 rpm, 1 min) using an Ultra-Turrax ® homogenizer (T18 Ultra Turrax, IKA, Wilmington, US). The foaming capacity was determined by comparing the foam volume at 1 min to the initial liquid volume. The foaming capacity (FC) was calculated as follows (Eq. (4)):

$$FC = \frac{V_1 - V}{V} \times 100\% \quad (4)$$

where V is the volume of the oat beverages, and V_1 is the volume of foam 1 min after whipping, respectively.

The foam volume was measured after storage at room temperature for 30 min. The foam stability (FS) was determined as the remained foam volume percentage using (Eq. (5)),

$$FS = \frac{V_2}{V_1} \times 100\% \quad (5)$$

where V_2 and V_1 represent the volume of foam after storage for 30 min and the initial volume of foam after whipping.

2.10. Statistical analysis

The experiments were preformed triplicate, and the data were presented as mean ± standard deviation. The statistical evaluations were computed by one-way analysis of variance (ANOVA) using Origin 2022 (Hampton, MA, USA). The statistical difference between samples was performed by the Tukey test with a p-value<0.05.

3. Results & discussion

3.1. Particle size distribution

Ultrasound can rupture plant cell walls through the generated high shear rate and microbubbles collapse in a liquid medium. Thus, the impact of ultrasound power levels (60, 240, and 960 W) and treatment durations (1, 5, and 9 min) on particle size change of the oat slurries before centrifugation and the oat beverages were studied. As shown in Fig. 1A, the oat slurries were a polydispersed system, which could be divided into two regions including one with small particle size (1–70 μm) and the other one with larger particle size (100–1600 μm). The ultrasound showed some impacts on the larger particle region in particle size distribution curves, leading to a left shifting of the size distribution. The more left shifting indicates the smaller size of the large particles. The pronounced peak left shifted from 709.68 to 329.93 μm as the ultrasound power level increased to 960 W for 9 min. In the small particle region, ultrasound treated oat slurries had distribution curves similar to that of the control.The effect of ultrasound on the large particle size reduction was also supported by the D_{43} (volume-based diameter) measurement, which is highly sensitive to any larger particles (Zhu et al., 2018). The ultrasound treated oat slurries also exhibited a reduction in D_{43} as the ultrasound exposure time increased to 9 min across all power levels when compared to the control without the

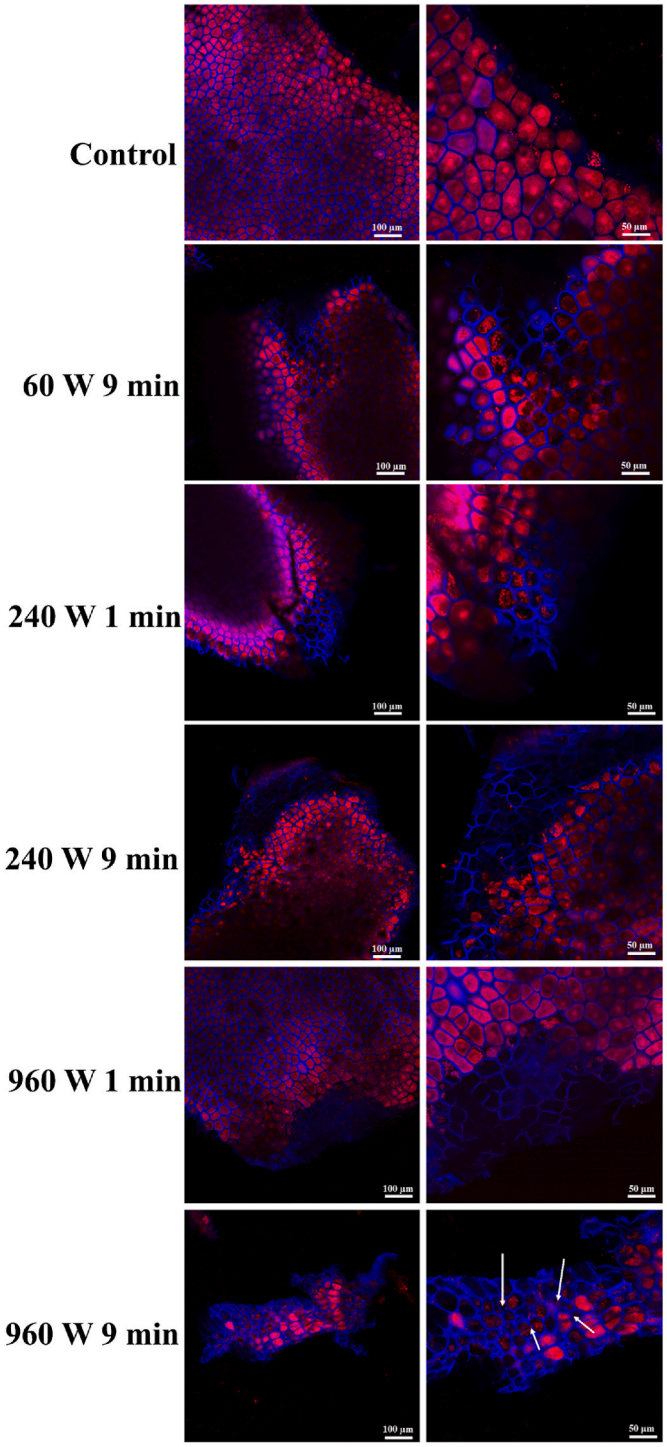

Fig. 2. Confocal micrographs of oat grain fibrous materials in the oat beverage residues undergo different ultrasound conditions. Protein bodies presented in red channel and polysaccharides (cell wall materials) are highlighted in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

treatment (Fig. 1B). As the ultrasound power level was raised to 960 W with 9 min treatment, the lowest D_{43} of 126.33 μm was achieved. From the optic microscopic observations, the larger particles were oat grain fibrous materials, and their size reduction was clearly observed when the ultrasound power levels were increased to 240 and 960 W with 9 min. This indicated the sizes of the fibrous materials had more impact at the higher ultrasound power with longer exposure time (Fig. S1). The

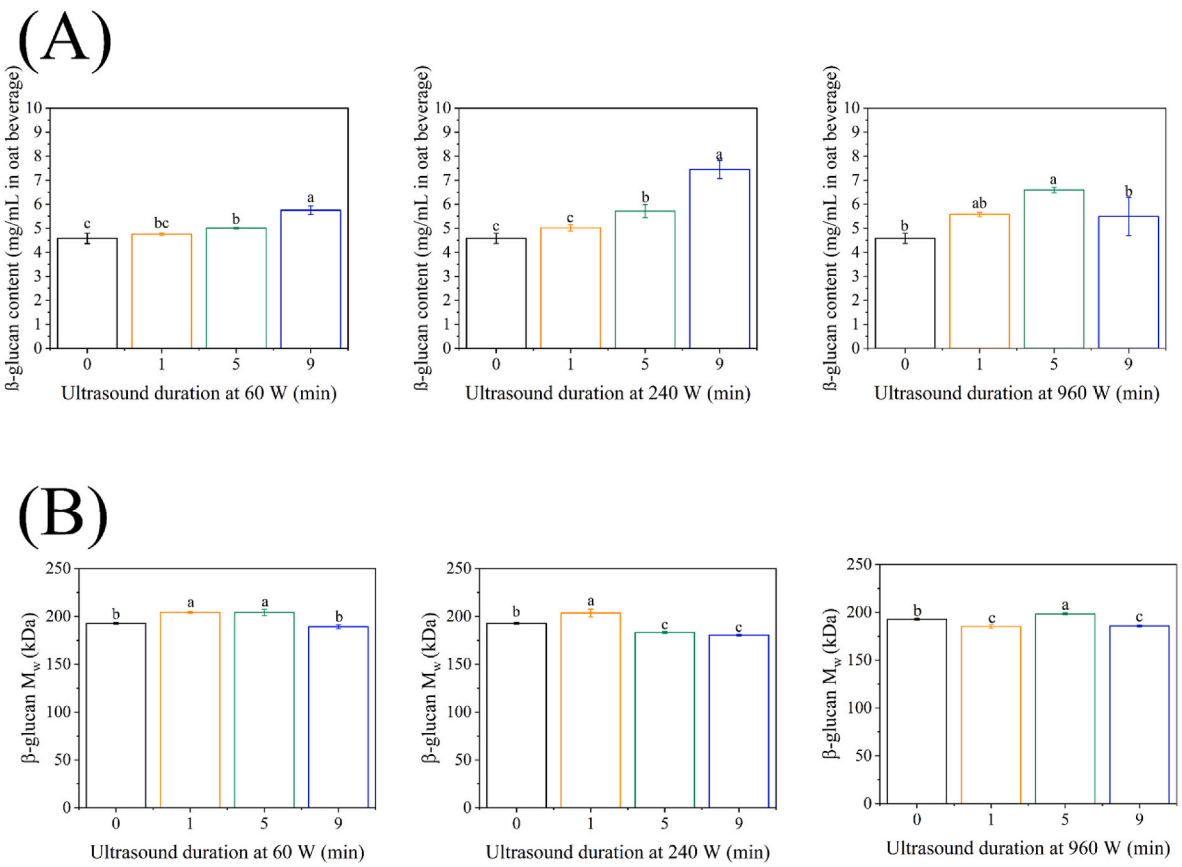

Fig. 3. Effect of ultrasound treatment (60, 240, and 960 W for 1, 5, and 9 min) on the (A) β-glucan content and (B) M_w in oat beverages. Control (0 min) was the sample without ultrasound treatment. Different letters above column denote significant differences ($p < 0.05$).

smaller particles were protein, polysaccharides and lipid (He & Xu, 2024). The size reduction of fibrous materials was due to cavitation resulting from bubble collapse, which generated micro-turbulence and hydrodynamic shockwaves, leading to the fibrous materials disintegration (Yusoff et al., 2022). The fibrous materials are in larger size, allowing more contact with the cavitation bubbles, thus resulting in greater hydrodynamic forces for the fragmentation. Furthermore, similar particle size distribution curves and no significant D_{43} was observed among the ultrasound treated oat beverages (Fig. S2). This indicated that ultrasound primarily affected the fibrous materials, potentially improving the embedded nutrient compounds to be available for extraction. Meanwhile, less impact on the small particles may allow better preservation of protein and β-glucan.

3.2. CLSM

The oat beverage residues were observed by the CLSM to further study the impact of ultrasound on the oat grain fibrous materials, as shown in Fig. 2. The protein bodies were stained with Acid Fuchsin (red), and the cell wall was stained with Calcofluor White (blue). Without the ultrasound treatment, large pieces of intact cell wall structure remained with protein bodies embedded in the cells. When 60 W was applied, the cells adjacent to the disintegrated edge became less packed, but the centre of the cells was remained intact and fully packed with protein bodies. When the higher ultrasound power level was applied, the destruction of cell walls not only limited to the edge of the residue fragment, with the observable damage towards its centre. This phenomenon was attributed to the greater exposure of cavitation effect on these cell walls. Prolonged treatment at 960 W for 9 min further intensified this impact, resulting in more damage in the centre of cell walls as indicated by the arrows in Fig. 2. The generated hydrodynamic shock wave can damage the cell structure including breakages and loosening of the cell structure (Yusoff et al., 2022). Although the cell walls were damaged, the overall shape of the cell walls was retained. Whereas several studies demonstrated completely rupture of cell walls when fruit juices were treated by a high-power level of ultrasound with a longer duration (Rojas, Leite, Cristianini, Alvim, & Augusto, 2016; Campoli, Rojas, do Amaral, Canniatti-Brazaca, & Augusto, 2018). Due to the difference in rigidity of the plant cell walls, ultrasound had less destructive effects on oat cell walls than those of fruits. The mechanism of intercellular materials extraction through ultrasound involves cell disruption, resulted in increasing the contact area of intracellular materials and extraction solvent (water) to promote mass transfer (Yusoff et al., 2022). Since the oat cells were not fully disrupted, the improvement of mass transfer of proteins into the water was limited. It was observed that some of the cells still contained fully packed protein bodies, even after treatment with 960 W for 9 min. Similar result was observed from the study of Preece, Hooshyar, Krijgsman, Fryer, and Zuidam (2017) in which protein bodies remained within the cells when the soy slurry was treated with 1700 W for 2.3 min.

3.3. The effect of ultrasound treatment on β-glucan content and M_w in oat beverage

The β-glucan content of oat beverages by ultrasound treatments is shown in Fig. 3A. The oat beverages treated with ultrasound of 60 and 240 W showed gradually increased β-glucan content. At 240 W for 9 min the highest content of β-glucan of 7.45 mg/mL was observed in the oat beverage which increased by almost 2 folds compared to the control (4.58 mg/mL). The explosions of bubbles generate energy to loosen the outer layer of oat cell walls, allowing more water to penetrate to the cell wall inner layer where β-glucan is located (Hematian Sourki et al.,

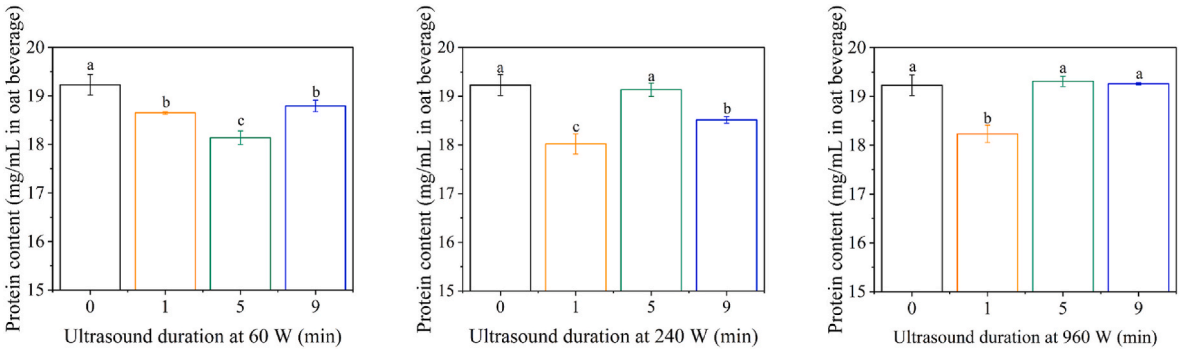

Fig. 4. Effect of ultrasound (60, 240, and 960 W) for 1, 5, and 9 min on the protein content of oat beverages. Control (0 min) was the sample without ultrasound treatment. Different letters above column denote significant differences ($p < 0.05$).

2017). However, when the ultrasound power level was further raised to 960 W and time was prolonged to 9 min, the β-glucan content was reduced to 5.49 mg/mL, even no significant difference compared to the control sample ($p > 0.05$). This reduction could be attributed to more insoluble materials such as cellulose and arabinoxylan were also released and suspend in the solvent (water), which possibly lower the permeability of water to extract β-glucan (Prakash Maran, Manikandan, Thirugnanasambandham, VignaNivetha, & Dinesh, 2013). The prolonged exposure time (9 min) may cause excessive release of these insoluble materials, leading to the further reduction of the β-glucan content in the oat beverage. Similar results were observed by the work of Hu et al. (2022), in which reduced extraction yield of polysaccharides from coix seeds was observed when ultrasound time was extended to 15 min at the high power level (480 W). Meanwhile, only a minor change was observed for the β-glucan M_w. The samples showed β-glucan M_w range of 180.51–204.23 kDa. Even with the highest ultrasound power level with prolonged treatment time (960 W for 9 min), only approximately 4.2% decrease of β-glucan M_w when ultrasound timing was extended from 0 to 9 min at 960 W. Since the large amount of microbubble formation at a higher ultrasound power level which could act as a barrier of energy transmission within the system. These bubbles may not effectively to produce the required energy to further decrease the M_w (Muñoz-Almagro, Montilla, Moreno, & Villamiel, 2017). Comparing to ultrasound, multiple enzymatic (e.g. the combination of α-amylase, protease and xylanase) and chemical (alkaline and acidic solutions) extraction methods can lead to extensively fragmentation of β-glucan M_w into shorter chains, which may interfere its blood-glucose and blood-cholesterol lowering abilities (Bozbulut & Sanlier, 2019; Goudar, Sharma, Janghu, & Longvah, 2020). Significantly increase in extraction of β-glucan content by the ultrasound treatment (240 W for 9 min) without extensively reducing its M_w makes this technology favorable for improving the nutritional quality of oat beverage.

3.4. The effect of ultrasound on protein content in oat beverage

The effect of ultrasound on protein content in the oat beverages is presented in Fig. 4. Comparing with the control, the ultrasound treated oat beverages showed either no significant difference or a reduction in the protein content. One explanation could be due to the rigid cell wall protected the intercellular proteins from cavitation effects, which limiting the extraction of cellular proteins (Karabulut, Yildiz, Karaca, & Yemiş, 2023). Another explanation was ultrasound not only released the protein, other components including dietary fibers such as β-glucan and oil were also being released in the oat beverages. These released components might interact with the oat protein which inhibited the dissolution of protein during extraction, leading to its precipitation by centrifugation (Byanju, Rahman, Hojilla-Evangelista, & Lamsal, 2020; Jaramillo, Roberts, & Coupland, 2011). Similar findings were found when using ultrasound to extract protein from chickpea as the authors suggested high contents of carbohydrates and lipids lowered the protein yield in the products (Byanju et al., 2020). An increase in water ratio might increase protein content in the oat beverages as water allowed more cavitation effects. However, from the industrial perspective, increase in water usage is not desirable because the oat beverage will be diluted, which also leads to diminish of its nutritive quality and desirable characteristics such as mouthfeel.

3.5. The effect of ultrasound on protein structure

3.5.1. SDS-PAGE

SDS-PAGE profiles of protein in control and oat beverages treated by ultrasound are shown in Fig. 5A. In oat protein, the major protein is 12S globulin. The former is the 320 kDa hexamer is comprised of 54 kDa subunits that bind non-covalently. Each subunit is disulfide bonded, which separated into acidic (32 kDa) and basic (22 kDa) subunits by 2-mercaptoethanol (Li & Xiong, 2021). Other bands at 50–75 and 15–17 kDa correspond to 7S and 3S subunit of oat globulin, respectively (Nieto-Nieto, Wang, Ozimek, & Chen, 2014). Comparison with the untreated oat beverage showed that ultrasound did not induce major changes in oat protein subunits or break down the disulfide bonds in protein structure. Similar results were observed when using ultrasound to hemp protein (Liu, Wang, Xue, & Adhikari, 2022) and quinoa protein (Li et al., 2023). In other studies, modification of extracted plant-based proteins subunits by ultrasound were observed. For example, bitter melon seed and olive leaf proteins were broken down into smaller structures through extra bands were revealed at the lower molecular weight ladder (Naik, Natarajan, Modupalli, Thangaraj, & Rawson, 2022; Ortega et al., 2024). This result suggests that the modification of protein molecular weight using ultrasound extraction is contingent on the specific plant sources employed.

3.5.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR was used to understand the secondary structure of protein. The absorption changes in amide region (amide I) were mainly studied and the results are shown in Fig. 5B. The amid I band with and without ultrasound treatment showed 7 bands, including 1691 cm^{-1} (β-sheets/turns), 1670 cm^{1} (β-turns), 1656 cm^{-1} (α-helix), 1641 cm^{-1} (random coil), 1629 cm^{-1} (β-sheet), 1618 cm^{-1} (intermolecular β-sheet), and 1609 cm^{-1} (vibration of amino acid residues) (Kong & Yu, 2007; Liu et al., 2009; Nieto-Nieto, Wang, Ozimek, & Chen, 2015). When oat grains underwent ultrasound treatment, no changes in amid I region were observed. This result suggests that ultrasound leave the secondary structure of oat protein intact.

3.5.3. Intrinsic fluorescence spectroscopy

Intrinsic fluorescence can be applied to characterize the tertiary structure changes of protein, mainly due to the presence of chromophores (tryptophan, tyrosine, and phenylalanine residues). The

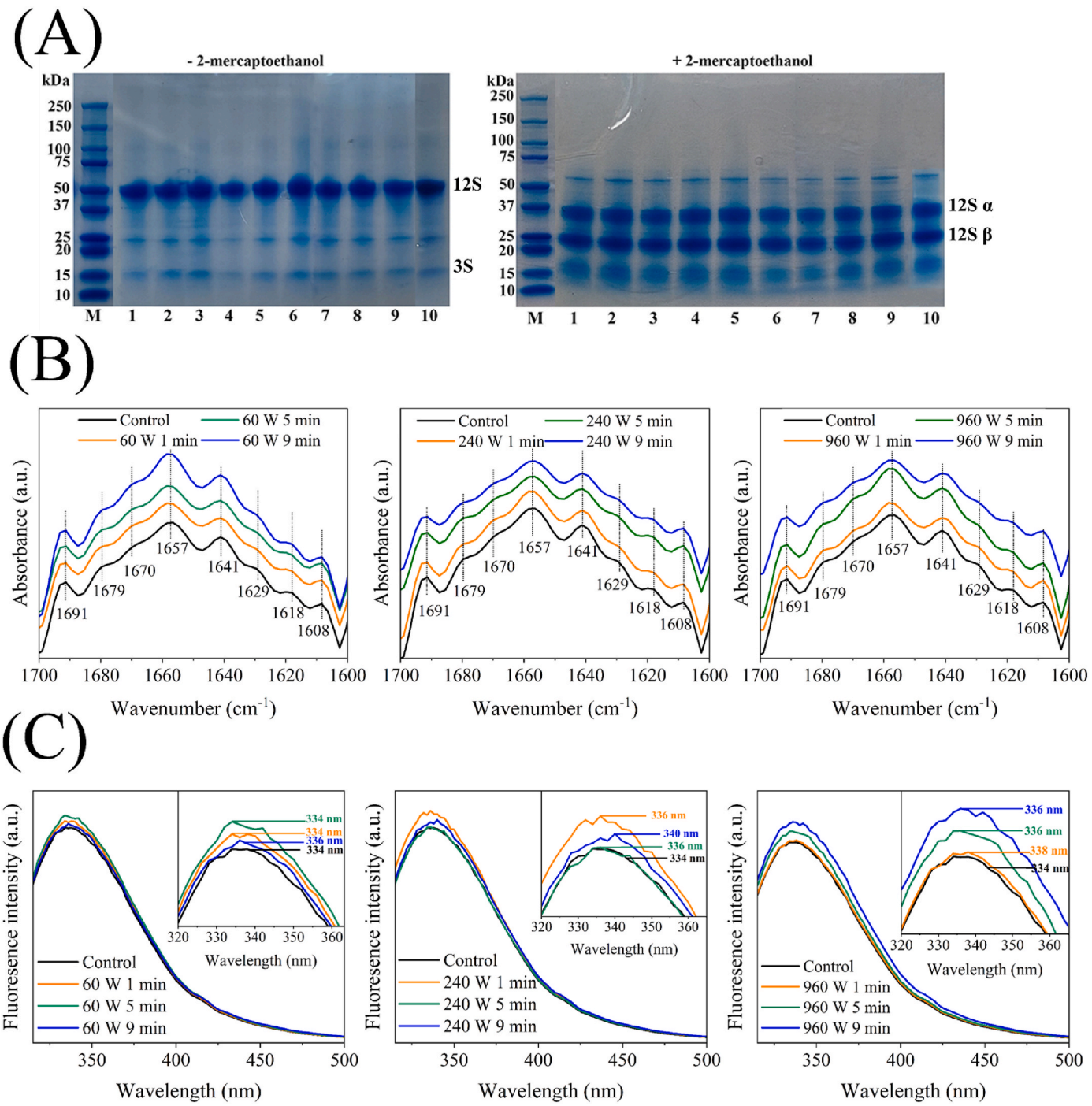

Fig. 5. Effect of ultrasound treatment (60, 240, and 960 W for 1, 5, and 9 min) on the (A) SDS-PAGE profiles (Marker, M; Control, lane 1; 60 W–1 min, lane 2; 60 W–5 min, lane 3; 60 W–9 min, lane 4; 240 W–1 min, lane 5; 240 W–5 min, lane 6; 240 W–9 min, lane 7; 960 W–1 min, lane 8; 960 W–5 min, lane 9; 960 W–9 min, lane 10), (B) deconvoluted FT-IR spectra, (C) intrinsic fluorescent spectra of oat beverages.

fluorescence spectra particularly reflect tryptophan as its intensity is greater than tyrosine and phenylalanine residues (Li & Xiong, 2021). The position of tryptophan in a protein can be analyzed through study of the λ_{max} emission; if the λ_{max} is less than 330 nm, tryptophan is buried in a non-polar region in a protein; if λ_{max} is greater than 330 nm, tryptophan is exposure to a polar environment (Vivian & Callis, 2001). As presented in Fig. 5C, the λ_{max} of control was 334 nm and ultrasound treated oat beverages were between 334 and 340 nm. When the ultrasound duration was increased to 9 min at 60 W, the λ_{max} was shifted to 336 nm. Further peak shift was observed as the oat beverage was treated with 240 W for 9 min. The shift of λ_{max} to the longer wavelength wave (red shift) indicates that tryptophan side chains in protein were exposed to the polar environment. This was aligned with the results of surface hydrophobicity (H_0) measurement, as shown in Fig. S3. H_0 indicates the number of hydrophobic groups exposed to the protein molecular surface. Treatment by 240 W for 9 min resulted the significant increased in H_0 when compared to the samples treated by 1 min and the control. Both results indicates that ultrasound partially unfolded oat protein, led to exposure of buried hydrophobic groups to the protein surface (Arranz et al., 2023). When the power level increased to 960 W, a reduction in H_0 was observed when the treatment duration was prolonged to 9 min (Fig. S3). The reduction in H_0 was also observed for back bean and hemp

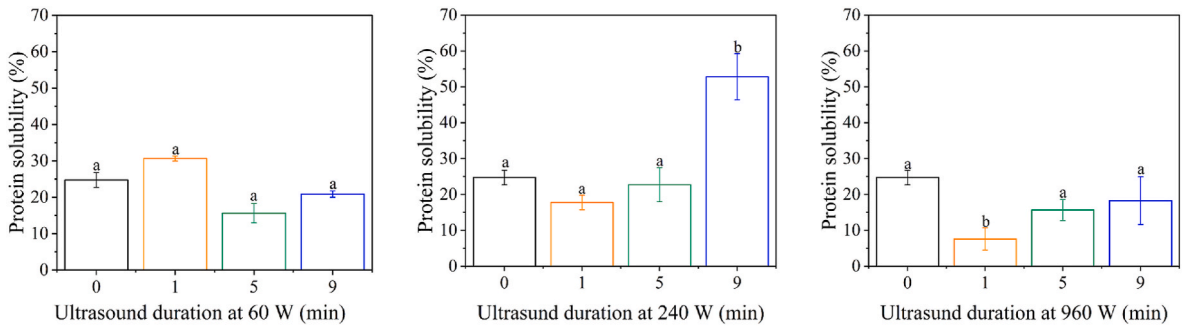

Fig. 6. Effect of ultrasound (60, 240, and 960 W) for 1, 5, and 9 min on the protein solubility of oat beverages. Control (0 min) was the sample without ultrasound treatment. Different letters above column denote significant differences ($p < 0.05$).

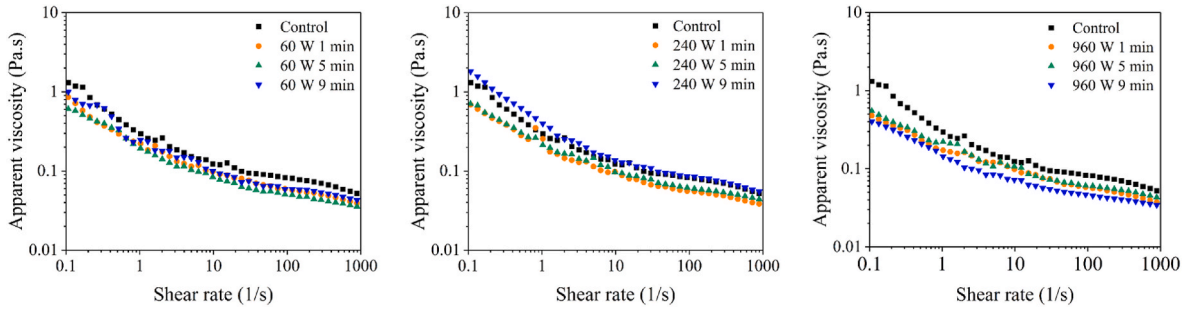

Fig. 7. Effect of ultrasound treatments on the apparent viscosity of oat beverages. Control (0 min) was the sample without ultrasound treatment.

proteins when treated by a high power ultrasound for a longer exposure time. This could be related to the aggregation of partially unfolded proteins through hydrophobic and other interactions, and thus the exposed hydrophobic groups were buried inside the protein aggregates (Liu et al., 2022; Mir, Riar, & Singh, 2019). Consequently, a blue shift occurred as the λ_{max} shifted back from 338 to 336 nm when the ultrasound treatment was prolonged from 1 to 9 min (Fig. 5C).

3.6. The effect of ultrasound treatments on protein solubility

Protein solubility is a critical factor in plant-based beverages as having a good protein solubility will improve physiochemical properties, including foaming properties of plant-based beverages. In this study, the oat beverage samples have a range of pH from 6.26 to 6.40, and oat protein is known to have limited solubility in such pH range (Yu et al., 2023). As displayed in Fig. 6, the protein solubility showed no statistical change compared with the control at low power ultrasound treatment. As the power level was increased to 240 W and treated for 9 min, the protein solubility was significantly enhanced from 24.7 to 52.8%. This could be attributed to partial unfolding structure of oat protein, increasing the interaction between the protein and water molecules. When the ultrasound power level and treatment time increased, more hydrophobic groups were exposed that could caused protein to reaggregate through non-covalent interactions and thus, reduce its solubility (Li & Xiong, 2021; Mir et al., 2019). Despite an increase in the solubility at 240 W, the protein recovery did not improve. This contracts with the expectations, as higher water solubility usually correlates with a better recovery (Karabulut et al., 2023; Li et al., 2023). Further studies are needed to understand this phenomenon.

Table 1
Consistency index (k) and flow behavior index (n) of untreated and ultrasound treated oat beverages using the Power Law model fitting.

Samples	Duration (min)	Consistency coefficient (k, Pa.s^n)	Flow behavior index (n)	R^2
60 W	0	0.333 ± 0.009^{a}	0.679 ± 0.013^{a}	0.988
	1	0.313 ± 0.012^{ab}	0.641 ± 0.020^{a}	0.984
	5	0.277 ± 0.007^{a}	0.663 ± 0.011^{a}	0.999
	9	0.299 ± 0.014^{a}	0.644 ± 0.008^{a}	0.986
240 W	0	0.333 ± 0.009^{ab}	0.679 ± 0.013^{a}	0.988
	1	0.266 ± 0.002^{b}	0.685 ± 0.012^{a}	0.992
	5	0.289 ± 0.020^{b}	0.666 ± 0.087^{a}	0.974
	9	0.358 ± 0.038^{a}	0.637 ± 0.066^{a}	0.979
960 W	0	0.333 ± 0.009^{a}	0.679 ± 0.013^{a}	0.988
	1	0.263 ± 0.020^{bc}	0.664 ± 0.027^{a}	0.983
	5	0.287 ± 0.008^{b}	0.654 ± 0.004^{a}	0.991
	9	0.248 ± 0.010^{c}	0.774 ± 0.011^{b}	0.995

Data were expressed as mean ± standard deviation. 0 min indicated the oat beverage was not treated with ultrasound and only enzymatic hydrolysis. Different superscripted letters indicate significant differences ($p < 0.05$).

3.7. Rheological properties

The rheological properties of oat beverage are important for its processing and sensory quality. Too high viscosity can result in inefficiency in operation such as low extraction yield of oat beverage and high energy usage. In addition, oat beverage with a high viscosity will have adverse impacts on its sensory properties such as mouthfeel, resulting in a distinct difference from bovine milk. Consumers often anticipate plant-based beverages to exhibit sensory characteristics similar to bovine milk, as they are perceived as beverage substitutes. The apparent viscosities of untreated and ultrasound treated oat beverages were presented in Fig. 7. At 60 W, the ultrasound treated oat beverages showed a reduction in apparent viscosity than that of the control. This could be explained by the collapse of the cavitation bubbles that caused physical disintegration of starch granules (Falsafi et al., 2019), thus allowing easier access of α-amylase to starch granules for a more completed hydrolysis. When the treatment time was extended to 9 min, a slight increase of the viscosity was observed, which was due to the increase of β-glucan content. As the ultrasound power level and timing increased to 240 W and 9 min, the apparent viscosity was even slightly higher compared to the control due to the highest β-glucan content in

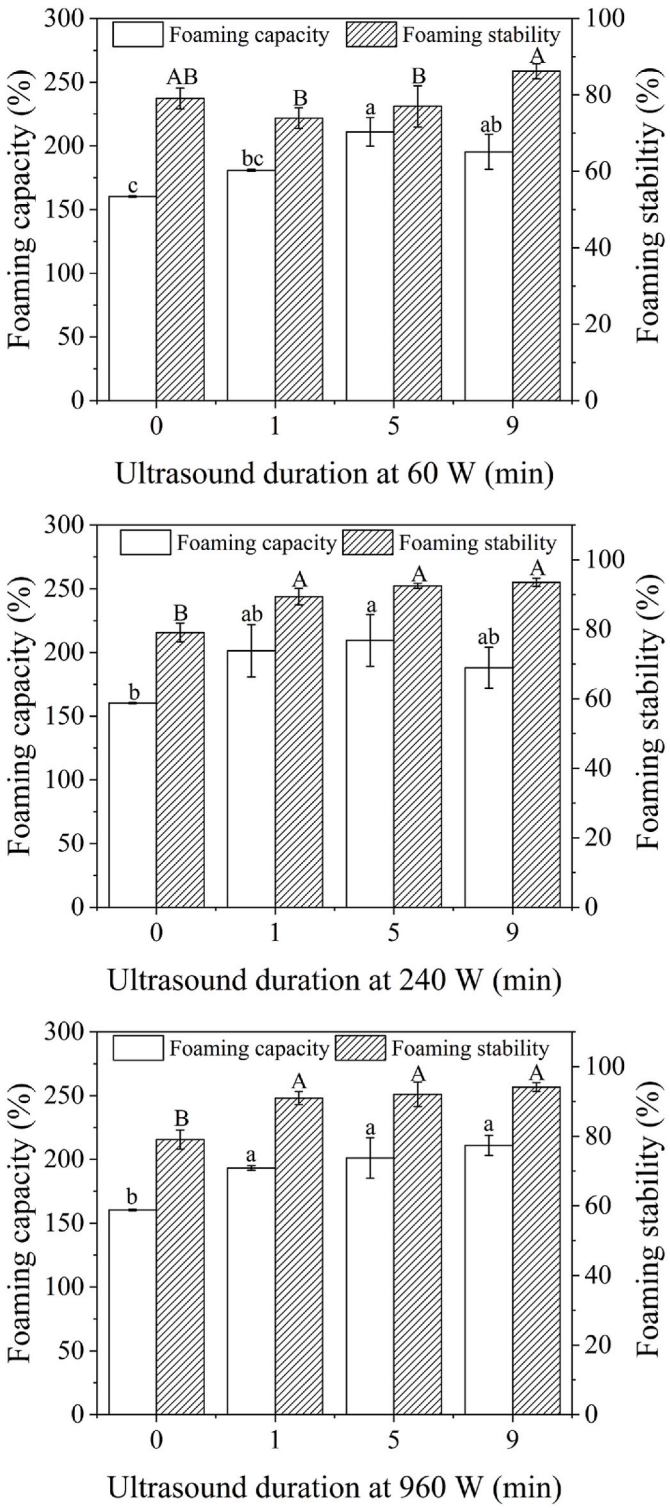

Fig. 8. Impact of ultrasound treatment (60, 240, and 960 W for 1, 5, and 9 min) on the foaming properties of oat beverages. Control represented no ultrasound treatment. Different letters above column indicate significant differences in the same characteristic ($p < 0.05$).

the oat beverage (Fig. 3A). β-Glucan has steric hindrance effects on water molecules and reduces its mobility, leading to a higher viscosity (Hematian Sourki et al., 2017). For the oat beverages treated by the ultrasound under 60 W for 1–9 min and 240 W for 1–5 min, the viscosities were reduced with the increasing shear rate because the entanglements formed by β-glucan were disrupted by the imposed movement (Papageorgiou, Lakhdara, Lazaridou, Biliaderis, & Izydorczyk, 2005). However, the viscosity of ultrasound treated oat beverage under 240 W for 9 min exhibited a different trend. A higher viscosity compared to the control and other samples was observed especially at high shear rates (100–1000 1/s). This could be related to the increased amount of β-glucan extracted into the oat beverage under such conditions, leading to formation of stronger entanglements that were more resistant to deformation by the imposed movement (Yuan, Ritzoulis, & Chen, 2019). Nevertheless, its consistency coefficient (k) was not significantly different than that of the control ($p > 0.05$) (Table 1), indicating their viscosities were still similar and the increase in β-glucan in this study would not necessarily change its viscosity to a level that can impact the sensory properties (Rao, 2014). At 960 W, the apparent viscosities were further reduced compared with other ultrasound treated samples. Moreover, their consistency coefficients (k) were significantly lower compared to the control ($p < 0.05$) (Table 1), indicating the oat beverages became less viscous. The ultrasound with the highest power level (960 W) caused more destructive effects on starch granules, allowing enhanced accessibility of α-amylase to hydrolysis starch and hence, significantly decreased in viscosity of oat beverages. Starch hydrolysis and β-glucan play important roles to determine the viscosity of oat beverage, that subsequently affect the operation processing of oat beverage and the sensory quality of the final products.

In Table 1, flow behaviour index reflects the rheology of a fluid system; when $n = 1$ indicates the fluid has a Newtonian, the fluid with $n < 1$ is shear-thinning (pseudoplastic) and $n > 1$ is shear thickening (dilatant) in nature (Rao, 2014). The control and samples by ultrasound treatment showed $n < 1$, indicating ultrasound did not alter the fluid types of oat beverage as all the samples exhibited shear-thinning behaviour. The positive impacts of shear thinning behavior on operational processing including ease of mixing and pumping when subjected to mechanical forces, and product sensory quality as desirable mouthfeel in the oat beverage development.

3.8. *Foaming properties*

The values for foaming capacity and stability are presented in Fig. 8. Notably, the foaming capacity of oat beverages showed an improvement after ultrasound treatments. This could be attributed to the reduction in larger particle size as larger particles in wet foam systems can decrease in foaming capacity. It was observed in a foam system with silica particles of different sizes, larger particles became pinned to the well of film between air bubbles, limiting their diffusivity, while the smaller particles continued to diffuse freely (Dickinson, 2015). This creates an osmotic pressure difference, pulling water from regions with larger particles, thinning the film in those areas and causing rupture. Moreover, the foaming capacity can be impacted by the viscosity of oat beverages. For example, the oat beverages treated at 960 W showed a significant decrease in viscosity, making proteins easier to absorb to the air-water interfaces (Sheng et al., 2018). Furthermore, the foaming stability was also significantly improved when ultrasound was applied than that of the control, typically at 240 and 960 W ($p < 0.05$). The increase of foaming stability could be due to the enhanced β-glucan content, providing a certain level of viscosity to lower the mobility of air bubbles (Eghbaljoo et al., 2022). More importantly, ultrasound induced the partial unfolding of oat proteins, increasing the number of hydrophobic groups exposure to surface, which enhanced their ability to absorb at the air-water interface and formation of a cohesive layer around the air bubbles to prevent coalescence and thereby increase the foaming ability (Mir et al., 2019). The improved foaming capacity and stability of ultrasound treated oat beverages will be desirable when using as an ingredient in such as coffee-based beverages and desserts.

4. Conclusion

This research has provided scientific insight for a fundamental

understanding of the impact of ultrasound as a pre-treatment technique on the nutritive quality and physicochemical properties of oat beverage. Ultrasound effectively reduced the fibrous materials into the smaller size, and partially disrupted oat grain cell wall structure. This resulted in up to 39% enhancement in β-glucan recovery when treated at the ultrasound power level at 240 W for 9 min, but the content was reduced when the greater power level was implied (960 W). Interestingly, ultrasound exhibited minimal impact on the β-glucan M_w that could preserved its inherent health benefits. Conversely, the protein recovery in oat beverage was not improved by ultrasound treatment, instead, ultrasound treatment led to increased hydrophobic amino acid side chains exposure to the surface. This led to enhanced foaming properties, which would be advantageous for developing coffee-based beverages and desserts. Furthermore, shear-thinning behavior was retained in ultrasound treated oat beverages, offering advantages for operational efficiency, and maintaining desirable mouthfeel. Therefore, ultrasound is a potential pre-treatment technology to improve the nutritive values and physicochemical properties of oat beverage. To further enhance the extractability of protein in oat beverage, it will be worthy of investigation to combine ultrasound with other technologies such as cell wall degrading enzymes for a better disruption of the rigid cell walls, thereby recovering more nutritious components to improve the overall nutritional quality of oat beverage.

CRediT authorship contribution statement

Esther Kwok: Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Jingqi Yang:** Methodology, Conceptualization. **Parinaz Taheri:** Writing – review & editing, Resources. **Lingyun Chen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116303.

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