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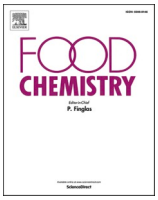

Ultrasonication-assisted enzymatic bioprocessing as a green method for valorizing oat hulls

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ABSTRACT

Ultrasonication-assisted enzymatic treatments using Viscozyme®, Alcalase®, and feruloyl esterase were applied to recover proteins, avenanthramides, phenolic acids, free sugars, and organic acids from oat hulls (OH). The profiles of the chemical compounds in OH were markedly influenced by the nature of enzymes, ultrasonication frequency, and processing time. A significant increase in the contents of proteins and phenolic acids was observed in the liquid fraction of all enzymatic treatments, which was 2–19 folds higher than those detected in untreated OH. In contrast, avenanthramides were mostly degraded during enzyme hydrolyses. The highest content of proteins (68.9 g/100 g DM) was found in the liquid fraction after the feruloyl esterase treatment assisted with 90 min of ultrasonication at 25 kHz. This fraction also contained 0.07% phenolic acids, 14.1% free sugars, and 1.8% organic acids, which can be potentially used as the ingredient of novel food products.

1. Introduction

During the past decade, the expanding market of oats (*Avena sativa*) is reflected by a nearly 20% increase in global oat production, which has risen from 21 million tons in 2012 to 25 million tons in 2022 (FAOSTAT, 2021). Consequently, as the primary side stream of oat production, enormous volumes of oat hulls (OH, the outer shell of oat, accounts up to 35% of the entire grain) are generated globally (Schmitz et al., 2021).

As agro-industrial wastes, OH is often undervalued. These materials are commonly disposed in landfills or used directly as animal feeds. As reported recently, a variety of valuable components were found in OH, mainly including proteins (1–7%, dry weight basis), cellulose (16–26%), hemicellulose (24–35%), and lignin (13–25%) (Schmitz et al., 2020, 2021). Bioactive components, such as avenanthramides and phenolic acids are also present in OH, which have various health-promoting functions, such as antioxidation, anti-inflammatory, and anti-cancer (Bratt et al., 2003; Grundy et al., 2018; Habschied et al., 2021). The research on OH valorization has focused mostly on utilizing lignocellulose with the purpose for biofuel production or livestock feed, due to the abundance of fiber in OH (Adewole et al., 2020; Dziki et al., 2022; Skiba et al., 2017). However, the usage as livestock feed increases animal husbandry carbon emissions, posing potential risk on environment. Converting OH to novel food products may be a more environmental-friendly way to valorize these materials. The OH-derived food products are expected to contain multiple essential nutrients. An optimal level of bioactive compounds (avenanthramides and phenolic acids) should also be retained in the products, to possess health-beneficial functions and to avoid negative effects on sensory attributes of the products. Yet, up to date, little has been done in this field other than usage as the source of dietary fibers (Galdeano & Grossmann, 2006).

The challenge of developing OH-derived food products lies in enhancement of OH nutrition quality. In comparison with fibers, proteins and bioactive phytochemicals are present in the OH at relatively low levels. Enzymatic hydrolysis has been proved as a feasible approach for altering chemical composition of OH, mostly used to degrade fibers to oligomeric and monomeric saccharides. Commercial enzymes such as Celluclast® (cellulase) and enzyme cocktails consisting of various combination of cellulase, xylanase, β-glucanase were applied to hydrolyze lignocellulose into glucose in renewable agro-industrial side streams such as OH (Dall Cortivo et al., 2020; Makarova et al., 2017). Recent studies on other agro-industrial wastes suggested that enzyme

Abbreviations: OH, oat hulls; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrum; PCA, principal component analysis; AV, avenanthramides; PA, phenylalkenoic acid; AA, anthranilic acid.

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treatments can also enhance the extractability of proteins and the release of bioactive compounds from side-stream matrix (Tian et al., 2022). Comparing to the conventional treatments using strong alkaline or acids, the use of enzymes avoids protein denaturation as well as the reduction of protein digestibility and levels of essential amino acids (Demirel et al., 2018; Schmitz et al., 2021; Skiba et al., 2017). Nevertheless, no research has been carried out to systematically investigate the impact of different enzymes on chemical profiles of OH.

Therefore, our study was designed to employ enzymatic bioprocessing to convert OH into fractions rich in multiple nutrients and bioactive compounds which can be used as readily available ingredients in food innovations. Different enzymes (food-grade carbohydrase, protease, and feruloyl esterase) were compared in terms of their efficiency in releasing nutrients and bioactive compounds. To promote the efficiency of enzymatic hydrolysis, ultrasonication was introduced into the enzyme treatments. Frequencies and processing time of ultrasonication were studied as key factors of enhancing the enzymatic release of these components from the bounded form in OH, in comparison with conventional shaking. Modern chromatographic and mass spectrometric analysis methods were applied to study the changes in the compositional profile of OH and the extraction efficiency of proteins and phenolic acids as the results of different treatments. The focus was on the release of soluble proteins as the major nutrient, as well as phenolic acids and avenanthramides as the main bioactive components. In addition, free sugars and organic acids were also monitored as important contributors to sensory properties of food. Moreover, effective release of free sugars as carbon sources by microbes is a necessary pretreatment of OH to facilitate further bioprocessing using fermentation. The novelties of our research include providing new insights into the valorization of OH, offering the reference for transforming other crop side streams towards food products fortified with multiple nutrients and bioactive compounds, as well as promoting the resilience of food system and circle economy.

2. Materials and methods

2.1. Materials and chemicals

Ground OH were provided by Raisio Oy (Raisio, Finland). Viscozyme® (a mixture of β-glucanase, pectinase, hemicellulase and xylanase) and Alcalase® were purchased from Sigma-Aldrich (St. Louis, MO, United States). Feruloyl esterase was provided by Novozymes (Bagsvaerd, Denmark). Sodium hydroxide and boric acid were from Sigma-Aldrich (Darmstadt, Germany). Trimethylsilyl reagent was ordered from Thermoscientific (Bellefonte, United States). All reference standards of sugars, organic acids, avenanthramides and phenolic acids and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, United States). Hydrochloric acid, diethyl ether and ethyl acetate were purchased from Honeywell (Espoo, Finland). Other chemicals of LC and MS grade were purchased from VWR International Oy (Espoo, Finland).

2.2. Enzymatic treatments of OH

The detailed information of enzymatic treatments was described in Fig. 1 and Supplemental Table 1. Three enzymatic treatments were performed by using Viscozyme, Alcalase, and feruloyl esterase. Approximately 4 g of OH was mixed with deionized water at a ratio of 1:10 (*w/v*). The mixtures were incubated in the water bath (Elma Schmidbauer GmbH, Singen, Germany) at the optimum temperatures for individual enzyme for 20 min. The incubation temperature was set according to the manufacturer's suggestions. The enzymatic treatments started immediately after the addition of Viscozyme, Alcalase, or

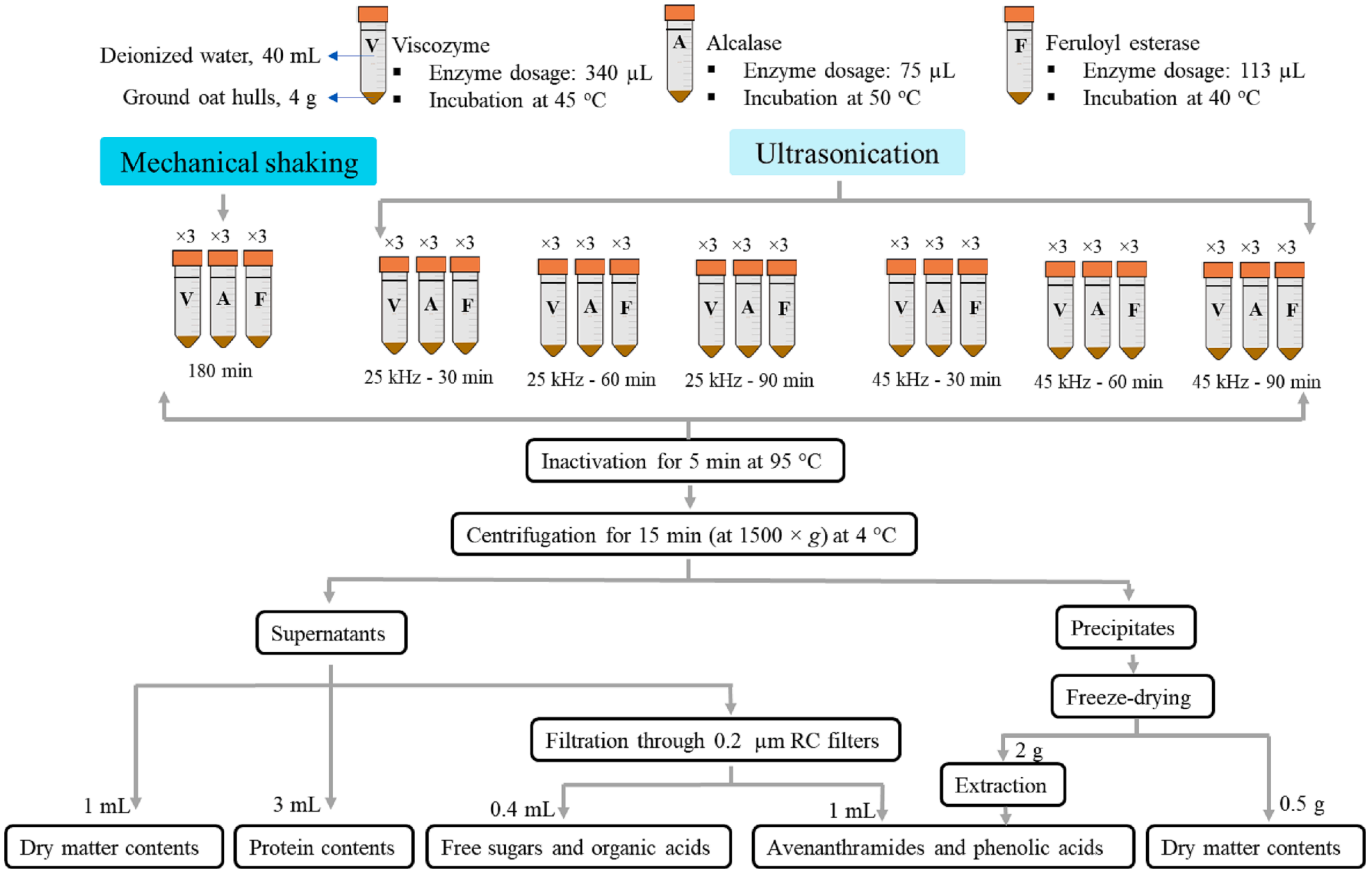

Fig. 1. Flow chart of enzymatic treatments and sample preparation.

feruloyl esterase. Each treatment was assisted with ultrasonication for 30, 60 and 90 min under the frequencies of 25 kHz and 45 kHz. In order to investigate the efficiency of ultrasonication in enzyme treatments, the control groups were incubated with same dosage of enzymes at same condition but assisted by mechanical shaking for 3 h (Lauda Ltd, Lauda-Königshofen, Germany). Each treatment was conducted in triplicates. After the treatments ended, all enzyme-treated samples were boiled at 90 °C for 5 min to deactivate the enzymes. The samples were centrifuged for 15 min (1500 × *g* at 4 °C), both supernatants and precipitates were collected. The precipitates were freeze-dried using freeze dryer (Buchi, Flawil, Switzerland). The supernatants and freeze-dried precipitates were stored at −20 °C for further analyses.

2.3. Dry matter content of OH raw material, enzyme-treated supernatants and precipitates

Dry matter content was determined at 105 °C. Approximately 1 g OH raw material and 1 mL enzyme-treated supernatants, 0.5 g freeze-dried precipitate were dried in the oven (~24 h) until they reached constant weights.

2.4. Total content of proteins in OH raw material and enzyme-treated supernatants

The content of proteins of raw material (1.5 g) and enzyme-treated supernatants (3 mL) were determined by a Kjeldahl autoanalyser (Foss Tecator Ab, Höganäs, Sweden). The conversion factor of N × 6.25 was chosen for calculating contents of proteins (International Organisation of Standardization, 2009).

2.5. Free sugars and organic acids in OH raw material and enzyme-treated supernatants

The raw material of OH (4 g) were extracted with 3 × 10 mL aqueous methanol (methanol: water, 8:2, *v/v*) by 20 min ultrasonication, followed by 15 min of centrifugation (1500 × *g* at 4 °C). All three-time extracts were collected and combined. The combined supernatant was diluted to 50 mL with extraction solvent. OH raw material extract and supernatants of enzyme-treated samples (1 mL) were filtered by 0.22 μm PTFE and RC syringe filters (Phenomenex) respectively for further analysis.

The analysis of the studied compounds was performed using a Shimadzu GC-2010 coupled with flame ionization detector (Shimadzu corp., Kyoto, Japan) equipped with a SPB-1 column (30 m × 0.25 mm i.d., 0.25 μm, Supelco, Bellefonte, PA, United States). The analytical method was described in our previous study (Tian et al., 2022). Briefly, 0.4 mL of each sample was mixed with 0.2 mL of each standard (approximately 0.5 g/100 mL of arabinose, xylose, xylitol, fructose, glucose, mannose, mannitol, inositol, sucrose, maltose, rhamnose, galactose, galacturonic acid, malic acid and citric acid). After drying by nitrogen flow, approximately 600 μL of trimethylsilyl (TMS) was added to derivatize the sample before GC analysis. The injection volume was 1 μL. The compounds were identified by comparing the retention times with those of external standards. Sorbitol (for sugars) and tartaric acid (for organic acids) were used as internal standards for quantitative analysis.

2.6. Avenanthramides and phenolic acids in OH raw material and enzyme-treated samples

Aqueous methanol was used to extract free phenolic acids and avenanthramides in OH. The OH raw material (approximately 2 g) were mixed with aqueous methanol (methanol: water, 8:2, *v/v*) at a ratio of 1:10 (*w/v*), followed by 20 min of ultrasonication and 15 min of centrifugation (1500 × *g* at 4 °C). The extraction was conducted three times. The supernatants from three-time extraction were combined together and evaporated to completely dry at 30 °C by using vacuum rotary evaporation (Heidolph, Germany). The residue was re-dissolved in 1.5 mL of methanol and filtered through 0.22 μm PTFE syringe filters (Phenomenex) before HPLC and MS analysis. The freeze-dried precipitates obtained from the enzyme treatments were extracted with the same procedure of OH raw material. For enzyme-treated samples, supernatant (1 mL) was directly used for analysis after being filtrated by 0.22 μm RC membrane filters (Phenomenex).

Total phenolic acids (free and bound) were extracted by both acid and alkaline hydrolysis (Tang et al., 2016). For acid hydrolysis, the OH raw material (approximately 2 g) were mixed with 25 mL of 2 M HCl, heated at 85 °C for 1 h, adjusted pH to 2 with 10 M NaOH, and then centrifuged at 6000 *g* for 5 min. The supernatant was extracted with 15 mL of diethyl ether/ethyl acetate (DE/EA, 1:1, v/v) four times. The solvent phase was combined and evaporated to completely dry at 30 °C by using vacuum rotary evaporation. The residue was re-dissolved in 1.5 mL of methanol and filtered through 0.22 μm PTFE syringe filters (Phenomenex) before HPLC. For alkaline hydrolysis, 2 g OH were treated with 25 mL of 2 M NaOH, hydrolyzed for 4 h at room temperature under a stream of N_2, acidified to pH 2 with 6 M HCl, and then centrifuged at 6000 *g* for 5 min. The supernatant was extracted with 15 mL of diethyl ether/ethyl acetate (DE/EA, 1:1, v/v) four times. The solvent phase was combined and evaporated to completely dry at 30 °C by using vacuum rotary evaporation. The residue was re-dissolved in 1.5 mL of methanol and filtered through 0.22 μm PTFE syringe filters (Phenomenex) before HPLC.

The identification of the studied compounds was performed on a Bruker ultra-high performance liquid chromatography (UPLC) system, equipped with a diode-array detector (DAD), an Apollo II electrospray ion source (ESI), and a quadrupole time-of-flight tandem mass spectrometer (Q-TOF) (Bruker Corp., Billerica, MA, United States). The chromatographic separation was conducted at room temperature using a Phenomenex Kinetex C18 column (100 × 4.60 mm, 2.6 μm, Torrance, CA, United States). The total flow rate was 1 mL/min. The injection volume was 10 μL. LC gradient program was set as: 0–5 min with 5% solvent B, 5–10 min with 10–15% B, 10–12 min with 15–20% B, 12–17 min with 20–23% B, 17–22 min with 23–27% B, 22–34 min with 27–35B, 32–37 min with 9% B, 34–36 min with 35–60% B, 36–38 min with 60–5% B, 38–40 min with 5% B. The chromatogram was monitored at wavelength of 320 nm (for phenolic acids) and 350 nm (for avenanthramides). The eluents of 0.3–0.4 mL/min were flown into MS system. Mass full scan was operated under both positive and negative ionization modes. The MS^2 scan was conducted with an auto MS/MS program in Q-TOF system. The parameters of MS analysis are given in Supplemental Table 2.

The quantification of studied compounds was carried out on Shimadzu LC-30AD liquid chromatograph system equipped with an SPD-M20A photodiode array detector (Shimadzu Corp., Kyoto, Japan). The chromatographic separation parameters were same as that in UPLC-QTOF analysis. All identified compounds were quantified by the calibration curves of commercial standards as shown in Supplemental Table 3.

2.7. Statistical analysis

The contents of compounds (expressed as mean ± standard deviation) were calculated both from 100 g DM raw material OH and 100 g DM supernatants/precipitates (Eq. (1)).

$$\text{Compound content}[\text{mg}/100\text{gDM}] = \frac{\text{C}\,[\text{g}/100\text{mL}] \times \text{V}\,[\text{mL}]}{\text{M}\,[\text{g}] \times \text{DM}[\%] \times 100} \times 1000 \times 100 \quad (1)$$

C: compound content in the extract; V: required sample volume for analysis; M: sample mass of the required volume; DM: sample dry matter.

The content of solubilized protein was calculated using the following equation (Eq. (2)).

$$\text{Solubilized protein}\,[\%] = \left(\frac{\text{C1}[\text{g}/100\text{g}]/100 \times \text{M1}\,[\text{g}]}{\text{M2}\,[\text{g}]} \times 100\right)/\text{C2}\,[\text{g}/100\text{g}] \times 100 \quad (2)$$

C1: protein content in the supernatant; M1: total supernatant mass; M2: analyzed OH mass; *C2*: protein content of raw material OH.

The extraction efficiency was calculated on the ratio of the content of phenolic acids from enzymatic treatments to the total contents obtained by acid or alkaline hydrolysis (Eq. (3)).

$$\text{Extraction efficiency}\,[\%] = \left(\frac{\text{C}\left[\frac{\text{mg}}{\text{mL}}\right] \times \text{V1}\,[\text{mL}]}{\text{M2}\,[\text{g}]}\right)/\text{C3}[\text{mg}/\text{g}] \times 100 \quad (3)$$

C: the contents of phenolic acids in the supernatant; V1: volume of the supernatant; M2: analyzed OH mass; C3: the contents of phenolic acids in acid or alkaline hydrolyzed OH.

One-way ANOVA with Tukey's post-hoc was applied to indicate statistical differences by IBM SPSS Statistics 26 (SPSS Inc., NY, United States). Two-way ANOVA test was applied to investigate the synergistic effects of enzymes, extraction method and ultrasonication conditions. For ANOVA tests, a p-value < 0.05 was considered to be statistically significant. Principal component analysis (PCA) with full cross validation was applied by using Unscrambler 11 (Camo Process AS, Oslo, Norway) to determine the correlation between chemical compositions and enzymatic treatments.

3. Results and discussion

3.1. Chemical composition of OH raw material

3.1.1. Proteins, free sugars, and organic acids

The phytochemical compositions of the OH raw material was shown in Table 1. Determined by the Kjeldahl method, the total content of proteins in OH was 5.2 g/100 g DM. This result was consistent with the observation of Swedish OH (1.4–5.4 g/100 g DM) and Danish OH (7.4 g/100 g DM) in the previous study (Schmitz et al., 2020). Our studied OH contained 89.2 mg/100 g DM of organic acids, including malic acid and citric acid. Two di-saccharides (sucrose and maltose), five monosaccharides (arabinose, xylose, fructose, glucose and mannose), three sugar alcohols (xylitol, mannitol and inositol) and one sugar acid (galacturonic acid) were identified from the raw material of OH at a sum content of 830.8 mg/100 g DM. In comparison with Swedish OH, our data showed a wider range of free monosaccharides. Reported by Schmitz et al. (2020), xylose, arabinose, galactose, and mannose were identified in varying cultivars of Swedish OH. Xylose was the dominant monomer unit in hemicellulose polymers, which accounted for 4–10% of DM of OH from the grains of different oat cultivars in Sweden. In our study, glucose (532.4 mg/100 g DM) was dominant, which accounted for 64% of the sum free sugars. Our result of glucose content was consistent with the value in Canadian OH (600 mg/100 g DM OH) (Agu et al., 2017).

3.1.2. Phenolic compounds

Eleven avenanthramides (2c, 5p, 5f, 2p, 2f, 2 c_d, 4p, 4f, 3f, 2p_d and 2f_d) and three phenolic acids (caffeic acid, *p*-coumaric acid and ferulic acid) were identified in our study (Supplemental Table 4, Supplemental Fig. 1). Table 1 showed the sum content of identified avenanthramides (10.5 mg/100 g DM) in OH raw material. This value was is similar to that of eight Finnish cultivars of husked oat (2.7–18.5 mg/100 g DM), but higher than that in US OH (0.1–1.0 mg/100 g DM) (Emmons & Peterson, 1999; Multari et al., 2018). In our studied OH, the most abundant avenanthramides were 2f (1.8 mg/100 g DM), 2p (1.4 mg/100 g DM), and 2c (1.1 mg/100 g DM), the sum of which accounted for 41% of the sum avenanthramides. Several studies indicated 2f, 2p, and 2c as the most common avenanthramides in oats and oat bran (Bratt et al., 2003; de Bruijn et al., 2019; Multari et al., 2018; Schär et al., 2018). Our study confirmed that this conclusion also applied to OH. To date, no article has reported long-chained derivatives in OH though they were the major part (up to ~ 70%) of the avenanthramides in oat seedlings (de Bruijn et al., 2019). Our results showed that long-chained avenanthramides 2p_d (1.2 mg/100 g DM), and 2f_d (1.0 mg/100 g DM) were in high contents compared to the short-chained derivatives (except 2f, 2p and 2c), which accounted for 24% of the total avenanthramides.

In our study, free phenolic acids were obtained from OH by a mild process using ultrasonication-assisted aqueous methanol extraction, with the sum content of 4.3 mg/100 g DM. Among which, *p*-coumaric acid (3.1 mg/100 g DM) accounted for 72% of the sum contents of phenolic acids, followed by ferulic acid (16%) and caffeic acid (12%). In addition to aqueous methanol, aqueous ethanol was used in another study to extract phenolic acids from various cultivars of OH (Emmons & Peterson, 1999). Consisting with our findings, their results showed average contents of free *p*-coumaric acid, ferulic acid and caffeic acid to be 1.0, 0.2 and 0.1 mg/100 g DM respectively. The sum contents of phenolic acid in the OH raw material obtained by acid and alkaline hydrolysis were 433.8 and 326.3 mg/100 g DM, respectively. Our results

Table 1
Concentration of phytochemicals in OH raw material.*

Composition	Content
Dry matter, %	**94.0 ± 0.0**
Proteins, g/100 g DM	**5.2 ± 0.1**
Sugars (free), mg/100 g DM	**830.8 ± 29.3**
Arabinose	14.1 ± 0.8
Xylose	30.2 ± 0.2
Xylitol	25.4 ± 0.7
Fructose	119.1 ± 2.2
Glucose	532.4 ± 19.7
Mannose	13.7 ± 0.2
Mannitol	24.3 ± 0.6
Inositol	6.4 ± 0.2
Sucrose	53.8 ± 2.4
Maltose	0.5 ± 0.0
A/X ratio	0.5 ± 0.0
Galacturonic acid	1.8 ± 1.1
Organic acids (free), mg/100 g DM	**89.2 ± 5.5**
Malic acid	69.3 ± 5.1
Citric acid	20.0 ± 0.7
Avenanthramides (free), mg/100 g DM	**10.5 ± 0.1**
2c	1.1 ± 0.1
5p	0.5 ± 0.0
5f	0.7 ± 0.1
2p	1.4 ± 0.0
2f	1.8 ± 0.1
2 c_d	0.3 ± 0.0
4p	1.0 ± 0.1
3f	0.9 ± 0.1
4f	0.6 ± 0.1
2p_d	1.2 ± 0.1
2f_d	1.0 ± 0.1
Phenolic acids (free), mg/100 g DM	**4.3 ± 0.1**
caffeic acid	0.5 ± 0.1
p-coumaric acid	3.1 ± 0.1
ferulic acid	0.7 ± 0.0
Phenolic acids (after acid hydrolysis), mg/100 g DM	**433.8 ± 15.3**
caffeic acid	71.7 ± 3.3
p-coumaric acid	172.3 ± 5.5
ferulic acid	110.6 ± 3.4
Others	79.2 ± 3.2
Phenolic acids (after alkaline hydrolysis), mg/100 g DM	**326.3 ± 21.8**
caffeic acid	44.9 ± 9.3
p-coumaric acid	144.8 ± 4.1
ferulic acid	110.0 ± 6.2
Others	26.7 ± 2.2
β-glucans, g/100 g DM	**0.7 ± 0.5**

* Results were shown as means ± standard deviation of triplicate analyses. A/X ratio, arabinose to xylose ratio.

were in the similar level of phenolic acids in comparison with the data in previous research of Swedish OH (386.1–951.6 mg/100 g DM), in which the samples were hydrolyzed using strong alkaline (2 M NaOH) for 16 h (Schmitz et al., 2020).

3.1.3. β-Glucan content and molecular weight of polysaccharides

The β-glucan content in the studied OH was 0.7 g/100 g DM (Table 1). Compared to oat bran (4 g/100 g DM), OH contained lower amount of β-glucan, although a different extraction method was used (Chen et al., 2018). As shown in HPSEC chromatogram, most of polymers (31% of area) had $3.9 < \log M_p < 3.0$, with major populations of 1.1 and 1.8 kDa (Supplemental Fig. 2a). The high amount of xylans present in OH might affect the molecular weight profile of OH, due to their high polydispersity (Girhammar & Nair, 1992). Nevertheless, polysaccharides of higher molecular weight were also observed. Polymers with $\log M_p$ value in the range of 4.0–5.9 accounted for about 22% of total peak area. In the chromatogram, a population of 3.1×10^2 kDa was observed (Supplemental Fig. 2b). Similar molecular weight was observed in oat β-glucans (Shewry et al., 2008).

3.2. The effect of different enzymes on chemical compositions of OH

Shown in Table 2, OH chemical profiles were significantly changed after enzymatic treatments. In general, contents of proteins, free sugars and organic acids in the supernatant of enzyme-treated OH dramatically increased up to 68.9 g/100 g, 38.1 and 2.3 g/100 g DM $_{supernatant}$, respectively. The amounts of sum phenolic acids in the supernatant (12.7–83.3 mg/100 g DM $_{supernatant}$) were also much higher than that (free form) in untreated OH. In the precipitates, the content of sum avenanthramides and phenolic acids were in the ranges of 12.4–35.2 μg/g DM $_{precipitate}$ and 10.9–32.5 μg/g DM $_{precipitate}$, respectively (Table 3).

Among all the treatment factors, enzyme was shown as the main contributor if all compounds are investigated at the same time in PCA models or independently in two-way ANOVA models (Supplemental Table 5). As shown in Fig. 2a (86% of variation on PC-1 & PC-2), feruloyl esterase supernatant samples were separated from Viscozyme and Alcalase samples on PC-1, while Viscozyme and Alcalase were further divided into separate groups on PC-2. According to the loading plot, feruloyl esterase correlated positively to all the compounds except for some free sugars. Viscozyme was closely associated to free sugars, primarily glucose, fructose, arabinose and mannose. Alcalase was associated with disaccharide-maltose. Regarding to the precipitate results shown in Fig. 2b, feruloyl esterase samples were almost completely separated from Viscozyme and Alcalase samples on PC-1 (82% of variation), yet Viscozyme and Alcalase were mixed distributed on both PC-1 and PC-2 (15% of variation). Feruloyl esterase samples positively correlated to all the avenanthramides and phenolic acids, while both Viscozyme and Alcalase showed negative correlations.

3.2.1. Soluble proteins

As shown in Table 2, the application of ultrasonication resulted in the same level or higher content of proteins in the supernatants compared to mechanical shaking when Viscozyme and feruloyl esterase were applied. However, when treating with Alcalase, the yield of soluble proteins by ultrasonication can be lower than that by shaking. The most abundant contents of proteins were found in feruloyl esterase treated samples (48.1–68.9 g/100 g DM $_{supernatant}$). Surprisingly, these values were over 2 folds higher than protein contents with protease addition (Alcalase, 14.6–30.1 g/100 g DM $_{supernatant}$) when the same shaking/ultrasound method applied. The reason could be due to the superiority of cell disintegration property of feruloyl esterase on the ferulic acid-polysaccharide framework of OH. Ferulic acid is known as the most common hydroxycinnamic acid in the cell walls of cereal grains. It is covalently cross-linked to polysaccharides by ester bonds, forming thick cell walls. These cross-links increase the mechanical strength of the cell wall and trap phytochemical compounds inside the cells. Feruloyl esterase can break the ester linkage and loosen the recalcitrant structure of matrix, therefore more intercellular compounds such as proteins, β-glucans and phenolics are liberated (Grundy et al., 2018). So far, use of feruloyl esterase has not been reported for protein extraction of any agro-industrial side streams.

Alcalase is an *endo*-protease, which effectively reduces protein size and increases the protein solubility, enhancing extraction (García Arteaga et al., 2020). Although the accessibility of Alcalase to matrix could probably be limited by recalcitrant cell wall cross-linking in OH, Alcalase was found as an effective enzyme for protein extraction from other agricultural wastes and industrial by-products, such as rice bran, sesame bran, canola press cake and brewers' spent grain proteins (Görgüç et al., 2019; Hanmoungjai et al., 2002; Tian et al., 2022). Among all the enzymes used in the work of Hanmoungjai et al. (2002), Alcalase was the most effective one in enhancing protein extraction (over 60%) from rice bran. Similarly, Alcalase improved protein extraction yield up to 78% in sesame bran (Görgüç et al., 2019). In the research carried out by Tian and co-works (2022), Alcalase showed significant superiority on protein yield (82%) and solubility (86%) in comparison with Viscozyme (40% and 76%) and Viscozyme-Phyzyme co-treatment (45% and 86%). Consistent to our findings, proteins yielded by Viscozyme only reached 54% of that obtained by Alcalase. In another study, it was also proved that Viscozyme (12%) was not as efficient as amylase combined with protease (45%) on protein extraction in rice bran (Tang et al., 2003). On the other side, compare to the alkaline-extracted protein content (15%), protein yield by Viscozyme hydrolysis was higher (56%) (Guan & Yao, 2008).

3.2.2. Free sugars and organic acids

Sugars and acids are important contributors of sensory properties of food. The contents and types of monosaccharides also significantly influence the nutritional properties of food, which in turn impact on energy metabolism and gut microbiota. Shaking showed better or the same capacity as ultrasonication for releasing free sugars and organic acids in Viscozyme and feruloyl esterase treated samples, while opposite results were observed in Alcalase treatments (Supplemental Fig. 3). In general, more free sugars were quantified from Viscozyme treated samples. Shown in Table 2, as a monosaccharide from cellulose degradation, glucose presented 64–80% of the sum free sugars in Viscozyme treated samples, which were 3–11 folds higher than those in Alcalase and feruloyl esterase treated samples, indicating that the specificity of Viscozyme lies in breaking down lignocellulosic structure of OH (Tian et al., 2022). Higher sum contents of arabinose, xylose and mannose found in Viscozyme treated samples may also indicated the better degradation of hemicellulose since these monosaccharides are the main units of hemicellulose (Schmitz et al., 2021). Interestingly, lower arabinose and higher xylose contents were observed in feruloyl esterase treated samples compared to Viscozyme treated ones under each extraction method. Although the saturation of the arabinoxylans in the studied treated OH could not be confirmed by the arabinose to xylose (A/X) ratios, A/X values only decreased by feruloyl esterase activity comparing to the OH raw material. This indicated that extracts of feruloyl esterase treated OH had good potential in unsubstituted xylan applications, as lower A/X value favoring greater aggregation. As the non-starch polysaccharides and major component of hemicellulose, low A/X arabinoxylans has more potent on health-promoting properties such as immune-enhancing activity (Mendis & Simsek, 2014).

3.2.3. Phenolic acids and avenanthramides

In Table 2, with only half of the shaking time, ultrasonication was almost as effective as mechanical shaking in releasing phenolic acids into the supernatants for each of the enzymes studied. The addition of feruloyl esterase resulted in higher yields (approximately 2–5 folds) of phenolic acids in the supernatants than that of Viscozyme and Alcalase (Supplemental Fig. 4). Among all the phenolic acids, ferulic acid was enormously released in the supernatants by feruloyl esterase, the

Table 2
Concentration of proteins, free sugars, organic acids, phenolic acids in the supernatants of enzyme-treated OH samples.*

Composition	Shaking 3 h	Sonication 25 kHz 30 min	Sonication 25 kHz 60 min	Sonication 25 kHz 90 min	Sonication 45 kHz 30 min	Sonication 45 kHz 60 min	Sonication 45 kHz 90 min
Dry matter, %	**1.0 ± 0.0^{b}**	**0.8 ± 0.0^{c}**	**1.0 ± 0.0^{b}**	**1.2 ± 0.0^{a}**	**0.8 ± 0.0^{c}**	**0.8 ± 0.0^{c}**	**0.8 ± 0.0^{c}**
Contents of compounds from 100 g DM supernatant in Viscozyme treated samples							
Proteins, g	**9.0 ± 0.6^{d}**	**8.3 ± 0.3^{d}**	**8.2 ± 0.0^{d}**	**15.0 ± 0.1^{c}**	**23.4 ± 0.4^{b}**	**24.8 ± 0.5^{ab}**	**26.3 ± 0.4^{a}**
Sugars (free), mg	**35988.0 ± 1258.0^{a}**	**29766.7 ± 52.1 ^{cd}**	**37443.2 ± 39.5^{a}**	**38122.0 ± 989.7^{a}**	**28348.2 ± 652.9^{d}**	**31224.1 ± 653.2^{bc}**	**32531.8 ± 237.5^{b}**
Arabinose	344.6 ± 19.0^{a}	179.5 ± 3.1^{c}	140.6 ± 2.2^{de}	129.2 ± 3.9^{e}	154.3 ± 4.5^{d}	196.9 ± 3.8^{c}	226.1 ± 1.8^{b}
Xylose	414.3 ± 15.3^{a}	168.4 ± 7.2^{d}	171.8 ± 3.1^{d}	171.6 ± 7.9^{d}	129.8 ± 2.1^{e}	195.1 ± 4.2^{c}	257.7 ± 1.8^{b}
Xylitol	257.3 ± 8.2^{bc}	278.7 ± 8.2^{a}	223.7 ± 4.3^{d}	196.2 ± 8.8^{e}	279.2 ± 6.8^{a}	271.9 ± 5.4^{ab}	253.2 ± 2.4^{c}
Fructose	7085.7 ± 116.5^{bc}	7766.2 ± 116.6^{a}	6648.0 ± 81.6^{c}	5728.9 ± 42.1^{d}	8084.3 ± 277.6^{a}	7808.6 ± 251.0^{a}	7310.4 ± 94.3^{b}
Glucose	26315.4 ± 1109.8^{b}	19649.7 ± 842.5^{de}	28565.6 ± 1103.6^{a}	30412.8 ± 919.1^{a}	18098.7 ± 375.4^{e}	21103.4 ± 473.0 ^{cd}	22963.4 ± 103.7^{c}
Mannose	543.1 ± 21.7^{a}	411.3 ± 8.1^{c}	337.6 ± 9.0^{d}	299.3 ± 9.7^{e}	392.8 ± 8.4^{c}	449.3 ± 8.0^{b}	459.2 ± 1.8^{b}
Mannitol	244.9 ± 8.3^{bc}	269.0 ± 4.0^{a}	214.3 ± 4.8^{d}	188.9 ± 6.9^{e}	269.8 ± 3.8^{a}	258.4 ± 3.8^{ab}	240.0 ± 2.5^{c}
Inositol	76.9 ± 5.0^{a}	61.0 ± 2.3^{bc}	51.9 ± 1.0^{de}	45.4 ± 1.6^{e}	55.7 ± 1.0 ^{cd}	63.5 ± 1.6^{b}	63.9 ± 2.0^{b}
Sucrose	45.0 ± 37.7^{a}	64.8 ± 36.1^{a}	64.9 ± 1.0^{a}	49.6 ± 14.9^{a}	53.3 ± 5.2^{a}	74.8 ± 15.2^{a}	65.4 ± 9.7^{a}
Maltose	592.3 ± 66.5^{d}	852.2 ± 98.7^{ab}	991.1 ± 73.9^{a}	870.8 ± 21.0^{ab}	789.1 ± 27.1^{bc}	756.6 ± 37.9^{bcd}	646.0 ± 105.1 ^{cd}
Rhamnose	ND**	ND	ND	ND	ND	ND	ND
Galactose	ND	ND	ND	ND	ND	ND	ND
A/X ratio	0.8 ± 0.0^{d}	1.1 ± 0.0^{b}	0.8 ± 0.0^{d}	0.8 ± 0.0^{d}	1.2 ± 0.0^{a}	1.0 ± 0.0^{b}	0.9 ± 0.0^{c}
Galacturonic acid	68.4 ± 2.5^{a}	45.9 ± 1.0^{b}	33.8 ± 3.4 ^{cd}	29.2 ± 3.1^{d}	41.1 ± 6.8^{bcd}	45.6 ± 2.4^{bc}	46.5 ± 7.1^{b}
Organic acids (free), mg	**994.7 ± 31.4^{a}**	**895.8 ± 37.5^{ab}**	**706.8 ± 26.1^{c}**	**657.5 ± 15.5^{c}**	**983.0 ± 47.2^{ab}**	**947.6 ± 62.6^{ab}**	**857.0 ± 81.7^{b}**
Malic acid	790.1 ± 24.2^{a}	778.0 ± 52.1^{a}	636.8 ± 39.5^{bc}	573.2 ± 30.4^{c}	793.5 ± 34.9^{a}	758.2 ± 51.5^{a}	716.1 ± 44.9^{ab}
Citric acid	204.6 ± 9.7^{a}	117.8 ± 24.8^{bc}	70.0 ± 13.9^{c}	84.3 ± 44.4^{c}	189.5 ± 21.6^{ab}	189.4 ± 14.0^{ab}	141.0 ± 36.9^{abc}
Phenolic acids (free), mg***	**38.7 ± 1.9^{a}**	**30.1 ± 3.1^{c}**	**19.7 ± 0.9^{e}**	**22.9 ± 1.0^{de}**	**25.7 ± 0.5^{d}**	**31.2 ± 0.6^{bc}**	**34.2 ± 2.1^{b}**
caffeic acids	9.0 ± 0.7 ^{cd}	8.4 ± 0.2^{d}	9.6 ± 0.5^{c}	9.0 ± 0.2 ^{cd}	11.4 ± 0.3^{b}	13.0 ± 0.1^{a}	13.7 ± 0.6^{a}
p-coumaric acid	12.3 ± 0.9^{a}	11.4 ± 1.8^{a}	1.5 ± 0.4^{c}	6.0 ± 0.8^{b}	6.6 ± 0.4^{b}	6.5 ± 0.5^{b}	5.9 ± 1.3^{b}
ferulic acid	17.4 ± 1.3^{a}	10.3 ± 1.2^{c}	8.5 ± 0.2^{d}	7.9 ± 0.0^{d}	7.7 ± 0.2^{d}	11.7 ± 0.1^{c}	14.6 ± 0.3^{b}
Dry matter, %	**1.0 ± 0.1^{a}**	**0.8 ± 0.0^{bc}**	**0.8 ± 0.1^{bc}**	**0.8 ± 0.0^{b}**	**0.7 ± 0.0^{c}**	**0.7 ± 0.0^{bc}**	**0.7 ± 0.0^{c}**
Contents of compounds from 100 g DM supernatant in Alcalase treated samples							
Proteins, g	**23.0 ± 2.8^{b}**	**22.4 ± 0.6^{b}**	**26.3 ± 1.1^{ab}**	**30.1 ± 0.4^{a}**	**14.6 ± 0.8^{c}**	**14.7 ± 0.8^{c}**	**15.9 ± 0.8^{c}**
Sugars (free), mg	**10076.9 ± 3638.3^{b}**	**13324.9 ± 20.4^{ab}**	**11555.7 ± 152.5^{ab}**	**10657.2 ± 295.4^{ab}**	**17537.7 ± 521.9^{a}**	**14926.0 ± 196.8^{ab}**	**15682.9 ± 4754.2^{ab}**
Arabinose	115.6 ± 15.5^{a}	59.4 ± 18.4^{ab}	77.6 ± 5.5^{ab}	47.6 ± 27.2^{b}	86.8 ± 12.5^{ab}	96.8 ± 25.3^{ab}	62.1 ± 26.4^{ab}
Xylose	206.6 ± 73.9^{a}	81.8 ± 2.4^{b}	74.2 ± 3.0^{b}	75.1 ± 2.3^{b}	94.4 ± 10.9^{b}	115.0 ± 20.8^{ab}	149.5 ± 47.2^{ab}
Xylitol	226.3 ± 77.7^{ab}	301.9 ± 11.6^{ab}	230.3 ± 9.8^{ab}	189.0 ± 8.1^{b}	329.4 ± 10.4^{a}	299.3 ± 57.1^{ab}	292.8 ± 79.6^{ab}
Fructose	1168.6 ± 389.6^{a}	1145.1 ± 122.7^{a}	924.4 ± 71.8^{a}	797.1 ± 69.9^{a}	1398.5 ± 138.9^{a}	1366.3 ± 229.0^{a}	1313.6 ± 352.4^{a}
Glucose	5098.0 ± 1816.5^{a}	3285.5 ± 127.3^{a}	2849.8 ± 13.8^{a}	2846.7 ± 81.1^{a}	3675.8 ± 284.8^{a}	4181.7 ± 791.1^{a}	4706.5 ± 1405.3^{a}
Mannose	77.7 ± 31.0^{a}	38.6 ± 2.5^{ab}	32.2 ± 1.3^{b}	29.8 ± 1.7^{b}	48.8 ± 6.4^{ab}	57.1 ± 10.7^{ab}	63.5 ± 20.9^{ab}
Mannitol	196.3 ± 68.8^{ab}	262.6 ± 11.9^{ab}	200.7 ± 7.9^{ab}	166.1 ± 7.5^{b}	285.8 ± 16.3^{a}	260.6 ± 50.4^{ab}	256.7 ± 59.2^{ab}
Inositol	50.9 ± 19.6^{a}	51.8 ± 5.7^{a}	41.2 ± 2.4^{a}	35.9 ± 2.3^{a}	54.4 ± 5.4^{a}	56.4 ± 11.3^{a}	57.3 ± 17.3^{a}
Sucrose	148.7 ± 82.8^{bcd}	390.9 ± 16.5^{a}	249.4 ± 15.9^{b}	170.7 ± 10.3^{bcd}	213.4 ± 34.2^{bc}	101.1 ± 21.7^{d}	128.2 ± 44.7 ^{cd}
Maltose	2767.1 ± 1190.7^{c}	7694.3 ± 82.1^{b}	6863.1 ± 127.7^{b}	6284.0 ± 131.3^{bc}	11336.7 ± 97.6^{a}	8371.2 ± 1634.2^{ab}	8632.2 ± 2703.6^{ab}
Rhamnose	ND	ND	ND	ND	ND	ND	ND
Galactose	ND ND	ND	ND	ND	ND	ND	ND
A/X ratio	0.6 ± 0.2^{ab}	0.7 ± 0.2^{ab}	1.1 ± 0.1^{a}	0.6 ± 0.4^{ab}	0.9 ± 0.1^{ab}	0.8 ± 0.1^{ab}	0.4 ± 0.1^{b}
Galacturonic acid	19.1 ± 15.5^{a}	13.1 ± 10.1^{a}	12.8 ± 5.6^{a}	15.1 ± 6.9^{a}	13.6 ± 5.2^{a}	20.5 ± 16.3^{a}	20.3 ± 4.2^{a}
Organic acids (free), mg	**901.4 ± 314.1^{ab}**	**1135.0 ± 41.9^{ab}**	**892.8 ± 27.7^{ab}**	**735.8 ± 25.6^{b}**	**1290.3 ± 59.4^{a}**	**1156.9 ± 246.4^{ab}**	**1056.9 ± 292.3^{ab}**
Malic acid	722.1 ± 252.7^{ab}	913.1 ± 20.4^{ab}	712.6 ± 18.5^{ab}	594.6 ± 17.9^{b}	1031.9 ± 43.3^{a}	920.1 ± 196.8^{ab}	881.0 ± 236.7^{ab}
Citric acid	179.4 ± 61.5^{ab}	221.8 ± 21.8^{ab}	180.1 ± 9.8^{ab}	141.2 ± 10.5^{b}	258.4 ± 16.3^{a}	236.8 ± 49.7^{ab}	175.9 ± 68.9^{ab}
Phenolic acids (free), mg	**30.3 ± 3.4^{ab}**	**12.7 ± 0.2^{e}**	**21.9 ± 0.5 ^{cd}**	**17.5 ± 0.6^{de}**	**31.7 ± 2.0^{a}**	**26.2 ± 2.3^{bc}**	**28.8 ± 4.0^{ab}**
caffeic acids	9.6 ± 0.8^{b}	7.1 ± 0.1^{c}	8.9 ± 0.2^{b}	6.7 ± 0.2^{c}	13.1 ± 1.0^{a}	10.3 ± 0.7^{b}	9.8 ± 1.1^{b}
p-coumaric acid	4.0 ± 1.2^{b}	0.0 ± 0.0^{d}	6.4 ± 0.4^{a}	4.9 ± 0.5^{ab}	5.8 ± 0.8^{a}	1.8 ± 0.9^{c}	0.0 ± 0.0^{d}
ferulic acid	16.8 ± 1.4^{ab}	5.6 ± 0.2^{d}	6.6 ± 0.1^{d}	6.0 ± 0.1^{d}	12.8 ± 0.2^{c}	14.2 ± 0.8^{bc}	19.0 ± 2.9^{a}
Dry matter, %	**0.5 ± 0.0^{a}**	**0.5 ± 0.0^{a}**	**0.4 ± 0.0^{a}**	**0.4 ± 0.0^{a}**	**0.4 ± 0.0^{a}**	**0.4 ± 0.0^{a}**	**0.4 ± 0.0^{a}**
Contents of compounds from 100 g DM supernatant in feruloyl esterase treated samples							
Proteins, g	**52.5 ± 1.6^{b}**	**51.6 ± 2.0^{b}**	**62.5 ± 1.1^{a}**	**68.9 ± 1.6^{a}**	**50.1 ± 2.7^{b}**	**48.1 ± 2.3^{b}**	**48.4 ± 1.9^{b}**
Sugars (free), mg	**17332.1 ± 111.9^{a}**	**12728.9 ± 728.3 ^{cd}**	**14011.5 ± 556.7^{bc}**	**14144.6 ± 801.7^{bc}**	**11537.5 ± 189.7^{d}**	**13907.2 ± 336.5^{bc}**	**15136.1 ± 450.6^{b}**
Arabinose	260.2 ± 4.6^{a}	94.6 ± 15.8^{de}	109.1 ± 3.2 ^{cd}	105.2 ± 10.9^{bcd}	81.4 ± 8.7^{e}	121.3 ± 37.1^{c}	154.3 ± 6.4^{b}
Xylose	540.6 ± 3.5^{a}	220.4 ± 9.0^{d}	314.0 ± 7.1^{c}	331.1 ± 21.2^{c}	206.3 ± 11.0^{d}	322.6 ± 25.6^{c}	414.2 ± 23.0^{b}
Xylitol	544.0 ± 8.2^{a}	555.5 ± 33.6^{a}	558.4 ± 14.8^{a}	454.8 ± 8.2^{b}	572.2 ± 14.9^{a}	587.3 ± 10.8^{a}	565.0 ± 21.7^{a}
Fructose	3448.5 ± 52.8^{a}	3219.0 ± 137.6^{ab}	3170.0 ± 182.6^{ab}	2647.9 ± 97.7^{d}	2784.5 ± 93.1 ^{cd}	2975.9 ± 133.2^{bc}	2995.0 ± 69.0^{bc}

(*continued on next page*)

Table 2 (*continued*)

Composition	Shaking 3 h	Sonication 25 kHz 30 min	Sonication 25 kHz 60 min	Sonication 25 kHz 90 min	Sonication 45 kHz 30 min	Sonication 45 kHz 60 min	Sonication 45 kHz 90 min
Glucose	10100.9 ± 138.5^{a}	6479.7 ± 410.5^{d}	7778.9 ± 263.6^{c}	8130.9 ± 468.2^{bc}	5705.2 ± 83.1^{d}	7753.0 ± 325.2^{c}	8966.5 ± 298.4^{b}
Mannose	265.3 ± 8.1^{a}	119.5 ± 4.0^{e}	148.4 ± 5.4 ^{cd}	157.7 ± 15.6^{bc}	91.6 ± 1.2^{f}	136.5 ± 2.6^{de}	174.9 ± 6.3^{b}
Mannitol	464.9 ± 10.3^{a}	472.0 ± 29.3^{a}	474.5 ± 13.0^{a}	387.5 ± 5.0^{b}	481.8 ± 9.6^{a}	494.3 ± 7.2^{a}	480.1 ± 19.5^{a}
Inositol	134.4 ± 2.4^{a}	110.9 ± 3.6^{bc}	112.2 ± 6.9^{bc}	103.3 ± 4.2^{c}	100.9 ± 3.9^{c}	111.9 ± 2.6^{bc}	120.9 ± 4.0^{b}
Sucrose	280.1 ± 43.6^{bc}	396.8 ± 18.3^{b}	122.1 ± 18.9^{d}	257.0 ± 14.2^{bcd}	595.5 ± 111.3^{a}	203.2 ± 44.4 ^{cd}	121.2 ± 65.7^{d}
Maltose	1194.5 ± 44.6^{b}	993.9 ± 76.6^{bc}	1149.5 ± 96.8^{b}	1500.0 ± 201.2^{a}	865.1 ± 95.3^{c}	1120.9 ± 50.3^{bc}	1093.4 ± 30.2^{bc}
Rhamnose	ND	ND	ND	ND	ND	ND	ND
Galactose	ND	ND	ND	ND	ND	ND	ND
	ND						
A/X ratio	0.5 ± 0.0^{a}	0.4 ± 0.1^{ab}	0.3 ± 0.0^{bc}	0.3 ± 0.0^{c}	0.4 ± 0.0^{abc}	0.4 ± 0.0^{abc}	0.4 ± 0.0^{bc}
Galacturonic acid	98.8 ± 24.9^{a}	66.6 ± 4.5^{a}	74.5 ± 6.0^{a}	69.1 ± 5.8^{a}	53.0 ± 34.4^{a}	80.2 ± 6.7^{a}	50.6 ± 31.9^{a}
Organic acids (free), mg	**2156.8 ± 30.7^{a}**	**2195.6 ± 150.9^{a}**	**2197.5 ± 92.9^{a}**	**1804.1 ± 42.0^{b}**	**2223.3 ± 94.9^{a}**	**2319.3 ± 39.4^{a}**	**2251.6 ± 88.5^{a}**
Malic acid	1695.6 ± 22.0^{a}	1721.1 ± 106.9^{a}	1738.5 ± 71.4^{a}	1419.6 ± 29.4^{b}	1764.3 ± 70.1^{a}	1841.2 ± 37.1^{a}	1798.6 ± 68.2^{a}
Citric acid	461.2 ± 8.8^{a}	474.5 ± 44.2^{a}	459.0 ± 21.5^{a}	384.6 ± 12.7^{b}	459.0 ± 24.9^{a}	478.2 ± 2.4^{a}	453.0 ± 21.9^{a}
Phenolic acids (free), mg	**76.1 ± 3.0^{ab}**	**60.3 ± 2.1^{c}**	**66.6 ± 3.4^{bc}**	**65.9 ± 5.5^{bc}**	**74.9 ± 3.2^{ab}**	**83.3 ± 3.4^{a}**	**78.4 ± 6.9^{a}**
caffeic acids	25.8 ± 1.4^{a}	20.2 ± 1.1^{bc}	20.6 ± 0.7^{bc}	16.7 ± 1.3^{c}	25.8 ± 2.6^{a}	27.7 ± 2.0^{a}	24.8 ± 3.6^{ab}
p-coumaric acid	3.2 ± 0.6^{c}	5.3 ± 1.1^{bc}	4.4 ± 1.9^{c}	6.2 ± 0.6^{bc}	11.5 ± 0.6^{a}	8.1 ± 0.7^{b}	5.8 ± 2.9^{bc}
ferulic acid	47.2 ± 1.4^{a}	34.9 ± 1.3^{d}	41.6 ± 1.1^{bc}	43.0 ± 3.7^{ab}	37.6 ± 3.7 ^{cd}	47.5 ± 1.6^{a}	47.8 ± 0.6^{ab}

* Results were shown as means ± standard deviation of triplicate analyses. Statistical differences were conducted based on one way-ANOVA and Turkey's post hoc test ($p < 0.05$), and shown with superscript letters a-e. **ND, not detected. ***Sum contents of caffeic, p-coumaric and ferulic acids.

content of which was up to 7-fold higher than that by the additions of Viscozyme or Alcalase. Similar result was found in previous OH research, in which ferulic acid content was increased considerably by feruloyl esterase hydrolysis (Schmitz et al., 2022). As shown in Supplemental Table 6, our findings also showed that ferulic acid yields (1.7–2.5 mg/g DM OH) in our enzyme-treated (feruloyl esterase supernatant) samples were lower than that in alkaline-treated OH (14.1 mg/100 g DM OH) from another research (Schmitz et al., 2021).

As shown in Table 3, ultrasonication showed equal or lower capacity of extracting phenolic acids in the precipitates of all enzyme-treated samples. Similar to the results of supernatants, feruloyl esterase was the most potent in releasing phenolic acids in enzyme-treated precipitates (Supplemental Fig. 5). The contents of sum phenolic acids in the precipitate of feruloyl esterase-derived samples (20.0–32.5 μg/g DM $_{precipitate}$) were up to 3 folds higher than Viscozyme- (10.9–31.5 μg/g DM $_{precipitate}$) and Alcalase-derived ones (11.7–20.0 μg/g DM $_{precipitate}$). It is worth noting that although having the health-promoting properties such as antioxidation, anti-inflammatory, anti-microbial activities and anti-cancer, adding excess amount of ferulic acid-rich extract could influence the sensory quality of the final products due to its bitterness and astringency flavor (Habschied et al., 2021).

None of the identified avenanthramides in OH raw material was found in the supernatants of enzyme-treated samples. The sum contents of identified avenanthramides in the precipitates were in the range of 12.4–35.2 μg/g DM $_{precipitate}$ (Table 3, Supplemental Fig. 6). Compared to shaking, ultrasonication showed almost equal capacity to release avenanthramides in the precipitates of Viscozyme and Alcalase treated samples, while the sum contents of avenanthramides could be even higher by ultrasonication than that by shaking in feruloyl esterase treated samples. Short-chain avenanthramides (66–78%) were found to be dominant in all the treatments, compared to long-chain derivatives (22–34%). Among all the short-chain derivatives, 2c, 2p, 2f accounted for 37–46% of the sum avenanthramides. Although avenanthramides are present in small amounts in OH, 2c, 2p, and 2f have been shown to be related to the fresh taste of oat products and may thus function as antioxidants protecting against rancidification (Bratt et al., 2003). When applied with same extraction method/ultrasound condition, most abundant avenanthramides were obtained in feruloyl esterase treated samples (18.3–35.2 μg/g DM $_{precipitate}$), while the contents were relatively lower in the samples treated by the other two enzymes (12.4–25.4 μg/g DM $_{precipitate}$). Regardless, avenanthramides were degraded by 3–9 folds after all the enzymatic treatments. Therefore, avenanthramides need to be extracted before enzyme addition in further research due to their potential as health-promoting components in innovative foods.

3.3. Effect of ultrasonication-assisted enzymatic treatments

In order to compare the content of solubilized proteins and the extraction efficiency of phenolic acids, the contents of proteins and phenolic acids were also calculated based on 100 g DM OH raw material. As shown in Fig. 3, the contents of proteins and the sum contents of identified phenolic acids ranged in 0.7–2.9 and 1.0–4.0 mg/100 g DM OH, respectively after the enzymatic treatments. Addition of feruloyl esterase with ultrasonication of 25 kHz for 90 min resulted in the highest content of solubilized protein in supernatants (56.3%). Applying 30–90 min ultrasonication under 45 kHz enhanced the solubility of proteins (38.3–42.9%) in Viscozyme treated samples compared to shaking (18.3%). On the contrary, the solubilized protein obtained by shaking (47.0%) was higher than that by 45 kHz ultrasonication (20.8–22.7%) in Alcalase treated samples. Compared to the extraction efficiency of 3-hour mechanical shaking (0.9–1.1%), 30–90 min ultrasonication (45 kHz) of feruloyl esterase incubation also showed the good extraction efficiency (0.8–1.2%) of identified phenolic acids. After incubation using carbohydrase (Viscozyme) and protease (Alcalase), the extraction efficiencies of identified phenolic acids (sum contents) by shaking were significantly higher than that by ultrasonication. The extraction efficiency of individual phenolic acid varied by different ultrasonication conditions.

The impacts of the enzymes, ultrasonication frequencies, ultrasonication processing times and their interactions were investigated in mixed two-way ANOVA models. As shown in Supplemental Table 5, the yields of phytochemical compounds in the treated OH were highly dependent on the synergistic effects of the treatment conditions. In addition to the previously described enzyme as the main factor, the effects of ultrasonication processing time and frequency were significant in acids, most of the free sugars and phenolic compounds among the supernatant samples ($p < 0.05$). Interestingly, soluble protein contents were dependent on the frequency only within the different enzymes. At the same time, the ultrasonication main effects were not significant in the avenanthramides in the precipitate samples. However, the enzyme-frequency interaction was significant in many of the avenanthramides showing the effect of frequency being dependent on the enzyme used.

Table 3
Concentration of avenanthramides and phenolic acids in the freeze-dried precipitates of enzyme-treated OH samples.*

Composition	Shaking 3 h	Sonication 25 kHz 30 min	Sonication 25 kHz 60 min	Sonication 25 kHz 90 min	Sonication 45 kHz 30 min	Sonication 45 kHz 60 min	Sonication 45 kHz 90 min
Dry matter, %	**99.5 ± 0.1^{a}**	**99.6 ± 0.0^{a}**	**99.3 ± 0.1^{a}**	**99.3 ± 0.2^{a}**	**99.8 ± 0.1^{a}**	**99.7 ± 0.0^{a}**	**99.3 ± 0.3^{a}**
Contents of compounds from 1 g DM precipitate in Viscozyme treated samples							
Avenanthramides (free), μg	**25.4 ± 1.5^{a}**	**19.8 ± 4.8^{ab}**	**13.3 ± 0.2^{ab}**	**12.4 ± 4.4^{b}**	**20.7 ± 0.5^{ab}**	**22.3 ± 0.4^{ab}**	**19.1 ± 3.5^{ab}**
2c	4.7 ± 0.3^{a}	3.9 ± 0.6^{ab}	3.4 ± 0.1^{b}	3.2 ± 0.8^{ab}	4.1 ± 0.0^{ab}	4.2 ± 0.3^{ab}	4.1 ± 0.5^{ab}
5p	1.3 ± 0.4^{a}	0.5 ± 0.2^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.0^{a}	0.7 ± 0.0^{a}	0.6 ± 0.1^{a}
5f	0.7 ± 0.1^{a}	0.1 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.1^{a}
2p	1.5 ± 0.1^{a}	1.7 ± 0.6^{a}	0.7 ± 0.0^{a}	0.7 ± 0.4^{a}	1.9 ± 0.0^{a}	1.9 ± 0.4^{a}	1.3 ± 0.4^{a}
2f	3.1 ± 0.1^{a}	2.8 ± 0.8^{a}	1.8 ± 0.1^{a}	1.7 ± 0.6^{a}	2.7 ± 0.1^{a}	3.4 ± 0.3^{a}	2.6 ± 0.6^{a}
2 c_d	2.9 ± 0.1^{a}	2.7 ± 0.0^{a}	2.3 ± 0.0^{a}	2.3 ± 0.2^{a}	2.6 ± 0.1^{a}	2.8 ± 0.1^{a}	2.7 ± 0.2^{a}
4p	2.4 ± 0.1^{a}	2.4 ± 0.7^{a}	1.4 ± 0.1^{a}	1.3 ± 0.5^{a}	2.6 ± 0.0^{a}	2.5 ± 0.0^{a}	2.1 ± 0.3^{a}
3f	2.0 ± 0.1^{a}	1.4 ± 0.4^{a}	1.3 ± 0.0^{a}	1.1 ± 0.5^{a}	1.5 ± 0.0^{a}	1.8 ± 0.2^{a}	1.5 ± 0.2^{a}
4f	1.5 ± 0.1^{a}	1.1 ± 0.3^{a}	1.1 ± 0.1^{a}	0.9 ± 0.6^{a}	1.2 ± 0.0^{a}	1.1 ± 0.2^{a}	1.1 ± 0.1^{a}
$2p_d$	3.4 ± 0.9^{a}	2.1 ± 0.7^{ab}	0.6 ± 0.0^{b}	0.6 ± 0.3^{b}	2.2 ± 0.1^{ab}	2.3 ± 0.0^{ab}	1.7 ± 0.5^{ab}
$2f_d$	1.7 ± 0.2^{a}	1.1 ± 0.3^{ab}	0.6 ± 0.0^{ab}	0.5 ± 0.3^{b}	1.2 ± 0.2^{ab}	1.4 ± 0.1^{ab}	1.2 ± 0.4^{ab}
Phenolic acids (free), μg **	**31.5 ± 4.1^{a}**	**15.1 ± 2.3^{b}**	**10.9 ± 0.5^{b}**	**12.0 ± 2.8^{b}**	**13.3 ± 0.2^{b}**	**17.1 ± 0.1^{b}**	**19.5 ± 1.6^{b}**
caffeic acids	8.8 ± 0.9^{a}	4.0 ± 0.6^{b}	3.7 ± 0.2^{b}	3.9 ± 0.9^{b}	3.9 ± 0.0^{b}	5.3 ± 0.0^{b}	6.4 ± 0.5^{ab}
p-coumaric acid	12.1 ± 1.6^{a}	6.5 ± 1.1^{bc}	3.8 ± 0.1^{c}	4.4 ± 1.0^{bc}	5.6 ± 0.2^{bc}	6.5 ± 0.0^{b}	6.8 ± 0.6^{bc}
ferulic acid	10.6 ± 1.7^{a}	4.6 ± 0.7^{b}	3.5 ± 0.2^{b}	3.6 ± 0.8^{b}	3.7 ± 0.0^{b}	5.3 ± 0.1^{b}	6.4 ± 0.5^{b}
Dry matter, %	**99.5 ± 0.0^{a}**	**99.4 ± 0.0^{a}**	**99.5 ± 0.2^{a}**	**99.5 ± 0.0^{a}**	**99.5 ± 0.2^{a}**	**99.6 ± 0.1^{a}**	**99.4 ± 0.0^{a}**
Contents of compounds from1 g DM precipitate in Alcalase treated samples							
Avenanthramides (free), μg	**19.6 ± 1.2^{a}**	**24.1 ± 2.7^{a}**	**24.2 ± 0.7^{a}**	**21.3 ± 8.7^{a}**	**16.3 ± 2.1^{a}**	**15.2 ± 1.4^{a}**	**13.4 ± 0.1^{a}**
2c	4.7 ± 0.3^{a}	5.3 ± 0.4^{a}	5.4 ± 0.1^{a}	4.8 ± 1.3^{a}	3.9 ± 0.4^{a}	3.9 ± 0.2^{a}	3.5 ± 0.0^{a}
5p	0.8 ± 0.0^{a}	0.9 ± 0.1^{a}	1.1 ± 0.1^{a}	0.8 ± 0.7^{a}	0.2 ± 0.0^{a}	0.2 ± 0.1^{a}	0.0 ± 0.0^{a}
5f	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.7 ± 0.0^{a}	0.4 ± 0.5^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
2p	1.4 ± 0.1^{a}	2.3 ± 0.3^{a}	2.2 ± 0.0^{a}	1.7 ± 0.8^{a}	1.3 ± 0.4^{a}	1.1 ± 0.1^{a}	0.7 ± 0.0^{a}
2f	2.7 ± 0.0^{a}	3.4 ± 0.8^{a}	3.0 ± 0.1^{a}	2.3 ± 0.9^{a}	1.8 ± 0.3^{a}	1.7 ± 0.2^{a}	1.5 ± 0.1^{a}
2 c_d	3.1 ± 0.2^{a}	3.3 ± 0.1^{a}	3.3 ± 0.1^{a}	2.9 ± 0.7^{a}	2.6 ± 0.2^{a}	2.5 ± 0.1^{a}	2.5 ± 0.0^{a}
4p	1.9 ± 0.0^{ab}	2.8 ± 0.3^{a}	2.7 ± 0.0^{a}	2.5 ± 0.8^{a}	2.0 ± 0.2^{ab}	1.8 ± 0.1^{ab}	1.4 ± 0.0^{b}
3f	1.8 ± 0.2^{a}	2.0 ± 0.2^{a}	2.0 ± 0.1^{a}	1.8 ± 0.7^{a}	1.3 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.2^{a}
4f	1.4 ± 0.1^{a}	1.6 ± 0.2^{a}	1.6 ± 0.4^{a}	1.9 ± 0.9^{a}	1.3 ± 0.0^{a}	1.2 ± 0.1^{a}	1.0 ± 0.2^{a}
$2p_d$	0.8 ± 0.1^{a}	1.1 ± 0.1^{a}	1.4 ± 0.4^{a}	1.3 ± 0.9^{a}	1.1 ± 0.3^{a}	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}
$2f_d$	0.6 ± 0.0^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.8 ± 0.4^{a}	0.8 ± 0.2^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}
Phenolic acids (free), μg	**20.0 ± 0.7^{a}**	**14.8 ± 1.0^{bc}**	**15.7 ± 0.1^{b}**	**17.0 ± 1.3^{ab}**	**11.7 ± 1.0^{c}**	**14.6 ± 0.2^{bc}**	**15.5 ± 0.0^{bc}**
caffeic acids	7.5 ± 0.6^{a}	5.1 ± 0.4^{bc}	5.1 ± 0.1^{bc}	5.1 ± 0.7^{b}	3.9 ± 0.4^{c}	5.1 ± 0.1^{bc}	5.4 ± 0.0^{bc}
p-coumaric acid	5.3 ± 0.0^{bc}	5.2 ± 0.3^{bc}	6.0 ± 0.0^{ab}	7.0 ± 0.1^{a}	4.1 ± 0.3^{c}	4.5 ± 0.0^{c}	4.4 ± 0.0^{c}
ferulic acid	7.2 ± 0.2^{a}	4.5 ± 0.2 ^{cd}	4.6 ± 0.0^{bc}	4.9 ± 0.5^{bc}	3.7 ± 0.3 ^{cd}	5.0 ± 0.0^{bc}	5.7 ± 0.0^{ab}
Dry matter, %	**99.5 ± 0.1^{ab}**	**99.5 ± 0.2^{ab}**	**99.5 ± 0.1^{ab}**	**99.4 ± 0.0^{ab}**	**99.8 ± 0.1^{a}**	**99.4 ± 0.0^{ab}**	**99.3 ± 0.0^{b}**
Contents of compounds from 1 g DM precipitate in feruloyl esterase treated samples							
Avenanthramides (free), μg	**18.3 ± 0.8^{c}**	**24.7 ± 1.7^{bc}**	**21.0 ± 0.5^{c}**	**22.0 ± 0.9^{c}**	**23.3 ± 3.0^{c}**	**35.2 ± 2.4^{a}**	**29.9 ± 0.5^{ab}**
2c	3.9 ± 0.0^{b}	4.8 ± 0.2^{ab}	4.5 ± 0.1^{ab}	4.8 ± 0.0^{ab}	4.8 ± 0.7^{ab}	6.2 ± 0.3^{a}	5.5 ± 0.1^{ab}
5p	0.4 ± 0.0^{a}	0.6 ± 0.1^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	1.2 ± 0.1^{a}	0.9 ± 0.1^{a}
5f	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.2 ± 0.0^{a}	0.5 ± 0.2^{a}	0.4 ± 0.1^{a}	1.0 ± 0.0^{a}	0.9 ± 0.1^{a}
2p	1.1 ± 0.0^{b}	1.9 ± 0.2^{ab}	1.5 ± 0.1^{b}	1.4 ± 0.2^{b}	2.0 ± 0.2^{ab}	3.0 ± 0.3^{a}	2.3 ± 0.0^{ab}
2f	2.4 ± 0.0^{c}	3.5 ± 0.3^{ab}	2.8 ± 0.1^{bc}	2.9 ± 0.0^{bc}	3.1 ± 0.5^{bc}	4.6 ± 0.6^{a}	3.8 ± 0.1^{ab}
2 c_d	3.0 ± 0.1^{a}	3.2 ± 0.1^{a}	3.1 ± 0.1^{a}	3.2 ± 0.1^{a}	3.1 ± 0.3^{a}	3.8 ± 0.1^{b}	3.6 ± 0.1^{b}
4p	1.9 ± 0.1^{a}	2.7 ± 0.2^{a}	2.2 ± 0.0^{a}	2.1 ± 0.2^{a}	2.7 ± 0.2^{a}	4.1 ± 0.3^{b}	3.4 ± 0.0^{a}
3f	1.5 ± 0.1^{a}	2.2 ± 0.1^{a}	1.8 ± 0.1^{a}	2.0 ± 0.0^{a}	1.7 ± 0.3^{a}	2.9 ± 0.2^{a}	2.6 ± 0.3^{a}
4f	0.8 ± 0.1^{b}	1.4 ± 0.1^{b}	1.2 ± 0.0^{b}	1.6 ± 0.5^{b}	1.5 ± 0.1^{b}	2.9 ± 0.1^{a}	2.2 ± 0.4^{ab}
$2p_d$	1.9 ± 0.2^{a}	2.3 ± 0.2^{b}	1.8 ± 0.0^{b}	1.6 ± 0.1^{b}	2.2 ± 0.3^{ab}	3.2 ± 0.4^{ab}	2.9 ± 0.1^{ab}
$2f_d$	1.3 ± 0.1^{b}	1.8 ± 0.2^{a}	1.5 ± 0.0^{a}	1.4 ± 0.1^{a}	1.5 ± 0.2^{b}	2.3 ± 0.2^{a}	1.9 ± 0.1^{a}
Phenolic acids (free), μg	**28.3 ± 2.0^{ab}**	**22.7 ± 0.9^{bc}**	**28.3 ± 0.9^{ab}**	**32.5 ± 3.2^{a}**	**20.0 ± 1.3^{c}**	**30.3 ± 0.7^{a}**	**30.7 ± 0.9^{a}**
caffeic acids	8.9 ± 0.4^{a}	6.4 ± 0.3^{bc}	7.7 ± 0.3^{ab}	8.7 ± 0.9^{a}	5.7 ± 0.5^{bc}	9.0 ± 0.0^{a}	9.2 ± 0.2^{a}
p-coumaric acid	7.5 ± 0.6^{a}	6.3 ± 0.3^{a}	6.6 ± 0.4^{a}	7.9 ± 1.0^{a}	5.9 ± 0.3^{a}	8.4 ± 0.4^{a}	8.2 ± 0.5^{a}
ferulic acid	12.0 ± 0.9^{bc}	9.9 ± 0.3 ^{cd}	13.9 ± 0.2^{ab}	15.9 ± 1.3^{a}	8.4 ± 0.6^{d}	12.9 ± 0.3^{abc}	13.2 ± 0.2^{abc}

*Results were shown as means ± standard deviation of triplicate analyses. Statistical differences between treatments were conducted based on one way-ANOVA and Turkey's post hoc test ($p < 0.05$), and shown with superscript letters a-e. **Sum contents of caffeic, p-coumaric and ferulic acids.

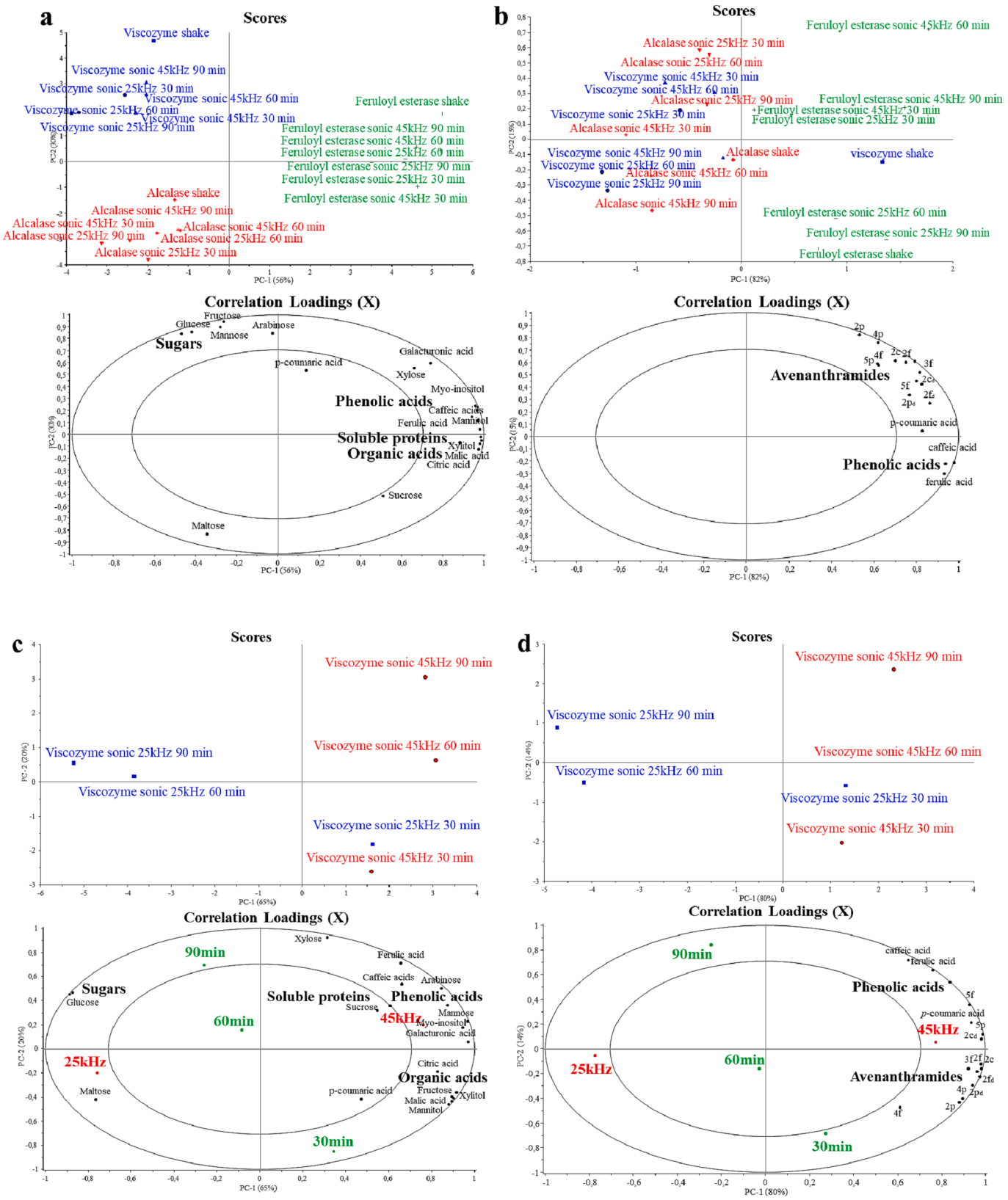

Fig. 2. PCA models for comparison of composition variables (n = 3) in all processed samples (colors in scores plots indicate different enzymes): a. supernatants, b. precipitates; within each set of enzyme-treated OH samples with ultrasonication frequency (red) and time (green) as down-weighted variables: c. supernatants of Viscozyme hydrolysis, d. precipitates of Viscozyme hydrolysis, e. supernatants of Alcalase hydrolysis, f. precipitates of Alcalase hydrolysis, g. supernatants of feruloyl esterase hydrolysis, h. precipitates of feruloyl esterase hydrolysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

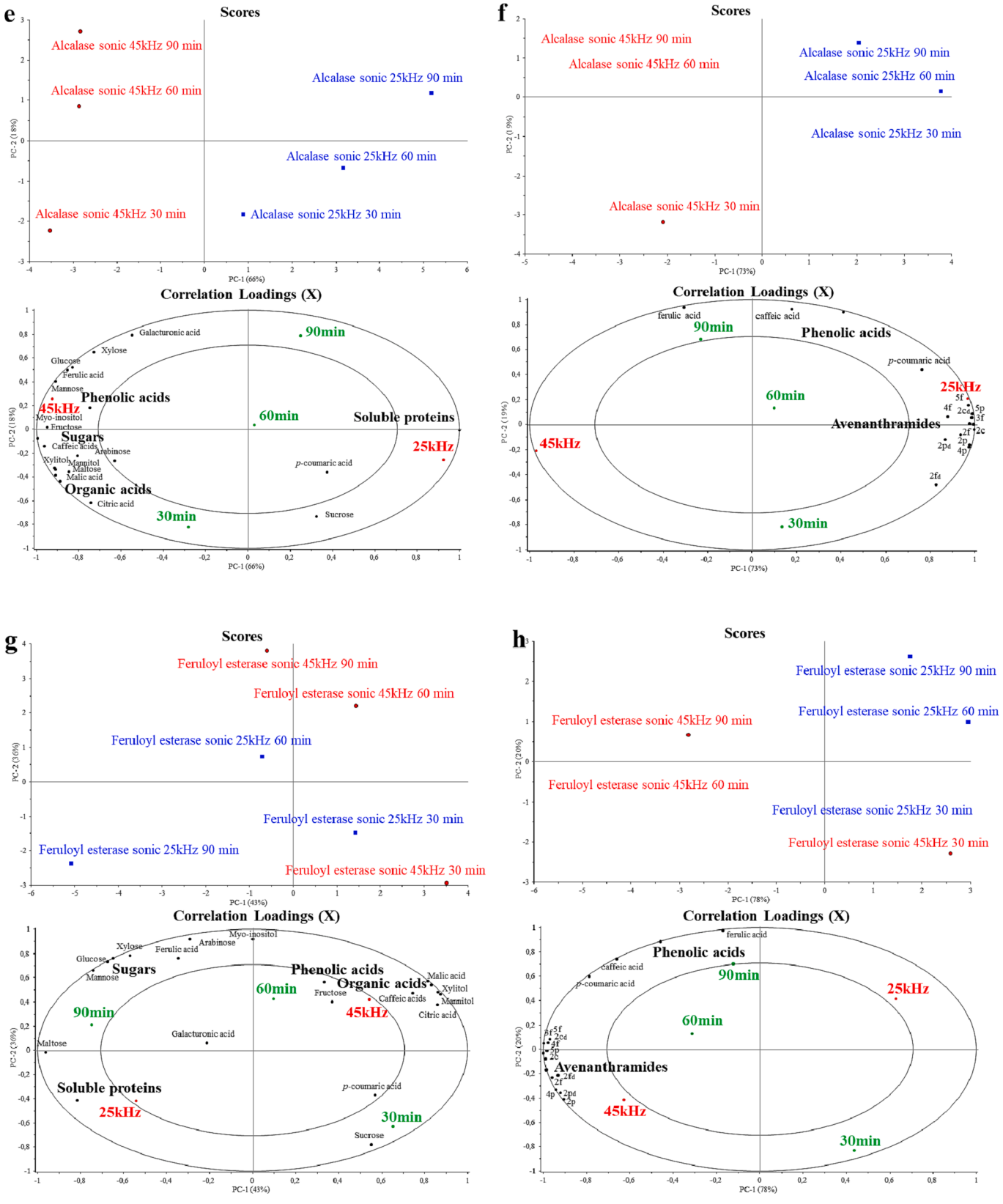

Fig. 2. (*continued*).

3.3.1. *Effects of ultrasonication processing time*

In our study, short ultrasonication processing time (30–90 min) had been proved to be efficient for enhancing the phytochemical extraction. As shown in Table 2, 30 min ultrasonication (25 kHz) yielded approximately the same amounts of soluble proteins as 3 h shaking did in all the enzyme-treated OH supernatants. Increasing shifts of solubilized protein and extraction efficiency of phenolic acids were observed with prolonged ultrasonication processing time in most ultrasonication-

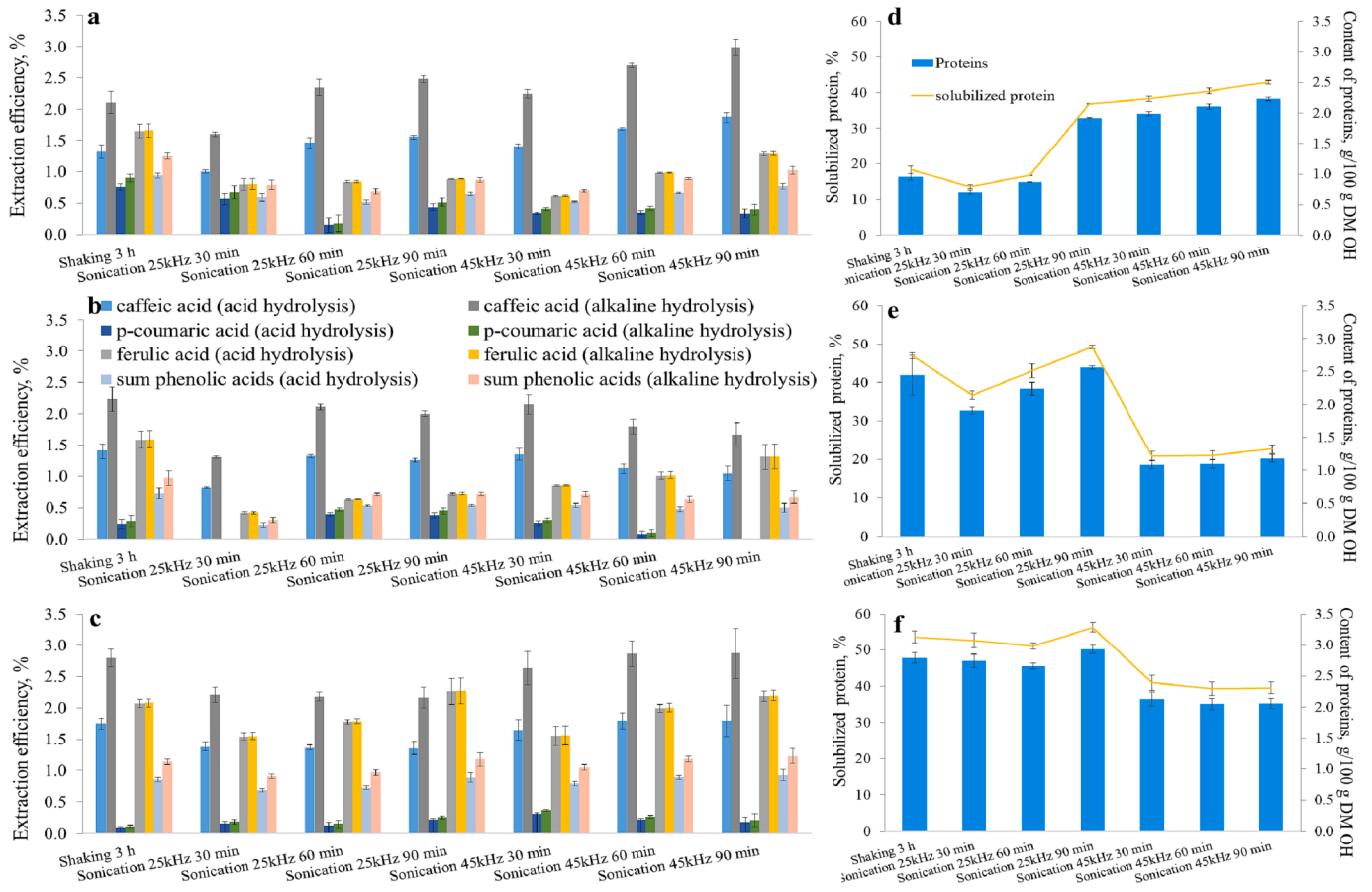

Fig. 3. Extraction efficiency of identified phenolic acids in the supernatants of OH treated by Viscozyme (a), Alcalase (b) and feruloyl esterase (c); contents of proteins from 100 g DM OH and solubilized protein in Viscozyme (d), Alcalase (e) and feruloyl esterase (f) treated samples.

assisted treatments (Supplemental Table 6). Likewise, ultrasonication for 90 min was confirmed to be efficient obtaining almost the same amount of free sugars as shaking did for 3 h for Viscozyme and Alcalase hydrolysis. Similar results were observed with sum organic acid and avenanthramide contents. In previous study, short-time ultrasonication (10 min) was confirmed to be efficient to solubilize hemicellulose in OH, with increasing yield of hemicellulose solubilization to 72% of the total hemicellulose (Schmitz et al., 2021). The shock waves generated by ultrasound create the cavitation phenomena. The cavitation results in the disruption and softening of plant tissues and makes the loose structure easier for enzymes to access, which eventually improves the extraction yield (Görgüç et al., 2019). In contrast, rather than breaking down sample structure, mechanism shaking only increases sample diffusion in the extraction solvent, allowing wider surface area between solid matrix and liquid solvent to contact.

3.3.2. *Effects of ultrasonication frequency*

Ultrasonication frequency had an important role in this study. As shown in Fig. 2c–h, ultrasonication frequency 45 kHz had better impact than 25 kHz on releasing most of the compounds (free sugars, sugar alcohols, organic acids, and phenolic acids) in the supernatants, the yields were highly depended on individual compound species and enzyme varieties. For example, with Viscozyme addition, the frequency 45 kHz showed higher yields of solubilized protein, while 25 kHz was preferred by Alcalase and feruloyl esterase. Additionally, for precipitates, 45 kHz was closely correlated to all the avenanthramides and phenolic acids when Viscozyme and feruloyl esterase applied to OH, but negatively related to these components in Alcalase treated samples. It could be explained that ultrasonication at frequencies lower than 166 kHz showed significant reductions in particle size of the substrate. The reason was due to the bubble size generated by different ultrasound frequencies. Lower frequency created larger bubbles, which caused more severe particle breakage when they imploded. This resulted in higher efficiency to reduce coarse particle size, leading to easier accessibility for enzymatic interaction. Oppositely, higher ultrasound frequency produced smaller bubbles, which led to a weaker effect on cavitation. Irregular small variations of compound yields could be observed among different low frequencies (Jordens et al., 2016).

3.3.3. *The interaction of ultrasonication time–frequency*

The synergistic effect of ultrasound processing time and frequency has also been confirmed in our study. Protein solubilization was significantly increased along with ultrasonication processing time under both ultrasound frequencies in Viscozyme samples (Fig. 3). The increasing contents of soluble proteins with prolonged ultrasonication processing time were only found under 25 kHz for Alcalase and feruloyl esterase samples, while longer ultrasonication processing time did not significantly influence the content change of soluble proteins under 45 kHz. Ultrasonication time–frequency interaction also affected the domination of the phenolic acid composition in the supernatants. The proportion of caffeic acid decreased while the proportion of ferulic acid increased under 45 kHz as longer ultrasonication processing time was applied for all the enzymes. However, regular pattern was not observed under 25 kHz. Moreover, the impact of ultrasonication time–frequency was not significant on phenolic acid yields in Alcalase and feruloyl esterase treated samples. Yet, sum phenolic acid contents in Viscozyme treated samples decreased at 60 min before slightly increased at 90 min under 25 kHz.

Interestingly, several previous articles reported the reduction contents of phenolic compounds in the extracts of crops with aid of

ultrasonication (without enzyme addition). For instance, the contents of phenolic acids in OH could be dramatically decreased by 15 times after ultrasonication processing followed by alkaline treatment (Schmitz et al., 2021). Content fluctuation of caffeic acid in defatted oat was also observed during 15 min ultrasonication-assisted extraction (Chen et al., 2018). Phenolic compounds were degraded in buckwheat hull after 10 min ultrasonication at both room temperature and 40 °C (Noore et al., 2022). This confirmed that phytochemical compounds could be degraded when they are exposed to ultrasound in excessive time. This is probably due to the production of free hydroxyl radicals, which allows highly active substances to break the chemical structure of phenolic compounds (Mikucka et al., 2022).

The impact of ultrasound processing time–frequency interaction on the precipitates was not as severe as to the supernatants. The differences were only observed in feruloyl esterase treated samples, in which the sum avenanthramides contents were significantly increased along with ultrasonication processing time under 45 kHz. Additionally, increased contents of phenolic acids were also found in feruloyl esterase treated precipitates under both ultrasonication frequencies (Table 3).

4. Conclusions

The present study compared the efficacy of varying treatments using enzymes with and without assistance of ultrasonication to release nutrients and bioactive compounds from OH. Enzyme being the key factor affecting chemical profiles, feruloyl esterase showed better capacity than Viscozyme and Alcalase in facilitating the recovery of most of the compounds (except free sugars) from OH. Ultrasonication significantly reduced the processing time needed for mechanical shaking during enzymatic treatment. Moreover, the compound yields and the extraction efficiencies of the compounds were highly dependent on the synergistic effect between ultrasonication processing time and frequency. To the best of our knowledge, our research is the first study to investigate the release of multiple nutrients and bioactive compounds from OH for designing novel foods. The findings provide important guidance for utilizing OH and serve as a useful reference for valorizing other currently under-exploited side streams.

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CRediT authorship contribution statement

Ying Zhou: Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Ye Tian:** Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Gabriele Beltrame:** Investigation, Data curation, Writing – original draft. **Oskar Laaksonen:** Project administration, Writing – review & editing. **Baoru Yang:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Baoru Yang reports financial support was provided by European Union. Baoru Yang reports financial support was provided by Academy of Finland.

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.foodchem.2023.136658.

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