Heliyon 10 (2024) e35166

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Synergistic effect of bath-ultrasonication and heating treatments on two-steps treatment of brewers' spent grain

Joncer Naibaho [a,b,*], Łukasz Bobak [a], Aneta Wojdyło [c], Małgorzata Korzeniowska [a,**], Yuyun Lu [d], Baoru Yang [e]

[a] Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, 51-630, Wroclaw, Poland
[b] Department of Food Chemistry and Technology, Ashtown Food Research Center – Teagasc, Ashtown, Dublin 15, D15 DY05, Dublin, Ireland
[c] Department of Fruit, Vegetable and Plant Nutraceutical Technology, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, 51-630, Wroclaw, Poland
[d] Department of Food Science and Technology, Faculty of Science, National University of Singapore, Singapore, 117542, Singapore
[e] Food Chemistry and Food Development, Department of Life Technologies, University of Turku, 20014, Turku, Finland

ARTICLE INFO

Keywords:
Value-added byproduct
Agro-based byproduct
Acoustics cavitation
Ultrasounds treatment
Thermal treatments

ABSTRACT

The study aimed to evaluate the chemical composition, antioxidant activity and techno-functionality of brewers' spent grain (BSG) treated with two-steps treatment involving 5, 15, and 25 min bath-ultrasonication (USB) continued with autoclave (AH) at 90, 110, and 130 °C and/or water-bath (CWH) at 80, 90, and 100 °C. The two-steps treatments slightly affected the water- and oil-holding capacity and extractable fat content. Most of the two-steps treatments increased the amount of flavan-3-ols and phenolic acids, up to 4 times higher compared to its control. The two-steps treatment involving CWH had no significant ($p > 0.05$) impact on fat content, antioxidants and techo-functionality of BSG. Up to 15 min USB increased the poly-unsaturated fatty acids and lowered the amount of saturated fatty acids. In conclusion, the two-steps treatment consists of USB (up to 15 min) continued with AH and CWH increased the amount of nutritional-related chemical composition such as UFA and phenolic acids as well as antioxidant activity of BSG.

1. Introduction

Global effort in valorizing agro-industrial and food processing side streams has been increasingly investigated [1]. Brewers' spent grain (BSG) is one of the most investigated side streams for its nutritional value thus its potential as a food and/or nutraceutical ingredient. BSG is the main waste of the brewery industry which represents 85 % of overall brewery byproducts [2]. Every 10 L of beer production generates around 20 kg of BSG; globally, approximately 1.9 billion hL of beer is produced every year [3]. By this, around 420 k tons of BSG has been generated worldwide. Besides the fact that improper handling of such a huge amount of side streams potentially damages the environment, it is widely well known that BSG contains high nutritional value which can provide a sustainable

* Corresponding author. Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, 51-630, Wroclaw, Poland.
** Corresponding author.
E-mail addresses: joncer.naibaho@upwr.edu.pl (J. Naibaho), malgorzata.korzeniowska@upwr.edu.pl (M. Korzeniowska).

https://doi.org/10.1016/j.heliyon.2024.e35166
Received 31 January 2024; Received in revised form 22 June 2024; Accepted 24 July 2024
Available online 25 July 2024

food ingredient. Therefore, several studies have investigated potential improvement of BSG in generating value-added by products such as polyphenolic, dietary fiber, protein, and biomaterials production [4–6].

In terms of side stream valorization, several emerging technologies have been applied in BSG. For instance, microwave-assisted extraction, supercritical, autoclave, and ultrasound [7–9]. The challenges arise due to the complex matrix structure of BSG, certain biological compounds become entrapped in the cell vacuoles and/or strongly bind to the main polysaccharide [10], thus impacting their bioavailability. Several methods, including thermal exposure, chemical and enzymatic treatments, have been applied to modify the structural properties of BSG, thus allowing for the release of bioactive compounds from the matrices [11,12]. Consequently, these methods enhanced yields of the extracts as well as their biological activities [11,12].

In addition to several applied methods in modifying composition of BSG, our previous study applied ultrasound bath-type (USB) at a range of time exposure continued with thermal exposure on BSG including autoclave and water bath treatments at a certain variety of temperature and time exposure. Cavitation from ultrasound treatment can degrade the matrix of plant biomass thus loosening the bond between polysaccharide and other compounds including lipid, polyphenolic, and protein [13]. Autoclave and water-bath are thermal processing which could modify dietary fiber composition of plant biomass thus releasing the polyphenolic compounds polysaccharide matrices [6,14]. A two-steps processing started with USB followed by thermal exposure could be expected to synergically improve the chemical composition of BSG. The study showed a modification in volatile composition of BSG [15]. However, the impact of the 2-steps treatments on other chemical composition, biological properties, and techno-functional properties remained unclear.

Therefore, the objective of the study was to evaluate the impact of 2-steps treatment on BSG which initially started with USB followed by autoclave or water bath at a certain variation in time and temperature exposure. It was hypothesized that the synergistic effect between certain USB exposures with thermal exposure might occur in enhancing the chemical composition, antioxidants, and techno-functional properties of BSG.

2. Materials and methods

2.1. Materials

BSG barley was obtained from a local brewery (light-beer producer) in Poland. Fresh BSG with 75–77 % moisture was then ground to pass 0.2 mm, followed by storage at −20 °C for further treatment. Trolox (6-hydro-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma-Aldrich (Steinheim, Germany). UPLC-grade water was prepared using an HLP SMART 1000s system (Hydrolab, Gdansk, Poland). Before use, the water was filtered by a 0.22 μm membrane filter immediately. All of the chemicals used were analytical grade.

2.2. Experimental design

The experiment was conducted following previously described [15]. All the controlled parameters such as time exposure, temperature, and ultrasound specification were based on the previous study: USB temperature was controlled at room temperature as acoustic cavitation is more efficient at low temperature. Dietary fiber modification at high temperature increased the solubility thus it was conducted at different temperature levels. There were 2 factors including USB time levels and thermal exposures temperature. Shortly, fresh BSG was milled, mixed with distilled water and then treated with USB. After that, it underwent to thermal treatments such as autoclave or water bath. Ground fresh BSG was mixed with distilled water (1:1) mixed properly in a beaker glass and closed with aluminum foil. After that, the mixture was treated with USB (Fisher Scientific, Elmasonic S10, Germany) for different exposure times (5, 15 and 25 min) at room temperature. The sonicated mixture was then treated with 2 different heating treatments, including conventional water bath heating (CWH) and autoclave heating (AH). CWH was carried out at different temperatures (80 °C, 90 °C and 100 °C) for 30 min, while AH was conducted at 90 °C, 110 °C and 130 °C for 12 min. Sonicated BSG without heating treatment was provided as a control. Therefore, a total of 21 samples were collected. The samples were then dried in an oven dryer at 70 °C-75 °C overnight to reach a moisture content below 6 %. The dried BSG was ground to obtain a particle size $\leq$0.2 mm and stored at 10 °C for further analysis.

2.3. Analysis of moisture and extracted fat content

Moisture content was measured using an oven method and fat content via the Soxhlet method (Buchi B-811), following procedures AOAC 2000, as described previously [16]. The measurements were carried out in triplicate.

2.4. Analysis of fatty acids composition by GC–MS

Fatty acid composition was assessed as follows, including lipid extraction, lipid derivatization and fatty acids analysis via GC–MS. Lipid was extracted following procedures described previously [17], followed by derivatization into fatty acid methyl esters (FAMEs); procedures were followed, as described in a previous study [18]. A gas chromatograph (GC6890) coupled with a mass spectrometer 5983 MS (Agilent Technologies Inc. CA, USA) equipped with a quadrupole mass detector was used for fatty acids identification. Separation was conducted using a capillary column HP-88 (0.25 mm × 100m) filled with 88:12 cyanopropyl-aryl poly-siloxane bed (grain size of 0.2 μm). Helium was used as a mobile phase, with a flow rate of 1 mL/min, and the sample was injected in the split mode 4:1. The program was set as follows: initial temperature 60 °C for 2 min, heating was carried out to reach 180 °C at a rate of 20 °C/min,

and then to 220 °C at a rate of 3 °C/min. After the temperature was achieved, it was maintained for 15 min. Heating continued to reach 250 °C at a rate of 5 °C/min, and the temperature was kept for 8 min. The spectra were identified using a search algorithm of the National Institute of Standards and Technology (NIST) library (2008 version).

2.5. Methanol extraction, antioxidant analysis and polyphenolic quantification

2.5.1. Methanolic extract preparation

An analysis of in vitro antioxidant activity and polyphenolic compounds was conducted in methanolic extracts. Methanol extraction was carried out following a previously described procedure [19] in duplicate. Briefly, 6 mL of 80 % methanol solution and 7 mL of 30 % methanol for measurement of antioxidant activity and polyphenolic identification, respectively, were added into 1 g of treated BSG and then vortexed for 1 min. The mixture was sonicated (Sonic 6D, Polsonic, Warsaw, Poland) for 20 min and left at 4 °C for 24 h. After that, the mixture was sonicated for 20 min, followed by centrifugation at 19000*g* for 10 min at 4 °C. The mixture was

Table 1
Physico-chemical and antioxidant properties of treated BSG with bath-ultrasound and its combination with autoclave and conventional water-bath heating treatments.

Bath ultrasonication	MC (%)	Fat (%)	WHC (g/g)	OHC (g/g)	Antioxidant activities (mmol Trolox/100 g)		Polyphenolic compounds (mg/kg)		
					ABTS	FRAP	Flavan-3-ols	Phenolic acid	Total polyphenolic
5 min									
Control	5.49 ± 0.20^{abcd}	6.33 ± 0.59^{de}	3.28 ± 0.04^{bcd}	2.21 ± 0.01^{a}	0.12 ± 0.00^{ghi}	0.05 ± 0.01^{h}	106.83 ± 9.87^{fgh}	18.53 ± 0.72^{gh}	125.37 ± 9.15^{ij}
AT 1	5.75 ± 0.05^{ab}	6.96 ± 0.46^{de}	3.45 ± 0.04^{abc}	2.22 ± 0.06^{a}	0.13 ± 0.00^{gh}	0.06 ± 0.00^{gh}	99.48 ± 3.95^{h}	17.58 ± 0.62^{gh}	117.06 ± 3.32^{j}
AT 2	5.28 ± 0.10^{abcde}	8.76 ± 0.29^{abc}	3.43 ± 0.03^{abc}	2.26 ± 0.05^{a}	0.25 ± 0.01^{cd}	0.18 ± 0.01^{cd}	171.74 ± 4.77^{e}	30.15 ± 2.78^{fg}	201.88 ± 1.98^{efg}
AT 3	5.45 ± 0.02^{abcd}	8.71 ± 0.06^{abc}	3.82 ± 0.04^{a}	2.19 ± 0.02^{ab}	0.29 ± 0.00^{b}	0.21 ± 0.01^{bc}	355.07 ± 16.38^{b}	25.20 ± 1.61^{gh}	380.27 ± 17.98^{c}
CWH 1	4.41 ± 0.03^{e}	6.76 ± 0.07^{de}	3.65 ± 0.05^{ab}	2.21 ± 0.01^{ab}	0.10 ± 0.00^{i}	0.04 ± 0.01^{h}	153.57 ± 7.34^{e}	13.28 ± 0.56^{h}	166.85 ± 7.90^{fghi}
CWH 2	4.82 ± 0.00^{bcde}	7.04 ± 0.19^{de}	3.71 ± 0.20^{ab}	2.20 ± 0.00^{ab}	0.12 ± 0.00^{ghi}	0.07 ± 0.00^{gh}	159.02 ± 8.25^{e}	14.15 ± 0.67^{h}	173.17 ± 8.92^{fgh}
CWH 3	5.18 ± 0.05^{abcde}	7.10 ± 0.24^{de}	3.74 ± 0.08^{ab}	2.17 ± 0.01^{abcd}	0.17 ± 0.00^{f}	0.13 ± 0.02^{fg}	153.80 ± 5.36^{e}	11.15 ± 0.58^{h}	164.95 ± 5.94^{ghi}
15 min									
Control	5.72 ± 0.31^{abc}	5.80 ± 0.47^{e}	3.05 ± 0.03^{cde}	2.18 ± 0.01^{abc}	0.10 ± 0.00^{hi}	0.04 ± 0.01^{h}	161.29 ± 6.59^{e}	22.91 ± 1.42^{gh}	184.20 ± 5.17^{fgh}
AT 1	5.94 ± 0.09^{a}	7.58 ± 0.20^{bcd}	3.37 ± 0.17^{abc}	2.19 ± 0.03^{ab}	0.10 ± 0.00^{hi}	0.06 ± 0.02^{gh}	168.68 ± 3.02^{e}	25.83 ± 2.35^{gh}	194.51 ± 5.37^{efgh}
AT 2	5.47 ± 0.04^{abcd}	9.16 ± 0.32^{a}	3.55 ± 0.11^{abc}	2.18 ± 0.00^{abcd}	0.21 ± 0.01^{e}	0.15 ± 0.01^{de}	312.74 ± 8.83^{c}	44.28 ± 1.93^{ef}	357.03 ± 6.90^{c}
AT 3	5.56 ± 0.18^{abcd}	9.26 ± 0.36^{a}	3.61 ± 0.13^{ab}	2.19 ± 0.02^{ab}	0.28 ± 0.01^{bc}	0.25 ± 0.02^{ab}	495.97 ± 14.91^{a}	46.38 ± 3.52^{e}	542.34 ± 18.43^{b}
CWH 1	4.67 ± 0.22^{de}	6.90 ± 0.25^{de}	2.27 ± 0.22^{g}	2.04 ± 0.02^{e}	0.10 ± 0.01^{hi}	0.06 ± 0.01^{gh}	141.10 ± 8.20^{efg}	70.10 ± 0.81^{cd}	211.20 ± 7.39^{def}
CWH 2	4.76 ± 0.13^{cde}	6.90 ± 0.16^{de}	2.68 ± 0.05^{efg}	2.05 ± 0.02^{e}	0.16 ± 0.01^{f}	0.06 ± 0.00^{h}	145.80 ± 6.89^{e}	43.76 ± 1.31^{ef}	189.56 ± 8.20^{efgh}
CWH 3	4.66 ± 0.15^{de}	7.16 ± 0.48^{de}	2.78 ± 0.01^{def}	2.04 ± 0.02^{e}	0.15 ± 0.01^{fg}	0.09 ± 0.02^{fgh}	173.46 ± 11.67^{e}	81.61 ± 7.64^{bc}	255.08 ± 4.03^{d}
25 min									
Control	5.54 ± 0.36^{abcd}	5.86 ± 0.21^{e}	2.46 ± 0.02^{fg}	2.07 ± 0.04^{e}	0.11 ± 0.01^{hi}	0.05 ± 0.00^{h}	103.55 ± 6.19^{gh}	47.49 ± 3.17^{e}	151.04 ± 9.36^{hij}
AT 1	5.18 ± 0.11^{abcde}	6.75 ± 0.35^{de}	3.31 ± 0.17^{bc}	2.09 ± 0.01^{cde}	0.12 ± 0.01^{ghi}	0.07 ± 0.01^{gh}	169.59 ± 4.25^{e}	65.49 ± 0.99^{65}	235.08 ± 5.23^{de}
AT 2	5.20 ± 0.07^{abcde}	8.83 ± 0.11^{ab}	3.58 ± 0.06^{ab}	2.11 ± 0.03^{bcde}	0.22 ± 0.01^{de}	0.18 ± 0.01^{cd}	266.06 ± 3.84^{d}	74.33 ± 5.33^{cd}	340.39 ± 9.17^{c}
AT 3	5.53 ± 0.06^{abcd}	9.21 ± 0.02^{a}	3.47 ± 0.25^{abc}	2.09 ± 0.01^{cde}	0.32 ± 0.00^{a}	0.27 ± 0.01^{a}	492.21 ± 12.63^{a}	106.39 ± 8.25^{a}	598.60 ± 20.88^{a}
CWH 1	4.89 ± 0.24^{bcde}	6.66 ± 0.52^{de}	3.49 ± 0.05^{abc}	2.09 ± 0.01^{cde}	0.10 ± 0.02^{hi}	0.05 ± 0.01^{h}	168.33 ± 13.04^{e}	82.56 ± 5.67^{bc}	250.90 ± 18.71^{d}
CWH 2	5.17 ± 0.02^{abcde}	6.80 ± 0.05^{de}	3.53 ± 0.05^{abc}	2.08 ± 0.00^{de}	0.12 ± 0.00^{hi}	0.07 ± 0.01^{gh}	143.38 ± 2.77^{ef}	46.35 ± 3.39^{e}	189.73 ± 0.61^{efgh}
CWH 3	4.90 ± 0.00^{bcde}	7.40 ± 0.46^{cd}	3.61 ± 0.08^{ab}	2.18 ± 0.02^{abc}	0.17 ± 0.00^{hi}	0.11 ± 0.01^{efg}	260.68 ± 15.92^{d}	93.54 ± 7.44^{ab}	354.22 ± 23.36^{c}

Note: CWH = conventional water-bath heating at 80 °C (1), 90 °C (2), and 100 °C (3) for 30 min; AT = autoclave treatment at 90 °C (1), 110 °C (2), and 130 °C (3); control = without heating treatment. The data is shown as mean ± standard deviation with at least duplicate analysis. Letters show the significant differences from other treatment in the same column ($p < 0.05$).

filtered using a 0.20 μm hydrophilic PTFE membrane (Millex Simplicity Filter, Merck, Germany) to obtain the methanolic extracts.

2.5.2. In vitro antioxidant activity

In vitro antioxidant activity was assessed for ABTS and FRAP [20,21] in triplicate, and the results were expressed as mmol Trolox equivalents/100 g of dry sample.

ABTS was conducted as follows. An amount of 3 mL of ABTS solution was added into a cuvette that contained 30 μL of methanol extract. The absorbance was measured at 734 nm with a wavelength spectrophotometer, exactly after 6 min. The blank measurement was prepared with 30 μL of distilled water. A curve standard was prepared and measured at 734 nm with the wavelength spectrophotometer, with absorbance range 0.700 ± 0.02.

The FRAP value was measured as follows: a mixture of acetate buffer of pH 3.6 and TPTZ (2,3,5-Triphenyltetrazolium chloride) was dissolved in 40 mM/L HCl, and $FeCl^*6H_2O$ dissolved in distilled water (10:1:1) was prepared on the same day for the measurement. A certain volume of the sample (0.1–1 mL) was mixed with distilled water to reach 1 mL of mixture. A volume of 3 mL of the reagent was added into the mixture, and the absorbance was measured at a wavelength of 593 nm, with an absorbance range of 0.200–0.800, exactly after 10 min. The result was calculated based on curve standard absorbance.

2.5.3. Identification of polyphenolic compounds

An identification of polyphenolic compounds was conducted for the total amount of flavan-3-ols and phenolic acids. Flavan-3-ols and phenolic acids were identified and quantified using Liquid Chromatography–Tandem mass Spectrometry (LC-MS-MS) following procedures as described previously [19]. Ultra-performance liquid chromatography (Acquity UPLC system) with binary solvent manager and photodiode array detector PDA (Waters Corp., Milford, MA. US) was used to determine the profile and content of flavan-3-ols and phenolic acids. The system was coupled to a Xevo™ G2 Q/TOF micro-mass spectrometer fitted with negative modes of electrospray ionization ESI source (Waters Corp., Manchester, UK). The analysis was carried out using full scan data-dependent MS from m/z 100 to 1700. The characterization of phenolic compositions was conducted according to the retention time and accurate molecular masses. The data were collected using MassLynx™ 4.1 ChromaLynx Application Manager software (Waters Corp. Milford, USA). Flavan-3-ols and phenolic acids were monitored at 280 nm and 320 nm wavelengths, respectively. Quantification was conducted based on the phenolic calibration standards, at concentrations ranging between 0.05 and 5 mg/mL ($R2 \geq 0.9995$). All of the samples were analyzed in triplicate, and the results were reported in mg/kg of dry weight sample.

2.6. Analysis of WHC and OHC

The techno-functionality of BSG, including water holding capacity (WHC) and oil holding capacity (OHC), were measured following the procedures described in a previous study [14], in triplicate.

2.7. Statistical analysis

Statistical analysis was conducted using the two-way analysis of variance (ANOVA) followed by Tukey's post hoc test in Statistica software, version 13.5.0.17. Principal component analysis was then conducted by using Minitab 17.

3. Results and discussion

3.1. Moisture and extractable fat content

The moisture and extractable fat content of treated BSG were measured, and the results are presented in Table 1. The results demonstrated that the moisture content of treated BSG ranged between 4.4 % and 5.8 %. The majority of the groups had no significant difference ($p > 0.05$) in moisture content. These results confirm the uniformity of the drying process on treated BSG, which was dried at 70–75 °C for ±16 h using an oven dryer. A slight significant difference ($p < 0.05$) was observed in extractable fat content. Different time exposures during USB (5, 15 and 25 min) without thermal exposure generated the same level of extractable fat content. However, continued autoclave treatment (110 °C and 130 °C) generated the highest fat content, regardless of time elevation, which ranged at 8.7 %–9.3 %. USB might have weakened the binding between polysaccharides and lipids during the ultrasound exposure as has been reported (Deng et al., 2022). After that, high temperature during autoclave allows migration of lipids from entrapped matrices. USB combined with conventional water bath treatments generated the fat content at the same level ($p > 0.05$) as in the control, which ranged at 5.8 %–7.6 %. The ability of autoclave treatment to increase fat content in BSG has been reported previously [5], from 10 – 11 % to 14–20 %. Thermal exposure on BSG increased the amount of extractable fat content. In the current study, BSG contained a lower amount of extractable fat content compared to that in the previous study. This phenomenon may have occurred during the multi-drying process, which increased the extractable fat content as a result of thermal exposure. Using a combination of ultrasound and microwave treatments for improving total lipid extraction has been identified previously [22], which aligned with the current study. The ability of thermal exposure in intensifying extractable fat content in BSG has also been identified previously [23]. BSG lipids are composed of fatty acids and tocotrienols, as well as volatile compounds including propionic, acetic, and butyric acids [17,22,24], which possess antioxidant activity [24]. USB continued with autoclave treatment (110 and 130 °C) improved fat extractability, which may be a sign of higher biological activity.

3.2. Polyphenolic compounds

The amounts of flavan-3-ols, phenolic acids and total phenolic compounds are presented in Table 1. The study demonstrated that the amount of flavan-3-ols fluctuated, regardless of the time and thermal level exposures of the treatments. The highest flavan-3-ol content was obtained in USB-AH (130 °C), regardless of time levels on USB. The same trend was identified in the total amount of

Table 2
Fatty acids composition (% of total fatty acids) of BSG treated with bath-ultrasound and its combination with autoclave and conventional water-bath heating treatments.

Fatty acids	Bath ultrasonication treatments						
	Control	Autoclave heating			Conventional water-bath heating		
		90 °C	110 °C	130 °C	80 °C	90 °C	100 °C
5 min							
C13:0	–	–	–	–	–	0.23 ± 0.01	–
C15:0	–	–	29.33 ± 0.08	–	–	–	–
C16:0	21.42 ± 0.18	21.67 ± 0.06	–	21.74 ± 0.12	21.39 ± 0.02	21.81 ± 0.01	21.83 ± 0.01
C18:0	3.17 ± 0.15	2.72 ± 0.07	–	3.13 ± 0.03	3.12 ± 0.07	3.27 ± 0.01	2.98 ± 0.02
C18:1 (n-9)	18.08 ± 0.17	16.71 ± 0.29	–	17.40 ± 0.00	17.75 ± 0.02	17.77 ± 0.01	17.53 ± 0.03
C18:2 (n-6)	48.70 ± 0.20	51.75 ± 0.11	70.67 ± 0.08	48.90 ± 0.03	49.10 ± 0.06	47.38 ± 0.02	49.90 ± 0.03
C18:3 (n-3)	5.54 ± 0.13	5.41 ± 0.01	–	5.93 ± 0.03	5.85 ± 0.07	5.85 ± 0.02	5.75 ± 0.01
C20	0.88 ± 0.04	–	–	0.84 ± 0.02	0.79 ± 0.03	0.83 ± 0.01	–
C20:1	2.19 ± 0.15	1.73 ± 0.04	–	2.05 ± 0.07	1.99 ± 0.07	1.97 ± 0.02	2.02 ± 0.04
C22:0	–	–	–	–	–	0.89 ± 0.02	–
SFA	25.48 ± 0.01^{n}	24.39 ± 0.13^{p}	29.33 ± 0.08^{h}	25.71 ± 0.07^{m}	25.30 ± 0.07^{n}	27.03 ± 0.01^{jk}	24.81 ± 0.03^{o}
MUFA	20.27 ± 0.32^{b}	18.45 ± 0.26^{f}	–	19.46 ± 0.06^{e}	19.74 ± 0.05^{cde}	19.74 ± 0.01^{cde}	19.55 ± 0.01^{de}
PUFA	54.25 ± 0.33^{g}	57.16 ± 0.13^{c}	70.67 ± 0.08^{a}	54.83 ± 0.00^{f}	54.95 ± 0.01^{f}	53.23 ± 0.00^{h}	55.64 ± 0.04^{e}
15 min							
C13:0	–	1.11 ± 0.00	0.86 ± 0.00	0.30 ± 0.01	–	0.46 ± 0.01	0.47 ± 0.01
C15:0	–	0.45 ± 0.01	0.35 ± 0.01	0.11 ± 0.01	–	0.57 ± 0.01	0.50 ± 0.01
C16:0	20.58 ± 0.01	20.51 ± 0.09	20.02 ± 0.06	20.08 ± 0.02	23.63 ± 0.07	20.97 ± 0.05	20.26 ± 0.01
C16:1	–	0.69 ± 0.00	0.53 ± 0.00	–	1.04 ± 0.02	–	–
C17:0	–	0.26 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.33 ± 0.01	0.30 ± 0.02	–
C18:0	3.27 ± 0.01	3.17 ± 0.01	2.97 ± 0.00	2.96 ± 0.01	3.77 ± 0.01	3.13 ± 0.03	3.36 ± 0.02
C18:1 (n-9)	17.87 ± 0.02	17.50 ± 0.01	17.42 ± 0.01	17.40 ± 0.01	0.54 ± 0.02	17.07 ± 0.02	17.28 ± 0.09
C18:2 (n-6)	50.43 ± 0.01	45.47 ± 0.13	47.39 ± 0.13	47.61 ± 0.05	56.83 ± 0.09	46.02 ± 0.00	46.23 ± 0.10
C18:3 (n-3)	5.65 ± 0.01	5.83 ± 0.05	5.57 ± 0.00	6.38 ± 0.01	6.92 ± 0.00	6.26 ± 0.02	6.10 ± 0.08
C20	–	0.87 ± 0.05	0.83 ± 0.04	0.82 ± 0.01	1.17 ± 0.03	0.87 ± 0.01	0.93 ± 0.02
C20:1	2.21 ± 0.02	1.99 ± 0.02	2.18 ± 0.07	2.01 ± 0.02	2.72 ± 0.01	2.09 ± 0.02	2.19 ± 0.07
C20:2	–	0.28 ± 0.01	0.29 ± 0.03	0.35 ± 0.02	0.41 ± 0.01	0.29 ± 0.00	0.36 ± 0.02
C22:0	–	0.89 ± 0.03	0.88 ± 0.01	0.82 ± 0.01	1.19 ± 0.01	0.92 ± 0.01	0.92 ± 0.02
C22:1	–	0.53 ± 0.02	–	0.49 ± 0.03	0.73 ± 0.01	0.55 ± 0.01	0.59 ± 0.04
C23:0	–	0.05 ± 0.01	–	–	0.04 ± 0.00	0.01 ± 0.00	0.13 ± 0.01
C24:0	–	0.38 ± 0.01	0.49 ± 0.01	0.42 ± 0.02	0.69 ± 0.02	0.49 ± 0.02	0.67 ± 0.02
SFA	23.84 ± 0.01^{q}	27.70 ± 0.04^{i}	26.62 ± 0.02^{l}	25.77 ± 0.04^{m}	30.81 ± 0.02^{g}	27.72 ± 0.00^{i}	27.25 ± 0.13^{j}
MUFA	20.07 ± 0.01^{bc}	20.72 ± 0.04^{a}	20.14 ± 0.08^{b}	19.90 ± 0.02^{bcd}	5.03 ± 0.07^{h}	19.71 ± 0.02^{cde}	20.06 ± 0.03^{bc}
PUFA	56.08 ± 0.02^{d}	51.58 ± 0.08^{k}	53.24 ± 0.10^{h}	54.34 ± 0.02^{g}	64.16 ± 0.09^{b}	52.57 ± 0.02^{ij}	52.69 ± 0.16^{i}
25 min							
C13:0	0.45 ± 0.01	–	–	–	–	–	–
C15:0	0.39 ± 0.02	–	–	–	84.48 ± 0.01	–	–
C16:0	20.64 ± 0.01	68.82 ± 0.08	82.42 ± 0.03	93.23 ± 0.06	–	46.12 ± 0.01	41.83 ± 0.02
C16:1	0.65 ± 0.01	–	–	–	–	–	–
C17:0	–	–	–	–	–	–	7.15 ± 0.01
C18:0	3.19 ± 0.01	–	–	–	–	5.64 ± 0.01	–
C18:1 (n-9)	17.31 ± 0.01	–	–	–	–	11.33 ± 0.04	18.56 ± 0.02
C18:2 (n-6)	45.79 ± 0.03	31.18 ± 0.08	17.58 ± 0.03	6.77 ± 0.06	15.52 ± 0.01	36.90 ± 0.02	32.47 ± 0.01
C18:3 (n-3)	6.15 ± 0.02	–	–	–	–	–	–
C20	0.85 ± 0.01	–	–	–	–	–	–
C20:1	1.98 ± 0.01	–	–	–	–	–	–
C20:2	0.33 ± 0.01	–	–	–	–	–	–
C22:0	0.85 ± 0.01	–	–	–	–	–	–
C22:1	0.53 ± 0.01	–	–	–	–	–	–
C23:0	0.04 ± 0.00	–	–	–	–	–	–
C24:0	0.50 ± 0.01	–	–	–	–	–	–
C24:1	0.36 ± 0.02	–	–	–	–	–	–
SFA	26.91 ± 0.01^{k}	68.82 ± 0.08^{d}	82.42 ± 0.03^{c}	93.23 ± 0.06^{a}	84.48 ± 0.01^{b}	51.77 ± 0.03^{e}	48.97 ± 0.01^{f}
MUFA	20.82 ± 0.04^{a}	–	–	–	–	11.33 ± 0.04^{g}	18.56 ± 0.02^{f}
PUFA	52.27 ± 0.013^{j}	31.18 ± 0.08^{n}	17.58 ± 0.03^{o}	6.77 ± 0.06^{q}	15.52 ± 0.01^{p}	36.90 ± 0.02^{l}	32.47 ± 0.01^{m}

Note: italic describes its presence in untreated BSG. The data is shown as mean ± standard deviation with at least duplicate analysis. Letters show the significant differences from other treatment in the same column ($p < 0.05$).

polyphenolics. The highest flavan-3-ols was reached as a result of the incorporation of AH. The amount of flavan-3-ols was higher than phenolic acids, which contributed to the fluctuation in the total polyphenolic content. A similar trend was observed in our previous study. However, a contrasting result has been reported, in which phenolic acids dominated polyphenolic compounds in BSG [6]. This phenomenon demonstrated the resistance level of flavan-3-ols and phenolic acids, depending on thermal exposure. A higher level of flavan-3-ols was observed in the treatment which applied less thermal exposure, while a higher level of phenolic acids was identified with the treatment that subjected BSG to thermal exposure multiple times [6]. The efficiency of ultrasonication depends on its combination with other methods, power and time exposure, as well as solvent incorporation [25]. In the current study, the highest flavan-3-ol levels were obtained in autoclave-combined treatments. According to previous studies, (+)-catechin and (−)-epicatechin are two compounds which are responsible for the amount of flavan-3-ols in BSG. This research indicated that bath ultrasonication may have a significant impact on the release and/or formation of (+)-catechin and (−)-epicatechin, due to synergistic effects with temperature and pressure, as has been emphasized previously [26].

The amount of phenolic acids is significantly ($p < 0.05$) affected by time exposure of USB. The higher the time exposure to USB, the higher the amount of phenolic acids. The 5 min USB generated phenolic acids in a range of 11.2–30.2 mg/kg, while 15 min and 25 min contained phenolic acids in a range of 22.9–81.6 mg/kg and 46.4–106.4 mg/kg, respectively. Thus, higher time exposure to USB may allow for a higher release of phenolic acids from the BSG matrix. The intensification of phenolic acids may have occurred as a result of the degradation of main dietary fiber in BSG, which solubilized cellulose from hemicellulose, as has been identified previously; this consequently enhanced the amount of phenolic acids [27]. Ultrasound treatment facilitated the breakdown of ester bonds between ferulic acids and the hemicellulose main chain, thus increasing hemicellulose solubility [26]. This phenomenon may increase the availability of ferulic acids. Ferulic acids are bound to the insoluble cellulose and/or hemicellulose by ester linkages. The breakdown of ferulic acids from the insoluble structure has been reported in BSG [28]. In addition to ferulic acids and their derivatives, such as di-ferulic acid dimer, decarboxylated diferulic acid and di-ferulic acid isomers, certain phenolic compounds including syringic acid, benzoic acid, *p*-coumaric acid, and sinapic acid, have been reported to be responsible for phenolic acids in BSG.

3.3. Fatty acids composition

The fatty acids composition of treated BSG is presented in Table 2 and the total amount of fatty acids is presented in Fig. 1. The results demonstrated that the treatments fluctuated the amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Moreover, the treatments altered the composition of fatty acids profiles by discharging and forming certain fatty acids. Those phenomena occurred, depending on the time in bath ultrasonication and the thermal level in autoclave and water bath heating treatments. USB for 5, 15 and 25 min without thermal exposures generated SFA in a range of 23.8–26.9 %, MUFA at 20.1–20.8 %, and PUFA at 52. – 56.1 %. Compared to our previous study [14], untreated BSG contained 45.2 % SFA, 19.4 % MUFA and 35.5 % PUFA. Thus, bath ultrasonication decreased the amount of SFA and increased the amounts of MUFA and PUFA. Compared to USB without thermal exposures, USB continued with CWH and AH increased the amount of SFA, which seems to be affected by period in USB treatment. The longer the USB, its combination with thermal exposures generated a higher level of SFA. The highest SFA was obtained in 25 min USB, which ranged at 49–93.2 %, followed by 15 min and 5 min at 25.8–30.8 % and 24.4–29.3 %, respectively. This phenomenon demonstrated that 25 min of USB continued with CWH and AH might have allowed a higher polymerization of fatty acids, thus reforming the SFA to reach a higher level compared to that in untreated BSG. Additionally, 5- and 15-min USB continued with CWH and AH generated a slightly higher SFA ($p < 0.05$); however, it was lower than that in untreated BSG.

Most of the 2-steps treatments significantly ($p < 0.05$) decreased the amount of MUFA to the same level as it was in untreated BSG. The 25 min USB followed by AH and CWH discharged the presence of MUFA, except at 90 and 100 °C CWH, which contained MUFA at 11.3 % and 18.6 %, respectively. Therefore, the formed MUFA due to USB may have undergone polymerization after continued with

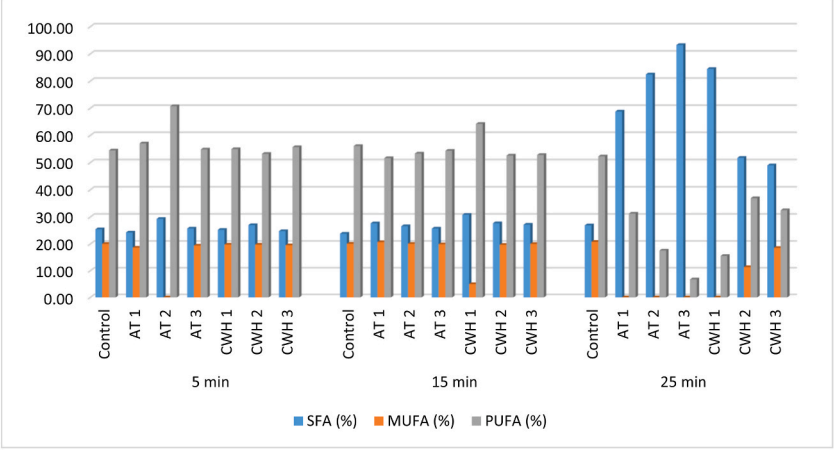

Fig. 1. Total amount of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poli-unsaturated fatty acids (PUFA).

CWH and AH. The impact of 2-steps treatments on PUFA was seemingly influenced by time exposures on USB. The longer the time exposure, the lower the amount of PUFA. This phenomenon confirmed that the increase in SFA may be due to the conversion of PUFA and MUFA, which resulted from thermal exposures after ultrasonication. However, thermal exposure combined with 5 and 15 min of bath ultrasonication resulted in PUFA at a higher level than that in control. USB continued with 110 °C AH reached up to 70.7 % of PUFA, which was two times higher than that in control. As mentioned previously, up to 25 min USB converted the SFA into MUFA and/or PUFA. However, the 2-steps (25 min USB) sharply decreased the amount of PUFA, showing the rearrangement of PUFA into SFA. AH enhanced the amount of PUFA and dropped the amount of SFA. Moreover, CWH has been observed to generate the same results on BSG [14]. Higher temperatures also have been identified to rearrange the fatty acid profile (increased UFA and decreased SFA) by increasing the transesterification rate [29]. It is widely accepted that SFA is responsible for non-communicable diseases, and UFA benefits human health. Thus, autoclave and water bath heating treatment are necessary to be combined with bath ultrasound at 5 and 15 min.

Fluctuations in the amounts of SFA, MUFA and PUFA are aligned with the rearrangement and/or discharging of fatty acid compounds, as can be seen in Table 2. Untreated BSG contains C16:0, C17:0, C18:1 (n-9), C18:2 (n-6) and C18:3 (n-3). The 5 min USB formed C20 and C20:1, while 15 min formed C20:1. In addition to C20 and C20:1, the 25 min USB formed C13:0, C15:0, C16:1, C20:2, C22:0, C22:1, C23:0, C24:0 and C24:1. The study demonstrated that USB tended to form UFA in BSG. The formation of UFA in the current study might be due to the rearrangement of SFA that has been released from the BSG matrix. Thermal exposures after USB showed a different trend in the fatty acids profile. AH and CWH after 5- and 15-min USB tended to form new fatty acids compared to those formed by the 25 min USB. However, thermal exposures after 25 min USB discharged the majority of formed fatty acids. The impact of ultrasound in specific profile of fatty acids composition, particularly on BSG has never been reported.

3.4. *In vitro antioxidant properties*

FRAP and ABTS were evaluated, which represented the in vitro antioxidant activity of treated BSG, and the results are shown in Table 1. Time differences USB had no significant ($p > 0.05$) impact on ABTS and FRAP, which showed ranges of 0.10–0.12 mmol Trolox/100 g and 0.04–0.05 mmol Trolox/100 g, respectively. However, USB continued with thermal exposures generated a significant ($p < 0.05$) difference in ABTS and FRAP, depending on the type and temperature level of the thermal treatments. USB continued with temperature below 100 °C had no significant impact on ABTS and FRAP, except the 15 min USB; 90 °C CWH. However, combinations with both CWH and AH with temperatures ≥100 °C significantly ($p < 0.05$) enhanced the ABTS and FRAP values to ranges of 0.15–0.32 mmol Trolox/100 g and 0.07–0.27 mmol Trolox/100 g, respectively. Compared to untreated fresh BSG in the preliminary results, the treatment significantly increases both ABTS and FRAP antioxidant activity. The higher amount of antioxidant properties in the current study revealed the synergistic effect of USB and thermal exposures.

3.5. *Techno-functional properties*

The impact of the 2-steps treatments on water-holding capacity (WHC) and oil-holding capacity (OHC) of BSG is presented in Table 1. In general, the 2-steps treatment generated the same levels of WHC and OHC, in which no significant differences ($p < 0.05$) were observed, except for the 10 min USB–CWH. The 10 min USB continued with CWH generated the lowest level of WHC and OHC compared to that of other treatments. The effect of single USB on techno-functional properties of BSG seems to depend on the time

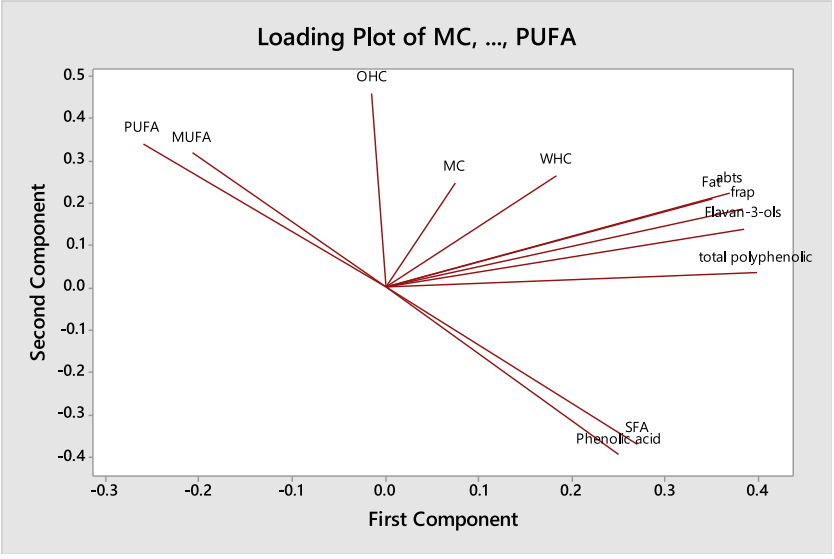

Fig. 2. Principal component analysis of ultrasound bath continued with thermal exposures on brewers' spent grain.

exposure. The results showed that lower WHC and OHC levels were observed in longer time exposures. The 5 min USB continued with thermal exposures generated the same levels of WHC and OHC, in ranges of 3.3–3.7 g/g and 2.2–2.3 g/g, respectively. The 10 min USB continued with CWH lowering the level of WCH. Although the single 25 min USB lowered the WHC value, the 2-steps treatment enhanced the WHC from 2.5 g/g to a range of 3.3–3.6 g/g. This phenomenon showed a synergistic effect between ultrasonication and water bath and autoclave heating, as has been emphasized previously [26].

WHC represents the ability of the BSG matrix to bind water molecules. The WHC level of BSG is highly influenced by the amount and/or the availability of arabinoxylans (Steiner et al., 2015). Consequently, the alteration of WHC in the current study may be due to the modification of dietary fiber composition, including arabinoxylans of BSG, because of the treatment. The decline in WHC due to the USB single treatment strengthens the possibility of cellulose and hemicellulose solubilization, as has been mentioned in the previous section. Moreover, OHC describes the ability of a BSG matrix to bind and hold oil fraction. The results revealed that 5 min USB generated a higher level of OHC compared to that of 25 min USB. However, 15 min USB fluctuated the OHC value, depending on its combination with thermal treatments. The modification in WHC and OHC in the current study demonstrated the modification of the characteristics of the hydrophobicity of BSG due to the loosening of the dietary fiber structure, as has been reported previously [30], in addition to improvements in cellulose solubility [31].

3.6. Principal component analysis (PCA)

The impact of USB continued with autoclave and/or water bath on the observed parameters is presented in PCA (Fig. 2). The PCA shows that the treatments divided the parameters into 4 groups of how the treatments affected the observed parameters. Total polyphenolic and flavan-3-ol were in the same area with ABTS, FRAP, and total fat content which might showed that the antioxidant capabilities of treated BSG (ABTS and FRAP) were highly affected by flavan-3-ols, total polyphenolic, and fat content. The levels of ABTS and FRAP seem to be aligned with the amount of flavan-3-ols and fat content. The highest antioxidant activity was identified in the samples which contained higher amounts of flavan-3-ols and extracted fat content. As has been discussed in Section 3.2, (+)-catechin and (−)-epicatechin are two compounds which are responsible for the amount of flavan-3-ols in BSG. Antioxidant properties demonstrate the availability of protons of certain compounds to neutralize reactive species [32]. It is widely accepted that ABTS possesses the ability to reduce molecular oxygen and hydrogen peroxide [20], while FRAP has the ability of the matrix to inhibit the reaction of lipid oxidation by reducing the catalyst action of the metal ion [10]. BSG is a complex material which consists of dietary fiber, polyphenolic compounds, protein, fat, and minerals. Fluctuations in ABTS and FRAP values in the current study demonstrated the release of polyphenolic compounds and fatty acids from the polysaccharides, due to the degradation as a result of bath ultrasonication combined with thermal exposures. Improvements in ABTS and FRAP seem to be the result of sono-chemical modification of polysaccharides, which destroy the cell wall and cleave ether linkages between lignin and hemicellulose, thus releasing fat and polyphenolic compounds [25,26]. Consequently, improvement in bioactivity such as FRAP and ABTS is expected in the current study.

SFA and phenolic acid were in the same area of the group, representing the conversion phenomenon of SFA into MUFA and PUFA as well as phenolic acids into other polyphenolic compounds in another group. Techno-functional properties such as OHC and WHC were in the same area with moisture level, describing the impact of moisture content value of BSG affecting the OHC and WHC of the treated BSG. In terms of its application in food processing, in addition to obtain a higher antioxidant property, it is important to maintain the polyphenolic composition. In terms of its techno-functionality particularly its behavior with food matrix (water and oil), it is important to maintain moisture content level.

4. Conclusion

The results revealed a synergistic effect due to the 2-steps treatment of USB continued with AH or CWH. The amount of flavan-3-ols fluctuated which was highly influenced by thermal treatments, including water bath and autoclave treatments. The 2-steps treatment intensified the amount of SFA and phenolic acids. Time differences in USB single treatment had no significant ($p > 0.05$) impact on ABTS and FRAP values, while two-steps treatment, particularly at temperatures $\geq$100 °C enhanced the antioxidant activity of methanol extracts of BSG. Further investigation into the identification of the treatments on specific compounds of polyphenolic compounds and polysaccharides composition is necessary.

Limitation of the study

The mechanism of action of the applied methods was unclear which however can be answered in further investigation.

Data availability

The data is available in the manuscript.

CRediT authorship contribution statement

Joncer Naibaho: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Łukasz Bobak:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Aneta Wojdyło:** Writing – review & editing, Methodology, Investigation,

Formal analysis, Data curation. **Małgorzata Korzeniowska:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yuyun Lu:** Writing – review & editing, Writing – original draft, Supervision. **Baoru Yang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition.

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.

Acknowledgements

This work was supported by UPWR 2.0, international and interdisciplinary program of development of Wrocław University of Environmental and Life Sciences, co-financed by the European Social Fund under the Operational Program Knowledge Education Development 2014–2020: Axis III Higher education for the economy and development; Action 3.5. Comprehensive program for schools of higher education (POWR.03.05.00–00-Z062/18).

It was also supported by ERA-NET CO-FUND Horizon 2020 - FACCE SURPLUS Sustainable and Resilient Agriculture for Food and Non-Food Systems and PROWASTE Protein-fibre biorefinery for scattered material streams (2019–2021).

References

[1] L.M. Rodriguez, J.L. Camina, V. Borroni, E.E. Pérez, Protein recovery from brewery solid wastes, Food Chem. 407 (2023) 134810, https://doi.org/10.1016/j.foodchem.2022.134810.
[2] M. De Paula, J.M. Latorres, V.G. Martins, Potential valorization opportunities for Brewer's spent grain, Eur. Food Res. Technol. 249 (2023) 2471–2483, https://doi.org/10.1007/s00217-023-04313-x.
[3] J. Conway, Beer Production Worldwide from 1998 to 2022, 2023. https://www.statista.com/statistics/270275/worldwide-beer-production/.
[4] J. Naibaho, M. Korzeniowska, A. Wojdyło, H. Muchdatul Ayunda, M. Foste, B. Yang, Techno-functional properties of protein from protease-treated brewers' spent grain (BSG) and investigation of antioxidant activity of extracted proteins and BSG residues, J. Cereal. Sci. 107 (2022) 103524, https://doi.org/10.1016/j.jcs.2022.103524.
[5] J. Naibaho, M. Korzeniowska, A. Wojdyło, A. Figiel, B. Yang, O. Laaksonen, M. Foste, R. Vilu, E. Viiard, Fiber modification of brewers' spent grain by autoclave treatment to improve its properties as a functional food ingredient, LWT 149 (2021) 111877, https://doi.org/10.1016/j.lwt.2021.111877.
[6] J. Naibaho, A. Wojdyło, M. Korzeniowska, O. Laaksonen, M. Föste, M.-L. Kütt, B. Yang, Antioxidant activities and polyphenolic identification by UPLC-MS/MS of autoclaved brewers' spent grain, LWT 163 (2022) 113612, https://doi.org/10.1016/j.lwt.2022.113612.
[7] E. Zago, C. Tillier, G. De Leener, R. Nandasiri, C. Delporte, K.V. Bernaerts, A. Shavandi, Sustainable production of low molecular weight phenolic compounds from Belgian Brewers' spent grain, Bioresour. Technol. Rep. 17 (2022) 100964, https://doi.org/10.1016/j.biteb.2022.100964.
[8] V.C. Roy, J.-S. Park, A.R. Haque, M.S. Ali, H.-J. Lee, B.-S. Chun, Bio-refinery of brewery spent grain utilizing natural deep eutectic solvent-induced subcritical water, J. Supercrit. Fluids 204 (2024) 106108, https://doi.org/10.1016/j.supflu.2023.106108.
[9] K.W.A. Al-Shwafy, M. Chadni, M.H. Hariz Abg Zamari, I. Ioannou, Enzymatic extraction of ferulic acid from brewer's spent grain: effect of physical pretreatments and optimization using design of experiments, Biocatal. Agric. Biotechnol. 51 (2023) 102779, https://doi.org/10.1016/j.bcab.2023.102779.
[10] MdJ. Rahman, L.N. Malunga, M. Eskin, P. Eck, S.J. Thandapilly, U. Thiyam-Hollander, Valorization of heat-treated brewers' spent grain through the identification of bioactive phenolics by UPLC-PDA and evaluation of their antioxidant activities, Front. Nutr. 8 (2021) 634519, https://doi.org/10.3389/fnut.2021.634519.
[11] S. Budaraju, K. Mallikarjunan, G. Annor, T. Schoenfuss, R. Raun, Effect of pre-treatments on the antioxidant potential of phenolic extracts from barley malt rootlets, Food Chem. 266 (2018) 31–37, https://doi.org/10.1016/j.foodchem.2018.05.110.
[12] A. Connolly, M. Cermeño, A.M. Alashi, R.E. Aluko, R.J. FitzGerald, Generation of phenolic-rich extracts from brewers' spent grain and characterisation of their in vitro and in vivo activities, Innovat. Food Sci. Emerg. Technol. 68 (2021) 102617, https://doi.org/10.1016/j.ifset.2021.102617.
[13] A. Taha, T. Mehany, R. Pandiselvam, S. Anusha Siddiqui, N.A. Mir, M.A. Malik, O.J. Sujayasree, K.C. Alamuru, A.C. Khanashyam, F. Casanova, X. Xu, S. Pan, H. Hu, Sonoprocessing: mechanisms and recent applications of power ultrasound in food, Crit. Rev. Food Sci. Nutr. (2023) 1–39, https://doi.org/10.1080/10408398.2022.2161464.
[14] J. Naibaho, A. Pudło, Ł. Bobak, A. Wojdyło, Á.A. López, L.M.W. Pangestika, S.N. Andayani, M. Korzeniowska, B. Yang, Conventional water bath heating on undried brewer's spent grain: functionality, fatty acids, volatiles, polyphenolic and antioxidant properties, Food Biosci. 53 (2023) 102523, https://doi.org/10.1016/j.fbio.2023.102523.
[15] J. Naibaho, A. Pudło, M. Korzeniowska, Y. Lu, B. Yang, Alteration of volatile compounds profile of brewers' spent grain by bath-ultrasonication and its combination with conventional water-bath and autoclave treatment, Ultrason. Sonochem. 90 (2022) 106192, https://doi.org/10.1016/j.ultsonch.2022.106192.
[16] O.O. Awolu, R.O. Osemeke, B.O.T. Ifesan, Antioxidant, functional and rheological properties of optimized composite flour, consisting wheat and amaranth seed, brewers' spent grain and apple pomace, J. Food Sci. Technol. 53 (2016) 1151–1163, https://doi.org/10.1007/s13197-015-2121-8.
[17] A.C. Fărcaş, S.A. Socaci, F.V. Dulf, M. Tofană, E. Mudura, Z. Diaconeasa, Volatile profile, fatty acids composition and total phenolics content of brewers' spent grain by-product with potential use in the development of new functional foods, J. Cereal. Sci. 64 (2015) 34–42, https://doi.org/10.1016/j.jcs.2015.04.003.
[18] D. Nowacki, H. Martynowicz, A. Skoczyńska, A. Wojakowska, B. Turczyn, Ł. Bobak, T. Trziszka, A. Szuba, Lecithin derived from ω-3 PUFA fortified eggs decreases blood pressure in spontaneously hypertensive rats, Sci. Rep. 7 (2017) 12373, https://doi.org/10.1038/s41598-017-12019-w.
[19] I.P. Turkiewicz, A. Wojdyło, K. Tkacz, P. Nowicka, T. Golis, P. Bąbelewski, ABTS on-line antioxidant, α-amylase, α-glucosidase, pancreatic lipase, acetyl- and butyrylcholinesterase inhibition activity of chaenomeles fruits determined by polyphenols and other chemical compounds, Antioxidants 9 (2020) 60, https://doi.org/10.3390/antiox9010060.
[20] I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76, https://doi.org/10.1006/abio.1996.0292.
[21] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.
[22] A. Patel, N. Arora, V. Pruthi, P.A. Pruthi, A novel rapid ultrasonication-microwave treatment for total lipid extraction from wet oleaginous yeast biomass for sustainable biodiesel production, Ultrason. Sonochem. 51 (2019) 504–516, https://doi.org/10.1016/j.ultsonch.2018.05.002.
[23] K. Kemppainen, K. Rommi, U. Holopainen, K. Kruus, Steam explosion of Brewer's spent grain improves enzymatic digestibility of carbohydrates and affects solubility and stability of proteins, Appl. Biochem. Biotechnol. 180 (2016) 94–108, https://doi.org/10.1007/s12010-016-2085-9.
[24] I. Parekh, A. Khanvilkar, A. Naik, Barley-wheat brewers' spent grain: a potential source of antioxidant rich lipids, J. Food Process. Preserv. 41 (2017) e13244, https://doi.org/10.1111/jfpp.13244.

[25] M. Buvaneshwaran, M. Radhakrishnan, V. Natarajan, Influence of ultrasound-assisted extraction techniques on the valorization of agro-based industrial organic waste – a review, J. Food Process. Eng. (2022), https://doi.org/10.1111/jfpe.14012.
[26] J. Chandrapala, C.M. Oliver, S. Kentish, M. Ashokkumar, Use of power ultrasound to improve extraction and modify phase transitions in food processing, Food Rev. Int. 29 (2013) 67–91, https://doi.org/10.1080/87559129.2012.692140.
[27] M. Sharma, K.K. Dash, Microwave and ultrasound assisted extraction of phytocompounds from black jamun pulp: kinetic and thermodynamics characteristics, Innovat. Food Sci. Emerg. Technol. 75 (2022) 102913, https://doi.org/10.1016/j.ifset.2021.102913.
[28] H.K. Sibhatu, S. Anuradha Jabasingh, A. Yimam, S. Ahmed, Ferulic acid production from brewery spent grains, an agro-industrial waste, LWT 135 (2021) 110009, https://doi.org/10.1016/j.lwt.2020.110009.
[29] E. Mallen, V. Najdanovic-Visak, Brewers' spent grains: drying kinetics and biodiesel production, Bioresour. Technol. Rep. 1 (2018) 16–23, https://doi.org/10.1016/j.biteb.2018.01.005.
[30] L. Yan, T. Li, C. Liu, L. Zheng, Effects of high hydrostatic pressure and superfine grinding treatment on physicochemical/functional properties of pear pomace and chemical composition of its soluble dietary fibre, LWT 107 (2019) 171–177, https://doi.org/10.1016/j.lwt.2019.03.019.
[31] R. Ravindran, S. Jaiswal, N. Abu-Ghannam, A.K. Jaiswal, Evaluation of ultrasound assisted potassium permanganate pre-treatment of spent coffee waste, Bioresour. Technol. 224 (2017) 680–687, https://doi.org/10.1016/j.biortech.2016.11.034.
[32] R. Abeynayake, S. Zhang, W. Yang, L. Chen, Development of antioxidant peptides from brewers' spent grain proteins, LWT 158 (2022) 113162, https://doi.org/10.1016/j.lwt.2022.113162.