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Antioxidant activities and polyphenolic identification by UPLC-MS/MS of autoclaved brewers' spent grain

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ABSTRACT

Autoclave treatment (AT) modified the dietary fiber composition of brewers' spent grain (BSG), impacting its techno-processing properties. However, its impact on antioxidant properties and stability of polyphenolic compounds remain unclear. This study aimed to evaluate the influence of AT on several antioxidant activities and polyphenolic composition. The results showed that AT increased ORAC (oxygen radical absorbance capacity), ABTS (2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid), and FRAP (ferric-reducing antioxidant power) value upto 7 folds compared to the untreated BSG. The lower temperature degraded the amount of flavan-3-ols and phenolic acids. However, AT at 110 °C and 130 °C upgraded the phenolic acids upto 4 and 11 folds respectively. Higher temperature also induced the formation of benzoic acid and (+)-catechin. UPLC-MS/MS identified several phenolic acids including syringic acid, benzoic acid, coumaric acid, ferulic acid and its derivatives, and flavan-3-ols such as (+)-catechin and (−)-epicatechin. In conclusion, AT improved the bioactivity of BSG, enhanced the amount of certains phenolic compounds and released the bioactive compounds from BSG matrices thus offering a higher benefit for industry to utilise autoclaved BSG.

1. Introduction

Potential of brewers' spent grain (BSG) as food and nutraceutical ingredients has been reported due to its biological properties (Bonifácio-Lopes, Teixeira, & Pintado, 2020; Connolly, Cermeño, Alashi, Aluko, & FitzGerald, 2021; Lynch, Steffen, & Arendt, 2016; Naibaho & Korzeniowska, 2021; Patrignani, Brantsen, Awika, & Conforti, 2021). BSG possesses several biological activities such as antioxidant activities, antimicrobial properties, DNA protective and antimutagenic, anti-inflammatory as well as maintaining colon health. Those capabilities are a result of a high amount of certain bioactive compounds such as polyphenolic compounds, protein and amino acids, lipid and fatty acids and dietary fibre (Naibaho & Korzeniowska, 2021; Nigam, 2017). Therefore, the improvement of chemical-related nutritional value and certain biological properties in food products such as bread, pasta, cookies, extruded products and yoghourt, has also been investigated (Heredia-Sandoval et al., 2020; Naibaho et al., 2022; Nocente, Taddei, Galassi, & Gazza, 2019; Torbica, Škrobot, JanićHajnal, Belović, & Zhang, 2019).

The addition of BSG in food products was observed to intensify the hardness of baked food products thus potentially to regulate semi-solid food products such as yoghourt (Naibaho et al., 2022; Naibaho & Korzeniowska, 2021). This phenomenon is due to the high amount of insoluble dietary fibre (IDF) which has a high ability to absorb high amounts of water (Steiner, Procopio, & Becker, 2015) thus disrupting the network formation in baked food products. However, autoclave treatment (AT) is able to modify the amount of IDF and convert to soluble dietary fibre (SDF) (Naibaho et al., 2021b). Consequently, it

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modified the physical properties of BSG such as water holding capacity and oil holding capacity (Naibaho et al., 2021b). AT degraded the hemicellulose and cellulose thus improving the amount of SDF (Li et al., 2019).

Several treatments have been reported for enhancing the yield of phenolic-rich extracts from BSG and its biological activities including pulsed electric field (Martín-García et al., 2020), solid state fermentation (Cooray & Chen, 2018; Tan, Mok, Lee, Kim, & Chen, 2019), enzyme treatments (Connolly et al., 2019, 2021), pH elevation (Connolly et al., 2021), and the combination of thermal and enzyme treatment (Budaraju, Mallikarjunan, Annor, Schoenfuss, & Raun, 2018). As mentioned earlier, AT transformed IDF in to SDF and modified the physical properties of treated BSG. Moreover, AT reduced the water activity (Aw) thus allowing a shelf life extension and safety during the storage. Surface chemistry study by Fourier transform infrared spectroscopy (FTIR) showed that AT changed the hydroxyl and acids functional groups of BSG (Naibaho et al., 2021b).

However, the influence of AT in biological activity of BSG such as antioxidant activities and polyphenolic composition remain unclear. Due to the decomposition and degradation of dietary fiber, certain polyphenolic compounds could be released from the cell wall and matrices of BSG thus improving the bioavailability. Major phenolic compounds in BSG are proto-catechuic, caffeic, *p*-coumaric and ferulic acids, catechin, and derivatives (Barbosa-Pereira et al., 2014; McCarthy et al., 2013; Moreira et al., 2013; Sibhatu, Anuradha, Yimam, & Ahmed, 2021). Polyphenolic compounds of BSG are responsible for several antioxidant activities including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) as well as non-enzymatic antioxidant activities such as total phenolic content (TPC), di(phenyl)-(2; 4;6-trinitrophenyl)iminoazanium (DPPH), ferric-reducing antioxidant power (FRAP), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), lipid peroxidation, and deoxyribose scavenging activity (Barbosa-Pereira et al., 2014; Crowley et al., 2017; Kumari et al., 2019; McCarthy et al., 2014; Moreira et al., 2013; Socaci et al., 2018; Verni et al., 2020). However, the composition and the amount of BSG polyphenolic extracts as well as its biological activities vary depending on several factors including source of origin, post handling process, extraction process and solvent used (Barbosa-Pereira et al., 2014; Bonifácio-Lopes et al., 2020; Meneses, Martins, Teixeira, & Musatto, 2013).

This study aimed to evaluate the impact of AT on BSG in terms of polyphenolic composition and its bioactivity of BSG. It is expected that several treatments might increase the antioxidant activities and improve the yield of polyphenolic compounds. Consequently, the improvement of biological properties of BSG will enhance the quality of BSG both as a food and nutraceutical ingredient. Thus, it will benefit the industry in providing a higher quality of BSG and its derivatives products.

2. Materials and methods

2.1. Materials and chemicals

BSG samples were collected from local breweries in Wroclaw, Poland. The samples were then handled as described in the previous study (Naibaho et al., 2021b). The BSG was dried using conventional drying methods at 75 °C to reach a stable moisture (approximately 2–5%). After that, the dried samples were ground by a laboratory scale mill and passed through a 385 μm laboratory scale sieve. The samples were kept in aluminium foil bags and stored at 4 °C before the treatment.

Trolox (6-hydro-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma-Aldrich (Steinheim, Germany). UPLC-grade water was prepared by using the HLP SMART 1000s system (Hydrolab, Gdansk, Poland). Before use, the water was filtered by a 0.22 μm membrane filter immediately. All chemicals used were analytical grade.

2.2. Experimental designs

AT was performed in an autoclave (ASL 100M, Poland) as described in the previous study (Naibaho et al., 2021b). A 75 g of distilled water was added into 25 g of BSG and mixed properly to obtain a homogenous mixture. The mixture was then packed into a polyethylene (PE) bag and vacuum sealed for the treatment. The treatment was carried out at 4 different temperatures (90 °C, 100 °C, 110 °C and 130 °C). The higher temperature (110 °C and 130 °C) was conducted with a set of 1 × 60 kPa/10 kPa pressure. All groups of treatment were conducted at different time exposure at 9, 12 and 15 min. Therefore, 12 samples were obtained in each group. After the treatment, all the samples were dried for 5 h using oven drying at 75 °C. After that, the dried samples were ground and passed through a 385 μm sieve and kept in aluminium foil bags at 4 °C before the preparation of methanol extracts. All analyses were performed in at least duplicate. BSG pale ale type from 2 different breweries was used as described in a previous study (Naibaho et al., 2021a). Therefore, the results from untreated BSG which has been reported in a previous study (Naibaho et al., 2021a) was provided as a comparison for those 2 types. BSG control was prepared by drying, milling and sieving as mentioned previously (Section 2.1). The comparison between those 2 types was provided based on the statistical analysis and the folds changes due to the treatment. The degree of changes in biological activities and phenolic compounds due to the autoclave treatment is performed in time folds.

2.3. Methanol extraction procedure

Methanol extracts from BSG were prepared for determination of antioxidant activities and identification of polyphenolic compounds. The extract was prepared following methods as described in previous studies (Turkiewicz et al., 2020). Briefly, 6 mL of methanol solution 80% (methanol/water: 80/20, *v/v*) and 7 mL of methanol 30% (methanol/water/acetic acid/ascorbic acid: 30/68/1/1, *v/v/v/m*) respectively for measurement of antioxidant activity and polyphenolic identification respectively, was added into 1 g of treated BSG and then mixed properly by using vortex for 1 min. After that, the mixture was then sonicated (Sonic 6D, Polsonic, Warsaw, Poland) for 20 min and the mixture was left 4 °C for 24 h. The mixture was then sonicated for 20 min followed by centrifugation at 19000*g* for 10 min at 4 °C. To obtain the methanol extract, the mixture was filtered using a 0.20 μm hydrophilic PTFE membrane (Millex Simplicity Filter, Merck, Germany).

2.4. Determination of antioxidants

Antioxidant activity of the extracts was assessed for ABTS, FRAP, and ORAC (Benzie & Strain, 1996; Ou, Chang, Huang, & Prior, 2013; Re et al., 1999) with slight modification with slight modification. The result was expressed as mmol Trolox equivalents/100 g dry sample.

2.4.1. ORAC

ORAC assessment was performed using fluorescence microplate method. 25 μL of samples, control, standard, and diluted buffer was added into wells containing 150 μL of fluorescein solution. The plate then was incubated for 30 min at 37 °C. After that, 25 μL of AAPH (2,2′-azobis(2-amidino-propane) dihydrochloride) solution was added to each well. As the timing is critical, the plate then immediately transferred to the plate reader and the fluorescence was measured every minute for 35 min. The calculation of standards and samples was done based on the area under the curve. The standard curve was obtained by plotting Trolox concentrations against the average of the area of two measurements for each concentration. The ORAC value was obtained using a regression equation from the standard curve.

2.4.2. ABTS

ABTS value was measured as follows: 3 mL of ABTS solution was

added into a cuvette containing 30 μL of methanol extract. Exactly after 6 min, the absorbance was measured at 734 nm wavelength spectrophotometer. The blank measurement was prepared with 30 μL of distilled water. A curve standard was prepared and measured at 734 nm wavelength spectrophotometer with absorbance range 0.700 ± 0.02.

2.4.3. FRAP

FRAP value was measured as follows. A mixture of reagent consists of acetate buffer pH 3.6, TPTZ (2,3,5-Triphenyltetrazolium chloride) dissolved in 40 mM/L HCl, and FeCl·6H20 dissolved in distilled water (10:1:1) is 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 mixture. 3 mL of the reagent was added into the mixture and the absorbance was measured exactly after 10 min at 593 nm wavelength with a range of 0.200–0.800 absorbance. The result then calculated as curve standard absorbance and the result was performed in Trolox equivalent.

2.5. Identification of polyphenolic compounds

Identification and quantification of flavan-3-ols and phenolic acids was conducted by Liquid Chromatography - Tandem mass Spectrometry (LC-MS-MS) following procedures as described in the previous studies (Tkacz, Wojdyło, Turkiewicz, & Nowicka, 2021; Turkiewicz et al., 2020, 2021). The profile and content were determined by ultra-performance liquid chromatography (Acquity UPLC system) with binary solvent manager and photodiode array detector PDA (Waters Corp., Milford, MA. US). The system was coupled to a Xevo™ G2 Q/TOF micro-mass spectrometer fitted with an electrospray ionisation ESI source (Waters Corp., Manchester, UK) which acts on negative modes. The analysis was carried out using full scan data dependent MS, scanning from *m/z* 100 to 1700. The characterisation of phenolic compositions was done according to the retention time and accurate molecular masses. The data were collected by a software, MassLynx™ 4.1 ChromaLynx Application Manager (Waters Corp. Milford, USA). Flavan-3-ols and phenolic acids were monitored at 280 nm and 320 wavelengths, respectively. Quantification was conducted based on the phenolic calibration standards at concentrations ranging between 0.05 and 5 mg/mL (R2 ≥ 0.9995). All the samples were analysed in triplicate and the results were performed in mg/kg dry weight sample.

2.6. Statistical analysis

The statistical analysis was carried out using Statistica software (version 13.5.0.17 by two-ways analysis of variance (ANOVA) for temperature and time exposure. Significant differences were evaluated at $p < 0.05$ by post-hoc Tukey HSD test assessment.

3. Results and discussion

3.1. Antioxidant activities

Antioxidant activities *in vitro* of BSG are shown in Table 1. In general, AT improved the antioxidant properties of treated BSG compared to that in control treatment. Furthermore, different temperatures and time exposure on AT significantly ($p < 0.05$) influenced the ORAC and ABTS value. However, different levels of temperature and time exposure had no impact on FRAP value ($p > 0.05$). ORAC value varied between 4.74 and 6.32 mmol Trolox/100 g dry weight; while ABTS and FRAP value ranged between 0.15 - 0.84 and 0.39–0.51 mmol Trolox/100 g dry weight respectively. Compared to the untreated BSG (Naibaho et al., 2021a), AT increased the antioxidant activities for ORAC, ABTS, and FRAP, and the level of improvement is shown in Fig. 1. As is shown in Fig. 1, higher temperature (110 °C and 130 °C) had the highest impact in the improvement of ORAC and ABTS value (Fig. 1a and b). Meanwhile, the highest enhancement impact on FRAP was given by the lower temperature treatments (90 °C and 100 °C). Thermal exposure on BSG by AT at 90 °C–130 °C increased the ORAC value more than 60% higher than that in control. AT was able to enhance the ABTS level remarkably up to 6–8 folds (>800%) than control, which was obtained at 130 °C. Although the combinations of temperature and time exposure statistically generated the same level of FRAP, treated BSG achieved FRAP value between 2 and 4 times higher (>250% higher) than that in untreated BSG. It was identified that untreated BSG had FRAP value at 0.1–0.2 mmol Trolox/100 g (Naibaho et al., 2021a) while current study revealed that AT-treated BSG generated FRAP level at a range of 0.39–0.51 mmol Trolox/100 g. FRAP value is reported to be related with the property of BSG in DNA protection effect (McCarthy et al., 2012).

Fig. 1 describes the level of improvement on antioxidant activities of BSG due to the AT on different temperature and time exposure. The figure shows that AT lower temperature (90 °C and 100 °C) increased FRAP higher than ABTS and ORAC value. FRAP demonstrated the ability of BSG in reducing the metal ion as a catalyst in lipid oxidation (Rahman et al., 2021). This result shows that AT at 90 °C and 100 °C improved the ability of phenolic compounds to diminish lipid oxidation. Higher temperature (110 °C and 130 °C) enhanced ABTS the most followed by FRAP and ORAC value. By this, AT improved the ability of BSG methanol extracts in electron donor for the reduction of molecular oxygen and

Table 1
Antioxidant activities *in vitro* of BSG treated with different temperature and time exposure by autoclave treatment.

Treatment	Antioxidant properties (mmol Trolox/100 g)			Polyphenolic compounds (mg/kg)		
	ORAC	ABTS	FRAP	Flavan-3-ols	Phenolic acids	Total
Group I						
Control*	2.615	0.086	0.106	824.95	100.55	925.50
90 °C; 9 min	4.738 ± 0.04^{h}	0.278 ± 0.03^{d}	0.450 ± 0.10^{a}	22.493 ± 0.01^{i}	30.021 ± 0.07^{l}	52.514
90 °C; 12 min	4.404 ± 0.09^{k}	0.303 ± 0.03^{d}	0.397 ± 0.07^{a}	22.980 ± 0.00^{h}	32.584 ± 0.00^{j}	55.563
90 °C; 15 min	4.898 ± 0.05^{g}	0.148 ± 0.03^{e}	0.390 ± 0.12^{a}	21.292 ± 0.13^{l}	30.512 ± 0.22^{k}	51.804
100 °C; 9 min	4.554 ± 0.16^{j}	0.250 ± 0.01^{de}	0.398 ± 0.07^{a}	21.774 ± 0.20^{k}	35.590 ± 0.14^{i}	57.363
100 °C; 12 min	4.572 ± 0.03^{i}	0.315 ± 0.01^{d}	0.444 ± 0.08^{a}	22.107 ± 0.01^{j}	38.478 ± 0.01^{g}	60.585
100 °C; 15 min	5.209 ± 0.05^{e}	0.283 ± 0.07^{d}	0.403 ± 0.03^{a}	24.435 ± 0.00^{g}	37.102 ± 0.01^{h}	61.537
Group II						
Control*	1.751	0.105	0.204	824.58	104.13	928.71
110 °C; 9 min	5.946 ± 0.29^{c}	0.503 ± 0.05^{c}	0.486 ± 0.04^{a}	38.230 ± 0.22^{f}	344.542 ± 0.25^{f}	382.772
110 °C; 12 min	5.956 ± 0.16^{b}	0.499 ± 0.02^{c}	0.486 ± 0.04^{a}	40.007 ± 0.09^{e}	374.444 ± 0.25^{e}	414.451
110 °C; 15 min	5.044 ± 0.15^{f}	0.511 ± 0.04^{c}	0.490 ± 0.03^{a}	41.929 ± 0.11^{d}	425.437 ± 0.19^{d}	467.366
130 °C; 9 min	5.764 ± 0.06^{d}	0.842 ± 0.03^{a}	0.509 ± 0.03^{a}	49.604 ± 0.15^{a}	1077.428 ± 0.33^{b}	1127.032
130 °C; 12 min	5.761 ± 0.12^{d}	0.683 ± 0.02^{b}	0.473 ± 0.00^{a}	46.339 ± 0.05^{c}	1057.985 ± 0.25^{c}	1104.324
130 °C; 15 min	6.321 ± 0.24^{a}	0.796 ± 0.05^{a}	0.508 ± 0.03^{a}	47.749 ± 0.01^{b}	1168.718 ± 0.39^{a}	1216.467

Note: the data is shown as mean ± standard deviation with triplicate analysis. *: the result is obtained from previous study (Naibaho et al., 2021b). except ORAC value was conducted in current study. Letters show the significant differences from other treatment in the same column ($p < 0.05$). Analysis was done in triplicate.

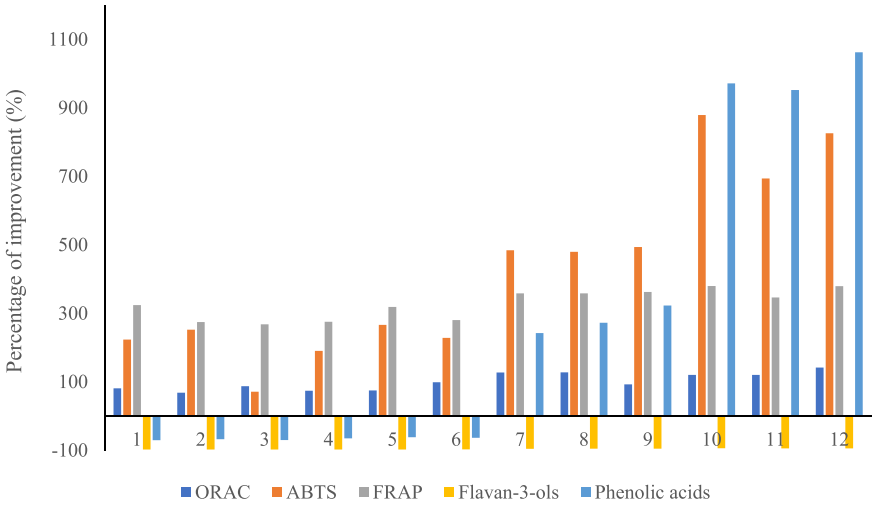

Fig. 1. The amount of improvement on biological activities and phenolic compounds of BSG due to the autoclave treatment at 90 °C (1: 9 min, 2: 12, 3: 15 min), 100 °C (4: 9 min, 5: 12, 6: 15 min), 110 °C (7: 9 min, 8: 12, 9: 15 min), and 130 °C (10: 9 min, 11: 12, 12: 15 min). The numbers were obtained based on the comparison of treated BSG (means) with untreated BSG from Table 1.

hydrogen peroxide. AT had the lowest enhancement on ORAC value regardless of the level of temperature and time exposure.

These results explain that AT strongly enhanced the biological activities of BSG. AT has been observed to enhance the amount of crude fat on BSG (Naibaho et al., 2021b) which might occur due to the release of the entrapped fat from BSG matrices. By this, the amount of extractable fat became higher. BSG consists of fatty acids such as palmitic, linoleic, oleic, and stearic acid which contribute to antioxidant properties in BSG (Fărcaş et al., 2015; Parekh, Khanvilkar, & Naik, 2017; Tan et al., 2019). The higher bioavailability of fat in BSG consequently improved its antioxidant properties. Several phenomena could occur due to the AT such as depolymerisation, debranching, and de-esterification of polysaccharides thus releasing the phenolic compounds which are responsible for biological activity. This phenomenon might be due to the transformation of insoluble dietary fibre into soluble dietary fibre (Naibaho et al., 2021b). Furthermore, FTIR analysis identified the microstructure changes in the acid functional group (Naibaho et al., 2021b) which might be the consequence of a higher presence of fatty acids or phenolic acids.

The improvement of biological properties of BSG has been observed due to the several treatments. Microwaves had 1.5 times higher %DPPH and %ABTS inhibition, and 3 times higher DPPH trolox equivalents than those in control (Budaraju et al., 2018). microwaves increased the antioxidant activity of BSG by inducing the formation of melanoidin which was formed due to the Maillard reaction (Patrignani et al., 2021). Fermentation improved the radical scavenging activity (Verni et al., 2020) and a 5.8 fold increase in total antioxidant activity has been observed (Tan et al., 2019). Aqueous pH-shift extraction increased the ORAC and TEAC (Trolox equivalent antioxidant capacity) value up to 4 and 3 times higher respectively; enzyme-aided generated 18 times higher FRAP value and had a higher *in vitro* ACE inhibitory activity (Connolly et al., 2021). Although it is reported that pH-shift improved the ORAC, TEAC, and FRAP more effectively than that in enzyme-aided treatment (Connolly et al., 2021).

However, some enzyme treatments could diminish the level of ABTS, FRAP, and ORAC (Connolly et al., 2019). Interestingly, pulsed electric fields on BSG had no impact on antioxidant activity and immunomodulatory effects (Kumari et al., 2019). Steaming (220 °C/2 min), roasting (60 °C/3 min), and autoclave (121 °C/20 min/15 psi) had no impact on the antioxidant activities and yield extracts (Budaraju et al., 2018). In current study, a fluctuation in antioxidant activities was observed. This phenomenon might depend on the stability of certain responsible compounds during the thermal exposure. Moreover, it might be also influenced by the capability of polyphenolic compounds in stabilising the specific free radical.

3.2. *Quantification and identification of flavan-3-ols and phenolic acid*

Quantitative analysis of phenolic compounds on treated BSG is presented in Table 1 and the level of modification in phenolic compounds due to the AT is performed in Fig. 1. The results showed that all the treatments significantly ($p < 0.05$) impacted the amount of flavan-3-ols and phenolic acids. Compared to the untreated BSG, AT at all temperature and time exposure ranges lowered the amount of flavan-3-ols up to −97% (Fig. 1). Interestingly, AT at 90 °C and 100 °C decreased the phenolic acid content up to −70% from the initial level, while temperature at 110 °C and 130 °C increased the amount of phenolic acids up to 1000% than that in control. The high reduction in flavan-3-ols shows that flavan-3-ols has a high instability in thermal exposure. The total amount of polyphenolic content depicts that only the highest temperature (130 °C) improved the amount of phenolic content. Although AT at 90 °C and 110 °C reduced the total amount of phenolic compounds compared to the control (untreated BSG), a significant improvement in antioxidant properties (ORAC, FRAP, and ABTS) was obtained as mentioned in the previous section. This phenomenon might be due to the depolymerisation of certain compounds such as caffeic acid into ferulic acid and led to its conversion into elementary units as reported previously (Wojdyło, Figiel, Lech, Nowicka, & Oszmiański, 2014). Moreover, a derivatization of ferulic acid into 4-vinylguaiacol due to a thermal exposure has been observed (Zago et al., 2022) and consequently declined the amounts of phenolic compounds while improving the antioxidant properties.

Higher temperatures (110 °C and 130 °C) multiplied the amount of phenolic acids due to its ability to disrupt the BSG matrices thus releasing the phenolic acids. It was observed that the high thermal exposure on BSG was able to disrupt the cell vacuoles and or cleaved the covalent bonds thus increasing the phenolic acids (Rahman et al., 2021). A reduction in the amount of phenolic acid by 4–6 times lower has been identified (Bonifácio-Lopes et al., 2020), brewing and roasting process lowered the hydroxycinnamic acid content (McCarthy et al., 2013). In addition, microwave treatment has been reported for increasing the antioxidant activity although it has no impact on the amount of bound

phenolic compounds (Budaraju et al., 2018). However, an improvement up to 2.7 folds in free phenolic acid and 1.7 folds in bound phenolic acid due to the pulsed electric field treatment has been reported (Martín-García et al., 2020) thus enhancing the amount of flavan-3-ols, phenolic acid derivatives, and flavonoids. Increase in free phenolic content has also been identified due to the thermal and enzyme treatment (Budaraju et al., 2018). The modification in phenolic compounds due to AT might be as an impact of dietary fibre transformation as reported previously (Naibaho et al., 2021b). Temperature exposure might induce the lignin solubility and polysaccharides degradation. Functional groups of lignin consist of guaiacyl and syringyl which similarly bind in all lignin. However, the amount of such compounds bound to the lignin varied depending on the extraction and isolation methods (Ohra-aho et al., 2016). By this, the variability in phenolic acids could occur due to the difference in strength binding between the matrices and phenolic compounds.

Tentative identification of phenolic compounds by LC-MS-MS is shown in Table 2. The result shows that the significant difference between flavan-3-ols and phenolic acid at 110 °C and 130 °C is due to the presence of benzoic acid and (+)-catechin which were not observed at 90 °C and 100 °C. As can be seen in Table 2, phenolic acid in BSG treated at 110 °C and 130 °C consists of syringic acid, benzoic acid, *p*-coumaric acid, ferulic-ferulic acid dimer, sinapic acid, ferulic acid, decarboxylated diferulic acid, and diferulic acid isomers; flavan-3-ols consists of (+)-catechin and (−)-epicatechin. The presence of benzoic acid and (+)-catechin was not identified at the treatment with a lower temperature (90 °C and 100 °C). According to a previous study, it was reported that hydroxybenzoic acid is the most dominant compound in BSG ohmic treated (Bonifácio-Lopes et al., 2022). By this, the low amount of phenolic acids in lower temperature treatment might be due to the absence of benzoic acid. The diminishing and inducing certain compounds have been observed in BSG due to the thermal treatment. Rahman et al. (2021) identified caffeic acid as absent in lower temperature treatment but it presented in higher temperature (>100 °C) and sinapic acid was identified at 160 °C oven heating (Rahman et al., 2021).

Furthermore, ferulic acid and its derivatives seems to be the most abundant compound which might contribute to the improvement in antioxidant properties. Ferulic acids are bound to insoluble structural cellulose or hemicellulose by ester linkages. The treatment might remove the ester-linked ferulic acid from the insoluble cellulose, insoluble hemicellulose, and lignin matrix as has been observed in previous study (Sibhatu, Anuradha Jabasingh, Yimam, & Ahmed, 2021). The temperature at 90 °C–100 °C does not efficiently rupture the cross-linking bond of ferulic acids and even caused a simplification in the structure, thus lowering the flavan-3-ols and phenolic acids. Higher temperatures allow the disruption of cross-linked bonds between insoluble ingredients and phenolic compounds thus creating the possibility to cleave the natural bonds and esterify ferulic acid. Therefore, the increase in yield extracts can be obtained (Sibhatu et al., 2021). Ferulic acid is responsible for DPPH capability due to the presence of phenolic nucleus and unsaturated side chain which form a resonance stabilised phenoxy radical (Connolly et al., 2021; Sibhatu et al., 2021). The role of hydroxycinnamic acid from BSG as an anti-inflammatory effect has been reported, due to its ability to reduce the stimulated cytokine production (McCarthy et al., 2014).

According to (Sibhatu et al., 2021), the major phenolic compounds in BSG are sinapic acid, p-coumaric acid and ferulic acid. Hydroxycinnamic acid is the most abundant phenolic acid from BSG including ferulic acid (FA), p-coumaric acid (p-CA) derivatives, FA derivatives, p-CA, caffeic acid (CA) and CA derivatives. CA regulated the antioxidant activity of phenolic extract from BSG (McCarthy et al., 2013). The presence of certain phenolic compounds in BSG depends on the solvent used (Bonifácio-Lopes et al., 2020). It is identified that extraction with 100% ethanol presented 4-hydroxybenzoic and syringic acids, while ethanol 60% identified the presence of catechin, vanillic acid, 4-hydroxybenzoic, vanillin and p-coumaric acid. Apparently, vanillin, p-coumaric acid, 4-hydroxybenzoic, catechin, ferulic acid and protocatechuic acid were observed in 80% ethanol extract. However, water extraction generated 4-hydroxybenzoic acid, p-coumaric and protocatechuic acid, vanillin, catechin, and vanillic acid. In the current study, methanol was used as a solvent during the extraction. Autoclave with different temperature and time exposure generated different levels of phenolic compounds, reduced certain compounds and induced the formation of certain compounds depending on the temperature used.

4. Conclusion

As hypothesised, AT improved the antioxidant properties in BSG including ORAC, ABTS and FRAP compared to that in untreated BSG. The study showed that the treatments at 90 °C and 100 °C enhanced the FRAP value the most while it reduced the amount of phenolic acid and flavan-3-ols. Meanwhile 110 °C and 130 °C gave the highest impact on ABTS level and multiplied the amount of phenolic acid. Tentative identification showed that AT induced depolymerisation which consequently induced certain compounds in a higher temperature such as benzoic acid and (+)-catechin. As a result, the declining of phenolic compounds at 90 °C and 100 °C was observed while at the same time it improved the antioxidant activities compared to the untreated BSG. Significant increase in antioxidant capacities and phenolic acid was observed at 110 °C and 130 °C treatments due to its ability in disrupting the BSG matrices and releasing the phenolic compounds. The study demonstrated a benefit effect in terms of the utilisation of BSG as food and or nutraceutical ingredients. Therefore, AT potentially provides the benefits for food industries with generating higher biological properties of BSG. Study on the quantitative and qualitative of observed phenolic compounds seems to be important in the near future in order to understand the impact of specific in certain biological properties.

Table 2
Identification of phenolic compounds by LC-MS-MS in treated BSG with different temperature.

Phenolic compounds	tentative identification	Retention time	MS	MS/MS	Temperature (°C)			
					90	100	110	130
phenolic acid	syringic acid	5182	197.08	153.07	✓	✓	✓	✓
	benzoic acid	5399	121.10	92.01	–	–	✓	✓
	p-coumaric acid	5576	163.06	119.04	✓	✓	✓	✓
	di-ferulic acid dimer	5731	387.10	149.07/134.01	✓	✓	✓	✓
	sinapic acid	6054	223.02	179.01	✓	✓	✓	✓
	ferulic acid	6248	193.02	134.01	✓	✓	✓	✓
	decarboxylated diferulic acid	6303	341.11	193.01/134.01	✓	✓	✓	✓
	di-ferulic acid isomers	7707	385.08	282.09/148.03	✓	✓	✓	✓
flavan-3-ols	(+)-catechin	3783	289.03	245.01	–	–	✓	✓
	(−)-epicatechin	5305	289.03	245.01	✓	✓	✓	✓

√ -present; - -absent.

CRediT authorship contribution statement

Joncer Naibaho: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, preparation, Writing – review & editing, Funding acquisition. **Aneta Wojdyło:** Methodology, Methodology, Validation, Formal analysis, Writing – review & editing. **Małgorzata Korzeniowska:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Oskar Laaksonen:** Writing – review & editing, Project administration, Funding acquisition. **Maike Föste:** Writing – review & editing, Project administration, Funding acquisition. **Mary-Liis Kütt:** Writing – review & editing, Project administration, Funding acquisition. **Baoru Yang:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

Authors declare no conflict of interest.

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