Journal of Cereal Science 107 (2022) 103524

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Techno-functional properties of protein from protease-treated brewers' spent grain (BSG) and investigation of antioxidant activity of extracted proteins and BSG residues

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ARTICLE INFO

Keywords:
Protein functionality
Antioxidant activities
Polyphenolic identification
Agro-industrial byproduct

ABSTRACT

The study aimed to investigate the biological properties of the protein fraction of brewers' spent grain (BSG) and its sediments as well as the techno-functional properties of BSG protein (BSGP). BSG was incubated with 0.5% protamex and a combination of protamex and flavourzyme, in addition to the control (incubation without protease). The results showed that enzymatic treatment enhanced the antioxidant activity of BSGP. Compared to the sediment fraction, BSGPs had higher antioxidant capacities than those in sediments. The current study demonstrated that the FRAP value is aligned with the amount of the polyphenolic compounds, while BSGP is responsible for ORAC and ABTS capabilities. Enzyme treatment on BSG enhanced the antioxidant properties of BSGPs and the amount of the phenolic compounds of the sediments. BSGPs treated with proteases possessed higher oil-holding capacity, foaming properties and lower emulsion capability. In conclusion, enzymatic treatment of BSG enhanced the protein functionality and bioactivity as well as intensified the antioxidant activity of its sediments allowing further valorization.

1. Introduction

Brewers' spent grain (BSG), a byproduct of the brewery industry, consists of 15–30% protein (Naibaho and Korzeniowska, 2021a; Wen et al., 2019). BSG protein is mainly dominated by hordein, glutelin, globulin and albumin (Wen et al., 2019), and its amino acids are mainly dominated by glutamine, proline and leucine (Connolly et al., 2013). The number of amino acids in BSG has been reported to be the same as that in barley (Cermeño et al., 2019). BSG protein is reported for its benefits to human health due to its biological properties including anti-inflammatory activity, antithrombotic and blood coagulation, angiotensin-converting enzyme activity, modulation of glycemic response, dipeptidyl peptidase IV inhibitory, and protective ability against oxidant such as DPPH (di(phenyl)- (2; 4;6-trinitrophenyl)iminoazanium), FRAP (ferric-reducing antioxidant power), ABTS (2, 2′-Azinobis-(3-ethylbenzthiazoline-6-Sulfonic Acid)) and ORAC (oxygen radical absorbance capacity) (Wen et al., 2019). Therefore, protein extraction from the BSG matrix is continuously examined and optimized (Wen et al., 2019).

One of the crucial factors in protein extraction from BSG is maintaining pH at approximately 8–9 (Vieira et al., 2016), thus improving the solubility of the protein. Consequently, a higher yield will be generated (Cian et al., 2018). Furthermore, incorporation of enzymes has been observed for its ability to drop the molecular weight of the protein, thus increasing the digestibility (Vieira et al., 2016; Wen et al., 2019). Several enzymes such as flavourzyme, corolase, alcalase and Promod 144 MG

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https://doi.org/10.1016/j.jcs.2022.103524
Received 1 April 2022; Received in revised form 1 July 2022; Accepted 7 July 2022
Available online 15 July 2022

with different concentrations were observed to enhance the yield of obtained protein at a broader pH range of 2.0–12.00 (Cermeño et al., 2019; Connolly et al., 2014, 2015). The studies revealed that different enzymes and their amounts influenced the biological properties of obtained proteins. Flavourzyme (1 and 2.5%) improved the biological properties of protein from BSG (McCarthy et al., 2013). A combination of flavourzyme with alkaline and neutral protease in BSG generated a protein with a higher biological activity (Cian et al., 2018). Flavourzyme promoted protein decomposition, thus enhancing more peptides and amino acids (Yang et al., 2020). Protamex lowered the degree of hydrolysis of protein obtained from Persian lime seeds while it had a higher functionality at a broader pH range (Fathollahy et al., 2021).

A combination of flavourzyme and protamex improved the quality of BSG as an animal feed and masked the bitter taste from protamex (San Martin et al., 2020). However, the study on its impact on the functionality of extracted protein has never been reported. The study aimed to compare and evaluate the impact of protamex, flavourzyme and combined protamex–flavourzyme on the functionality of BSG extracted protein. It was expected that different enzymes would generate protein with different biological properties, polyphenolic compositions and techno-functional properties such as water solubility, foaming and emulsion formation as well as stability. The results would be beneficial for industries and scientists in terms of the further utilization of protein extracts obtained from BSG.

2. Materials and methods

2.1. Materials

The BSG samples were collected from a light-type beer-producer brewery in Poland, dried with conventional drying methods at 70 °C–75 °C for approximately 3.5 h to reach a stable moisture (2–5%), ground to a 500 mm particle size, and kept at 10 °C prior to the extract preparation. 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 using a 0.22 μm membrane filter immediately. All chemicals used for analyses were analytical grade.

2.2. Experimental design

Firstly, BSG and water were mixed with a ratio of 1:10 and the mixture was separated into three group treatments: mixture without protease treatment as control (-C), treated with 0.5% protamex (-P) and treated with a combination of 0.5% protamex and 0.1% flavourzyme (-PF). The groups were treated at 50 °C for 3 h at pH 8.5, followed by heating at 90 °C in order to inactivate the enzymes. After that, the treated mixtures were cooled down to room temperature and centrifuged to separate the liquid fraction from the sediment. The sediment (BSGS) was dried using a conventional dryer at 75 °C to reach the moisture content <6%, sealed in an aluminum foil bag and stored at 10 °C for antioxidant analysis and polyphenolic identification. The liquid fraction, protein, (BSGP) was dried by a semi-pilot spray dryer (APV Anhydro A/S LAB S1 spray dryer, Denmark). The fraction was evaporated in hot air with an inlet temperature of 160–165 °C and outlet temperature of 82–85 °C. The instrument was operated with an air pressure nozzle at 2 bars and the velocity of the peristaltic pump at 2.5 L/h. The dried extract was packed into an aluminum foil bag, sealed and kept at a chilled temperature (10 °C) for further studies.

2.3. Antioxidant properties and polyphenolic identification of protein extracts and sediments

2.3.1. Methanol extraction

The extract was prepared following methods described in previous studies (Tkacz et al., 2021). The extracts were provided using methanol 80% in distilled water used for antioxidant analysis and methanol 30% (methanol/water/acetic acid/ascorbic acid: 30/68/1/1, *v/v/v/m*) for polyphenolic measurement. Antioxidant assessment and polyphenolic measurement were performed in methanol solution. Therefore, the extracts were prepared in a methanol solution. The solution was added into 1 g of sample, and it was mixed using a vortex for 1 min. The mixture was sonicated (Sonic 6D, Polsonic, Warsaw, Poland) for 20 min and left at 4 °C. After 24 h, it was again sonicated for 20 min followed by centrifugation at 19000×*g* at 4 °C for 10 min. The extract was separated using a hydrophilic PTFE membrane (0.20 μm) (Millex Simplicity Filter, Merck, Germany).

2.3.2. Assessment of antioxidants

Antioxidant activities were evaluated with 2,2′-Azinobis-(3-Ethylbenzthiazoline- 6-Sulfonic Acid) (ABTS), ferric reducing antioxidant potential (FRAP), and oxygen radical absorbance capacity (ORAC) (Benzie and Strain, 1996; Crowley et al., 2015; Ou et al., 2013; Re et al., 1999). The result was expressed as mm trolox equivalents/100 g dry sample.

2.3.3. Identification of polyphenolic compounds

Flavan-3-ols and phenolic acids were identified and quantified following procedures as described previously (Tkacz et al., 2021). The measurement was performed by ultra-performance liquid chromatography (Acquity UPLC system) with a binary solvent manager and a photodiode array detector PDA (Waters Corp., Milford, MA. US), coupled to a XevoTM G2 Q/TOF micro-mass spectrometer and fitted with an electrospray ionization ESI source (Waters Corp., Manchester, UK). A full-scan, data-dependent MS was used, scanning from *m/z* 100 to 1700. Phenolic composition was characterized according to the retention time and accurate molecular masses. Flavan-3-ols and phenolic acids were monitored at 280 nm and 320 wavelengths, respectively. The data were collected using the MassLynxTM 4.1 ChromaLynx Application Manager (Waters Corp. Milford, USA) software. Quantification was conducted based on the phenolic calibration standards at concentrations ranging from 0.05 to 5 mg/mL (R2 $\geq$ 0.9995). All the samples were analyzed in triplicate and the data were presented in g/kg of dry weight sample.

2.4. Techno-functional properties of protein extracts

2.4.1. Water solubility index (WSI)

WSI was assessed following the procedure as described previously (Jafari et al., 2017; Rashid et al., 2015) with a slight modification. A total of 2.5 g of the sample was suspended in 25 mL of distilled water at room temperature. The suspension was vortexed for 1 min and left for 30 min at room temperature (25 °C). The mixture was centrifuged at 4100×*g* for 15 min at 4 °C. The dissolved powder in the liquid fraction was dried at 105 °C to reach a constant weight. The dried supernatant was determined as the value of WSI.

2.4.2. Oil holding capacity (OHC)

OHC was determined by mixing 2.5 g of the sample with 6 mL of vegetable oil. The mixture was left at room temperature for 30 min followed by centrifugation at 3000×*g* for 15 min. The remaining oil was measured after removing the supernatant and described as OHC (Kteniouda et al., 2013).

2.4.3. Emulsifying activity (EAI) and stability index (ESI)

EAI and Esi were determined as described previously (Fathollahy et al., 2021) with a slight modification. The sample was suspended in distilled water (1 g/100 mL), mixed with 10 mL of vegetable oil and homogenized with a homogenizer at 8900×*g* for 1 min. An amount of 5 mL of 0.1% sodium dodecyl sulfate solution was added into 50 μL of the homogenized sample at 0 and 10 min after homogenization. The

absorbance was directly measured at a 500 nm wavelength. EAI and ESI were determined as Equations (1) and (2):

$$EAI = \frac{2\ x\ 2.303\ x\ A0}{0.25\ x\ sample\ (g)} \quad (1)$$

$$ESI = A0\ x\ [\frac{\triangle t}{A0 - A10}] \quad (2)$$

A0: absorbance at 0 min, A10: absorbance at 10 min, $\triangle t$: 10 min.

2.4.4. Foaming capacity (FC) and stability (FS)

An amount of 5 g/L sample in distilled water was prepared and homogenized for 10 min at 20000 rpm using a homogenizer. The change in volume before foaming, during foaming and after 30 min left at room temperature were recorded (Fathollahy et al., 2021). FC and FS were determined as Equations (3) and (4):

$$FC = \frac{B - A}{A}\ x\ 100\% \quad (3)$$

$$FS = \frac{C - A}{A}\ x\ 100\% \quad (4)$$

A: volume before whipping, B: volume just after whipping, C: volume after 30 min whipping.

2.5. Statistical analysis

Antioxidant activities and polyphenolic composition were assessed statistically by two-ways ANOVA (Analysis of Variance) while the techno-functional properties were determined by one-way ANOVA using Statistica software (version 13.5.0.17). The significant difference was determined at $p < 0.05$ followed by Tukey post hoc test.

3. Results and discussion

3.1. Antioxidant activities of protein and sediments

Antioxidant activities of BSGPs and the sediments were evaluated on ORAC, ABTS and FRAP and the results are presented in Table 1. The results revealed that BSGPs had a significantly ($p < 0.05$) higher capability in ABTS and FRAP in all observed treatments. However, only protamex treatment generated different ORAC levels, while control and protamex–flavourzyme treatment had similar levels of ORAC in protein extract and sediment. ORAC value on BSGPs ranged from 5.9 to 7.0 mmol Trolox/100 g, while its sediment had a range of 5.2–5.9 mmol Trolox/100 g. Moreover, BSGPs had ABTS capability at a range of 4.15–5.55 mmol Trolox/100 g, while its sediment was at a range of 0.32–0.63 mmol Trolox/100 g. From the perspective of FRAP value, BSGPs and sediments ranged between 0.44 and 0.53 mmol Trolox/100 g and 0.15–0.34 mmol Trolox/100 g, respectively. Within those three group treatments, BSGPs had a higher level of ORAC and ABTS. In sediments, the highest value of ORAC and ABTS was obtained in the BSGS-C and BSGS-PF, respectively. Different trends in FRAP value were identified. In protein, BSGP-C had the highest FRAP value, while its sediment (BSGS-C) had a lower FRAP capability. However, BSGP-PF had the lowest FRAP value and generated the highest FRAP value in its sediment.

The ability of protamex and protamex/flavourzyme in enhancing the ORAC and ABTS might be related to the protein content of the extracts. As mentioned above, those treatments generated a higher protein content of BSGPs. Flavourzyme and protease had been identified for their ability in reducing the molecular weight of peptides, thus generating higher amounts of low-molecular-weight amino acids (Yang et al., 2020). Therefore, they generated a higher ORAC and ABTS capability, as the current study showed. Previous study reported that untreated BSG contained ABTS and FRAP capability at a range of 0.09–0.24 mmol Trolox/100 g and 0.11–0.31 mmol Trolox/100 g, respectively (Naibaho et al., 2021). This capability is much lower than that in BSGPs and the sediments, as obtained in the current study. Additionally, it was observed that untreated dried BSG had only around 1.75–2.62 mmol Trolox/100 g ORAC value (Naibaho et al., 2022). Meanwhile, the current study revealed a higher capacity that was almost 3 times higher. The treatments on BSG, regardless of the enzyme's treatment, enhanced the antioxidant capabilities of BSGPs as well as in several BSGSs. The result revealed that protein fractions had the highest antioxidant activities compared to the sediments. This phenomenon describes the protein responsible for the antioxidant activity of BSG. The ability of protease in improving antioxidant activity of BSGPs might be aligned to its ability in generating a lower molecular weight of peptides (Abeynayake et al., 2022). Protease and flavourzyme increased the amount of amino acids in BSGPs with a lower molecular weight (Kriisa et al., 2022). FRAP presented the ability of the extracts in inhibiting lipid oxidation (Rahman et al., 2021) by reducing the metal ion. Moreover, antioxidant activity is also influenced by the ability of the extracts in donating its proton to neutralize reactive species (Abeynayake et al., 2022). ABTS demonstrated the ability of electron donors in reducing the molecular oxygen and hydrogen peroxide (Benzie and Strain, 1996). The protein generated by protease treatments in the current study revealed a higher performance in neutralizing free radicals and reducing metal ion. This performance might be aligned with the release of hydrophobic amino acids which exert proton donation capacity (Abeynayake et al., 2022). The ability of BSG protein as an antioxidant has also been reported in previous studies (Vieira et al., 2017). The antioxidant activity in the sediments might be due to the presence of dietary fiber, fatty acids and polyphenolic compounds (Naibaho and Korzeniowska, 2021b). BSG has been reported for its matrix complexity, which consequently led to an entrapment of certain bioactive compounds in the matrices (Naibaho and Korzeniowska, 2021b). The treatments in the current study might have allowed the release of bioactive compounds from the matrices, such as protein, as well as phenolic compounds. The obtained results in the current study show that both protein extracts and the sediments of the treated BSG are valuable and beneficial for food and nutraceutical

Table 1
Antioxidant properties and polyphenolic composition of the protein extracts (BSGPs) and the sediments (BSGSs) of BSG.

Treatments	ORAC (mmol Trolox/100 g)		ABTS (mmol Trolox/100 g)		FRAP (mmol Trolox/100 g)		Phenolic acids (mg/kg)		Flavan-3-ols (mg/kg)		Total polyphenolic (mg/kg)	
	BSGPs	BSGSs	BSGPs	BSGSs	BSGPs	BSGSs	BSGPs	BSGSs	BSGPs	BSGSs	BSGPs	BSGSs
Control	5.90 ± 0.00^{ab}	5.94 ± 0.00^{ab}	4.15 ± 0.03^{c}	0.59 ± 0.04^{d}	0.53 ± 0.00^{a}	0.17 ± 0.01^{e}	122.89 ± 0.00^{a}	17.05 ± 0.00^{f}	130.66 ± 0.00^{c}	73.85 ± 0.00^{f}	253.55	90.90
0.5% protamex	7.00 ± 0.48^{a}	5.52 ± 0.06^{b}	5.27 ± 0.04^{b}	0.32 ± 0.03^{e}	0.46 ± 0.02^{b}	0.34 ± 0.01^{c}	65.37 ± 0.00^{c}	26.26 ± 0.00^{d}	103.67 ± 0.00^{e}	315.22 ± 0.00^{a}	169.04	341.48
Prot + Flav	6.17 ± 0.03^{ab}	5.70 ± 0.56^{b}	5.55 ± 0.02^{a}	0.63 ± 0.01^{d}	0.44 ± 0.00^{b}	0.28 ± 0.01^{d}	70.82 ± 0.00^{b}	18.84 ± 0.00^{e}	118.42 ± 0.00^{d}	173.74 ± 0.00^{b}	189.23	192.59

Note: The data is shown as mean ± standard deviation of three replication. A different subscription letter shows a significant difference ($P < 0.05$) in the same observed parameter.

ingredients. Protein extracts can be applied into food products depending on their techno-functional properties, which will be discussed in further sections. Currently, the potential of plant-based protein as a food ingredient has been increasingly investigated due to the rise in demand in consumers' preferences (Beacom et al., 2021). The potential benefits of the biological properties of BSG protein can be incorporated into dairy products, meat analogue and other food productions as a substitution ingredient and or main ingredient. Taking into account that BSG protein might also influence the final products acceptability. According to a previous study, the incorporation of protease and flavourzyme declined the bitter taste compared to that in BSG protein prepared without enzyme incorporation (Kriisa et al., 2022). Further investigation of BSGP-added food products is seemingly important in terms of processing technology and final product acceptability. The valorization of BSG sediments into food products can be applied as a source of dietary fiber, mineral and phenolic compounds for bread, cookies, yogurts or the extrusion industry, as described in previous studies (Naibaho and Korzeniowska, 2021b).

3.2. Polyphenolic identification on protein extracts and sediments

Phenolic acids and flavan-3-ols of BSGPs and BSGSs were analyzed, and the results are presented in Table 1. The results showed that BSGPs contained a higher number of phenolic acids compared to that in sediments. The higher the phenolic acids in BSGP, the lower their number in BSGS. This phenomenon seems negatively related to the ORAC value, as described previously. BSGP-C had the highest number of phenolic acids compared to that in BSGP-P. However, its sediment obtained the lowest number of phenolic acids. BSGS-P contained the highest phenolic acid content compared to that in other sediment treatments. However, its protein extract (BSGP-P) had the lowest phenolic acids. The number of phenolic acids is seemingly related to the FRAP value both for BSGPs and BSGSs. The sample which had the highest level of phenolic acid had the highest FRAP capability. Interestingly, BSGP-P contained a lower amount of flavan-3-ols compared to that in its sediments, while BSGP-C had a higher amount of flavan-3-ols compared to its sediment. This phenomenon slightly aligned with the FRAP value as it was observed also in phenolic acid analysis. By this, polyphenolic compounds on BSGPs and BSGSs are responsible for FRAP capability. However, the ORAC and ABTS capability is aligned with its protein content, as described earlier.

BSGP-C contained a higher amount of total polyphenolic content compared to BSGS-C. In contrast, BSGP-P and BSGP-PF had a lower polyphenolic content compared to its sediments. By this, the utilization of enzymes optimized the protein extraction in BSG with a lower total polyphenolic content, particularly the 0.5% protamex treatment. Polyphenolics were entrapped in the sediment, which means that the sediment can be used as a polyphenolic source for food or nutraceutical purposes. By this, the treatment in the current study not only generated BSGPs that contained higher antioxidant capabilities, but it also produced a sediment with a higher phenolic content. Tentative identification of polyphenolic compounds was carried out in order to evaluate the presence of specific compounds both in BSGPs and BSGSs, and the result is shown in Table 2. The result showed that several phenolic acids were identified, including syringic acid, benzoic acid, coumaric acid, ferulic–ferulic acid dimer, sinapic acid, ferulic acid, diferulic acid and its isomers. Moreover, (+)-catechin and (−)-epicatechin were observed as a group of flavan-3-ols. The results explained that several compounds were absent in BSGPs as well as in BSGSs due to the treatments. Syringic acid, sinapic acid, diferulic acid and (+)-catechin were not present in BSGSs, while ferulic–ferulic acid dimer was absent in BSGPs. It has been reported that certain phenolic compounds are bound in the matrices, thus impacting their release from the BSG matrices. Sinapic acid is present in BSG in bound form, and it can be released at higher temperatures (Rahman et al., 2021). By this, the absence of mentioned compounds might be because those compounds are entrapped in BSG matrices.

Table 2
Tentative identification of polyphenolic compounds in BSGPs and BSGSs.

Tentative polyphenolic identification		BSGPs	BSGSs
phenolic acid	syringic acid	✓	–
	benzoic acid	✓	✓
	coumaric acid	✓	✓
	ferulic-ferulic acid dimer	–	✓
	sinapic acid	✓	–
	ferulic acid	✓	✓
	diferulic acid	✓	–
	diferulic acid isomers	✓	✓
flavan-3-ols	(+)-catechin	✓	–
	(−)-epicatechin	✓	✓

✓: present; -: absence.

Phenolic compounds in BSG are dominated by sinapic acid, p-coumaric acid, ferulic acid and caffeic acids as well as their derivatives (Sibhatu et al., 2021). According to Table 2, sinapic acid was not identified in the sediment while coumaric acid and ferulic acids were observed in BSGSs and BSGPs. Meanwhile, caffeic acid was not identified in either BSGPs or BSGSs. This phenomenon demonstrated the possibility of a conversion of caffeic acid into ferulic acid. It was reported that caffeic acid is responsible for the antioxidant activity of phenolic extract from BSG (McCarthy et al., 2013). By this, the antioxidant activity of the protein extracts and sediments in the current study might be ruled by ferulic acid. Previous studies have confirmed that the presence of phenolic nucleus and an unsaturated side chain in ferulic acid structure generated a resonance that stabilized the phenoxy radical, thus improving the DPPH capability of ferulic acid from BSG (Connolly et al., 2021; Sibhatu et al., 2021). Although several compounds were absent in the sediments, the total number of phenolic compounds in enzymes treated sediments was observed to be the highest compared to that in the extracts. By this, the high amount of total phenolic content in the sediments might be due to the higher content of benzoic acid, coumaric acid, ferulic acid and its isomer, as well as (−)-epicatechin.

3.3. OHC

The capability of BSGPs in binding and holding oil was measured, and the result is presented in Table 3. The OHC value in the current study ranged from 2.07 to 2.23 g/g sample. The result revealed that BSGP-P significantly ($p < 0.05$) had the highest OHC compared to other BSGPs. Meanwhile, BSGP-PF had the same OHC level as was in BSGP-C. BSGP-P contained more lipophilic fraction compared to that in other treatments. In other words, protamex increased the hydrophobicity of the BSGP. It was observed that the lipid and protein were linked by the aliphatic chain of lipids to the non-polar chain of amino acids, showing that a higher OHC described a higher hydrophobicity (Withana-Gamage et al., 2011). Therefore, the lower level of OHC control showed a higher hydrophilic fraction, which might lead to a higher water solubility. A higher OHC demonstrated a better performance in food stabilizing effect, mouthfeel and reducing loss during the food processing (Benitez et al., 2019). A higher OHC level on BSGP-P might show better performance from a food processing point of view.

The impact of protamex in enhancing protein functionality has also been identified which were extracted from persian lime. An improvement in OHC was reported due to the treatment by protamex (Fathollahy et al., 2021). The difference in OHC value might be an impact of different sources of material and extraction process. Higher OHC level might have a lower water solubility. BSGPs in the current study revealed a better performance in terms of water solubility due to it having a lower OHC, which might increase the solubilization rate during the mixing process.

Table 3
Functionality of proteins extracted from BSG.

BSGPs	Protein content (%)	Dry matter (%)	OHC (g/g)	WSI (%)	EAI (m^2/g)	ESI (min)	FC (%)	FS (%)
BSGP-C	12.6 ± 0.3	2.7 ± 0.1	2.074 ± 0.02^{b}	77.782 ± 0.28^{a}	233.065 ± 7.07^{a}	141.337 ± 7.22^{a}	27.084 ± 2.95^{b}	4.167 ± 0.00^{b}
BSGP-P	37.5 ± 0.0	3.7 ± 0.0	2.225 ± 0.03^{a}	80.967 ± 1.81^{a}	276.458 ± 0.39^{a}	74.543 ± 5.31^{b}	83.333 ± 0.00^{a}	18.750 ± 2.95^{ab}
BSGP-PF	31.4 ± 0.7	3.9 ± 0.1	2.074 ± 0.01^{b}	82.090 ± 0.96^{a}	268.675 ± 18.31^{a}	49.944 ± 3.16^{c}	81.250 ± 8.84^{a}	39.583 ± 8.84^{a}

Note: The data is shown as mean ± standard deviation of three replication. A different subscription letter shows a significant difference ($P < 0.05$) in the same observed parameter.

3.4. WSI

Although it had different levels in OHC value, BSGPs possessed the same level of WSI in which there was no substantial significant difference ($p > 0.05$). WSI was assessed to represent molecular degradation ingredient behavior in aqueous phase which describes the reconstitution of the powder (Jafari et al., 2017; Rashid et al., 2015). Difference in WSI is observed to occur due to the difference in moisture content and protein solubility (Tas et al., 2022). Since there was no difference in WSI, further investigation in protein solubility is not needed in this study.

3.5. Emulsion properties

Statistically, there was no significant difference ($p > 0.05$) in EAI in all observed groups and it ranged from 233.1 to 276.5 m^2/g. Meanwhile, a significant difference ($p < 0.05$) in ESI was identified. The difference in EAI was reported due to the elevation in hydrolysis degree (Fathollahy et al., 2021). By this, the obtained BSGPs in the current study might have the same level of hydrolysis degree. The difference in ESI is influenced by the molecular weight of amino acids (Fathollahy et al., 2021). Both BSGP-P and BSGP-PF might have had a lower molecular weight compared to the control. As reported previously that enzymatic treatment in protein extraction generated amino acids with lower molecular weight (Wen et al., 2019). Smaller molecular weight peptides had a lower efficiency in declining the interface tension. Thus, their unfolding and reorientation at the interface to stabilize the emulsion are restricted compared to the longer peptides (Fathollahy et al., 2021).

3.6. Foaming properties

The foaming properties of BSGPs are presented in Table 3. Protease-treated BSGPs had a significantly higher ($p < 0.05$) FC and FS level compared to that in control. By this, enzymatic treatments improved the foaming properties of BSGPs. The same phenomenon had been reported previously in BSG protein and lime seed protein (Connolly et al., 2014; Fathollahy et al., 2021). As foaming properties were aligned with the pH level, barley protein showed higher foaming properties at alkaline pH and a lower value at acidic pH value (Connolly et al., 2014). Foaming properties on BSGPs were not influenced by DH (Connolly et al., 2014). The foaming formation might be due to the higher solubility (WSI), as mentioned previously. This result is aligned with a previous study that reported that the foaming capacity is influenced by the protein solubility, hydrophobicity and tenderness (Li et al., 2021). The higher FS in protease-treated protein might be due to the higher amount of protein content. The current study revealed that enzymatic treatments enhanced the protein content, thus improving the foaming stability. Li et al. (2021) emphasized that the FS level is influenced by the protein concentration, hydration and molecular interaction. The increase in foaming properties is also impacted by protein flexibility, which allows the protein particle to encapsulate air particles and spread quickly on the air–water interface (Li et al., 2021). The interaction between proteins at the air–water interface is facilitated by the increase in net charge, which contributes to the higher foaming properties (Fathollahy et al., 2021). Foaming properties is an important parameter in the utilization of BSGPs as a food ingredient. Higher foaming properties show a better performance in food products. By this, the BSG-PHs obtained by protease and protease/flavourzyme potentially offer benefits for food producers.

4. Conclusion

The study revealed that enzymatic treatments on BSG improved the ORAC and ABTS value of BSG-PHs, which might be due to its impact in generating higher protein content. Protein fractions had higher antioxidant properties than that in their sediments. It was observed that polyphenolic compounds are responsible for the FRAP value of BSG and both protein fractions and sediments; meanwhile, protein content is responsible for ORAC and ABTS values. The results show that enzymatic treatments on BSG improved the antioxidant properties of its BSGPs and enhanced the phenolic compounds in its sediments. The techno-functional characterization of BSGPs demonstrated that enzymatic treatments generated a higher oil holding capacity, foaming formation capability and foaming stability, although they had a lower emulsion activity index. By this, protease-treated BSGPs might will a better performance in food processing. Further investigation on its impact on the mechanical processing of food products is seemingly important.

Author contributions

Joncer Naibaho: conceptualization, methodology, validation, formal analysis, investigation, writing-original draft preparation, writing-review and editing, funding acquisition.

Małgorzata Korzeniowska: conceptualization, methodology, validation, investigation, resources, writing - review and editing, supervision, project administration, and funding acquisition.

Aneta wojdyło: methodology, formal analysis, writing - review and editing.

Hanif Muchdatul: writing - review and editing.

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Declaration of competing interest

None.

Acknowledgements

Authors would like to thank Prof. Adam Figiel (Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland) for the extracts drying and Prof. Oskar Laaksonen (University of Turku) for project funding.

This work was supported by UPWR 2.0, international and interdisciplinary programme of development of Wrocław University of Environmental and Life Sciences, co-financed by the European Social Fund under the Operational Programme Knowledge Education Development 2014–2020: Axis III Higher education for the economy and development; Action 3.5. Comprehensive programmes 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).

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