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Soy-based yogurt-alternatives enriched with brewers' spent grain flour and protein hydrolysates: Microstructural evaluation and physico-chemical properties during the storage

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ABSTRACT

Current study evaluated the influence of brewers' spent grain (BSG) flour and its three different protease-treated protein extracts (BSGPs) in the production of soybean based yogurt-alternatives (SYA). Confocal laser scanning microscopy showed that protease-treated protein extracts from BSG generated a softer, more regular and denser network distribution while BSG flour generated an irregular and less fat particle distribution. Rheological properties showed that BSG derivatives significantly ($p < 0.05$) increased the viscosity index of the yogurt from a range of 44–80 at the first day to a range of 91–150 at 14 days of storage showing a strengthening effect on the formed network of SYA. It also preserved the flow behavior and consistency during the storage shown by syneresis value which was stable during the 14 days storage at a range of 270–380 g/kg. BSG derivatives enhanced the amount of lactic acid on SYA which increased from 4 g/kg at control to 6–11 g/kg at SYA with BSG derivatives. In conclusion, BSG derivatives modified the microstructural properties of SYA, improved rheological behavior and acidity during the refrigerated storage.

1. Introduction

Plant-based yogurt alternatives have been increasingly studied and developed for its health benefits, processing properties and commercial market trends (Boeck, Sahin, Zannini, & Arendt, 2021; Grasso, Alonso-Miravalles, & O'Mahony, 2020; Greis et al., 2020; Ningtyas, Hati, & Prakash, 2021). Soybean is in the second place of the most popular ingredient in the development of plant-based yogurt alternatives after coconut (Boeck et al., 2021). Compared to coconut, soybean contains much lower amounts of saturated fatty acids (Boeck et al., 2021) thus might have significant health influences. Soybean-based yogurt alternative (SYA) contains low amounts of cholesterol and saturated fat as well as being lactose-free (Xiao, 2011). Therefore, SYA maintains weight loss and lipid level reduction in obese mice (Li et al., 2020), acts as an antidepressant and tumor antiproliferation as well as alleviates hypercholesterolemia, cardiovascular risk, hepatic lipid accumulation, and tumor antiproliferation (Sengupta, Koley, Dutta, & Bhowal, 2018, 2019; Ko, Lin, & Tsai, 2013; Sengupta, Goswami, Basu, & Bhowal, 2016; Xiao, 2011). These properties are linked to the presence of gamma-aminobutyric acid, free amino acids, and isoflavones aglycones (Sengupta et al., 2018, 2019; Ko et al., 2013; Sengupta et al., 2016; Xiao, 2011). Furthermore, the fermentation process on SYA alters the protein digestibility (Rui et al., 2019).

Due to the health benefits of SYA, several studies have identified the possibility of the quality improvement of SYA. From a bioactivity point of view, the incorporation of *L. reuteri* and *Spirulina platensis* enhances the level of vitamin B12 and hypocholesterolemic properties respectively on SYA (Gu, Zhang, Song, Li, & Zhu, 2015; Sengupta et al., 2018). Different homogenization methods, blanching temperature and germination stage of soybean modify the biological properties of SYA (Cho, Chun, Kim H., Kim J.S., & Kim J.H., 2013; Mei, Feng, & Li, 2016; Peng & Guo, 2015). Aiming to improve the physical behavior and acceptability,

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the addition of apple juice, inulin and elimination of lipoxygenase has been evaluated for their influence on gel firmness, textural behavior, odor compounds, color and network structure formation of SYA (Rinaldoni, Campderrós, & Pérez, 2012; Zhou et al., 2019, ier, Gündüz, Yilmaz, & Memeli, 2015).

Currently, incorporation of brewers' spent grain (BSG) in SYA production has never been reported. BSG, a by-product of the brewery industry, contains a high amount of nutritional value including dietary fiber, protein and amino acids, fatty acids, polyphenolic compounds and minerals (Lynch, Steffen, & Arendt, 2016; Naibaho & Korzeniowska, 2021). Those chemical constituents are responsible for biological properties of BSG such as antioxidants, antimicrobial, and immunomodulatory effects (Cermeño et al., 2019; Kumari et al., 2019; Verni et al., 2020). Particularly protein and amino acids of BSG have antioxidant, anti-inflammatory and immunomodulatory activities, inhibit and protect against oxidative stress, modulate glycemic response, antithrombotic effects and promote angiotensin converting enzyme activity (Lynch et al., 2016; Naibaho & Korzeniowska, 2021). Furthermore, dietary fiber in BSG could act as prebiotic (Amorim, Silvério, & Rodrigues, 2019; Xiros & Christakopoulos, 2012).

From the perspective of food techno-processing, high amount of dietary fiber, particularly insoluble dietary fiber, regulates the textural behavior of BSG-added food (Föste, Verheyen, Jekle, & Becker, 2020; Fărcaş et al., 2015; Heredia-Sandoval et al., 2020; Ktenioudaki et al., 2015; Nocente, Taddei, Galassi, & Gazza, 2019). This phenomenon occurs due to the presence of arabinoxylans which possess high capability in binding water (Steiner, Procopio, & Becker, 2015). In other words, BSG has the potential to enhance the nutritional value of SYA and regulate its flow behavior. BSGF is mainly dominated by insoluble dietary fiber (IDF) up to 550 g/kg with crude protein content up to 300 g/kg (Naibaho et al., 2021). The high amount of IDF might have a negative impact on structure formation of food products (Naibaho & Korzeniowska, 2021). Protein compound in BSGF is mainly entrapped in the vacuole cell walls, therefore, its availability and its impact on food processing could be neglected. BSGPs provided by different enzymes incorporation, contained different profiles of amino acids and protein availability (Kriisa et al., 2022). BSGPs contained protein content at a range of 130–380 g/kg with different foaming properties and oil holding capacity (Naibaho et al., 2022a). Due to the separation of IDF from BSGPs, the presence of protein in BSGPs might play an important role in texture formation of yogurt. The main properties of BSGF are influenced by its IDF while the behavior of BSGPs is regulated by its protein content.

BSG flour (BSGF) has been incorporated previously into milk based yogurt and it was identified that 100 g/kg substitution of BSG flour maintained the flow behavior, preserved the consistency, regulate lactic acid bacteria (LAB) and lactic acid production during the 14 days of storage in addition to its impact in microstructural properties (Naibaho et al., 2022b). Seemingly challenging, protein hydrolysates from BSG (BSGP) were prepared by using different proteases (Naibaho et al., 2022a) and used as protein enrichment sources in coconut-based yogurt-alternatives production along with BSG flour (Naibaho et al., 2022c). The evaluation of BSG derivatives including BSGF and BSGPs in SYA has never been conducted. Therefore, the study aimed to evaluate the impact of BSGF and BSGPs in SYA production. It was hypothesized that different BSG derivatives generated a significant influence on microstructural properties and network formation, flow behavior, mechanical properties, consistency and lactic acid production.

2. Materials and methods

2.1. Materials and materials preparation

BSG was collected from a local light-beer type production in Wroclaw, Poland. The BSG was treated as described in a previous study (Naibaho et al., 2021). Briefly, BSG was dried by convective drying to reach a moisture content at a range of 2–5 g/100 g sample, milled by a laboratory scale milling and sieved to pass a 150 μm sieving. The BSG flour was then packed in aluminum foil bags and kept at 4 °C for further studies.

BSGPs were prepared as described in a previous study. Briefly, BSG was added into distilled water (1:10; *g:g*) and the mixture was prepared for incubation with the addition of Protamex (a serine endoprotease) and Flavourzyme (a mixture of endo- and exopeptidase) (Novozymes, Bagsvaerd - Denmark) as follows. The mixture without protease was described as control (BSGP-C), the mixture incubated with 5 g/kg of Protamex (BSGP-P) and incubated with a combination of 5 g/kg of Protamex and 1 g/kg of Flavourzyme (BSGP-PF). The mixtures were incubated at 50 °C for 3 h at pH 8.5 and then heated at 90 °C to inactivate the enzymes. After cooling down to room temperature, the mixture was then centrifuged at 1500×g at 24 °C (Fisher Scientific GmbH, Schwerte, Germany). The liquid fractions were separated and dried by using a semi-pilot scale spray dryer (APV Anhydro A/S LAB S1, Hirtshals, Denmark). The drying was carried out at inlet temperature 160–165 °C and outlet temperature at 82–85 °C, with the velocity of the peristaltic pump at 2.5 L/h and air pressure nozzle 200 kPa. The dried extracts, as BSGPs) were packed in a sealed aluminum bag and stored at 4 °C. The functional properties of those BSGPs as well as their biological properties is presented in Supplementary 1 as reported in a previous study (Naibaho et al., 2022a).

Water soluble soybean extracts (WSE – Alpro SOYA, Wevelgem, Belgium) and microbial culture (vivo - Zakwaska, Warsaw, Poland) were purchased from the commercial market in Wroclaw, Poland. According to the product label, WSE contained protein 3.0 g, sugar 2.5 g, glucide 2.5 g, fat 1.8 g, and fiber 0.5 g per 100 mL and microbial culture composed of maltodextrin, *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp *bulgaricus, Lactobacillus acidophilus*, and *Bifidobacterium lactis*. Rhodamine 123 and Nile Red with Cat No R8004 and 72485, respectively, were purchased from Sigma-Aldrich (Hamburg, Germany). All chemicals used for analyses were analytical grade.

2.2. Yogurt alternatives preparation

SYA were prepared following the instruction of microbial culture as follows. Each of BSG derivatives (BSGF and BSGPs) was added to WSE with a ratio 1:9 (*g/g*) and mixed properly. After that, the mixture was heated at 90 °C for 15 min and cooled down to reach 43 °C. Microbial culture (0.5 g/kg) was added into the mixture and the temperature was kept at 43 °C to reach pH range at 4.3–4.7. The pH was observed periodically and recorded; the mixture was homogenized slowly during the pH observation for 10 s using a laboratory scale mixer at the lowest speed (260 rounds/min; 4 cm gap). The fermentation was ended by mixing using a laboratory scale mixer followed by cooling down to 15 °C. SYA was removed into a cup, closed properly with top lid, and stored at 4 °C for 18 h prior to the analysis as day 1. The same process was carried out as a control without any addition of BSG derivatives. Therefore, 5 different formulations were obtained, and duplicate samples were prepared.

2.3. Confocal scanning laser microscopy (CLSM)

CLSM was conducted following the method described in the previous studies as follows (Naibaho et al., 2022b) with some modifications using a Leica SP8 MP confocal microscope (Leica Microsystems, GmbH, Wetzlar, Germany). The staining solutions (Nile Red and Rhodamine 123) were prepared at a concentration of 10 μg/ml in water; samples were provided in freeze-dried form. Approximately 9–30 mg of the sample was suspended in the staining solution (1:4; *g:mL*). The stained sample was transferred onto a glass slide and covered with a coverslip. The images were presented using a 20x (NA 0.75) air objective. A reflection of laser light was used to visualize the structure of the sample, in addition to the fluorescence of a given dye. The excitation was done at 488 and 561 nm laser for Rhodamine 123 and Nile Red, respectively; the reflected light channel was generated at 638 nm laser for Rhodamine 123 and 488 nm for Nile Red. Three representative fields of view were imaged for each sample. ImageJ/Fiji software in a fire look-up table was used to present the maximum intensity projections of scanned volumes (10–80 μm thick, 0.68 μm intervals).

2.4. Fourier transform infrared spectroscopy (FTIR)

FTIR was carried out using IRSpiritTM, Shimadzu (Shimadzu Europe, GmbH, Duisburg, Germany) following the instruction of the instrument. The measurement of the absorbance was conducted on freeze-dried samples and observed at 4000 and 400 cm^{-1}.

2.5. Rheological behavior

The analysis of rheological properties was carried out as described (Naibaho et al., 2022b) using a rotational Haake RheoStress 6000 rheometer (Thermo Electron GmbH, Karlsruhe, Germany) equipped with a thermostatic bath Haake A10 and a UTM Controller (Thermo Electron GmbH, Karlsruhe, Germany). The measurement was done at 20 °C using a cone/plate (C60/1° Ti L no.222-1868/stainless steel plate TMP60 no.222-1891) in a geometry system with a gap of 1 mm. One -mL of the sample was added into the plate surface at shear rate from 0 to 2000/s. The data was presented at shear rate 10 to 2000/s. The flow curve was fitted to Power model of Ostwald de Waele in order to obtain flow behavior related value, with equation:

$$\eta_{50} = \mathrm{k}\mathrm{x}\gamma^{n-1}$$

η_{50} = apparent viscosity (Pa.s); k = consistency index (Pa.s); γ = shear rate (1/s); n = flow behavior index.

2.6. Syneresis

A 5 g of sample was centrifuged at 2700×g (Fisher Scientific GmbH, Schwerte, Germany) and 10 °C for 15 min. After that, the supernatant was separated and the sediment was weighed (Bouaziz et al., 2021; Khubber, Chaturvedi, Thakur, Sharma, & Yadav, 2021). Syneresis value was calculated as the equation:

Syneresis (g/kg sample) = Weight of supernatant (g) / Weight of yogurt (kg)

2.7. Lactic acid production and pH

Lactic acid was assessed by the titration method with NaOH (0.25 mol/L). Distilled water was added to the yogurt (1:1) and 3 drops of indicator phenolphthalein was added. The amount of lactic acid was obtained following the equation:

$$Lactic\ acid\ (mL\,/\,kg\ sample) = \frac{volume\ of\ NaOH\ (mL)\ x\ N\ x\ 90}{Sample\ x\ 1000}\ x\ 100$$

The pH was measured by InoLab pH-meter according to the instructions of the instrument.

2.8. Statistical analysis

Statistical analysis was conducted in order to determine significant differences for the quantitative collected data from at least duplicate analysis for each replicate sample during the 14 days of refrigerated storage. It was performed in two-ways analysis of variance (ANOVA) using Statistica software (version 13.5.0.17). The significance difference was determined at $p < 0.05$ followed by Tukey post-hoc test.

3. Results and discussion

3.1. Fermentation period

Fermentation time was observed regarding the pH derivation to reach a range of pH at 4.3–4.7 and the result is presented in Fig. 1. In general, different types of BSG derivatives generated different patterns in pH change during the fermentation in order to reach the pH compared to the control. Fig. 1 shows that SYA prepared with BSGF had the shortest fermentation time (3 h) while SYA prepared with BSGPs had the longest fermentation time (4.5 h). The pH difference between the observed group was identified from the initial process before the incubation stage. Compared to the control, BSGF slightly reduced the initial pH of the mixture while BSGPs increased it. This phenomenon also occurred in the incorporation of BSG derivatives in coconut extracts based yogurt alternatives (Naibaho et al., 2022c). However, it did not occur in the milk based yogurt prepared with BSG flour (Naibaho et al., 2022b). BSGPs were prepared by an incubation process with a pH of 8.5. Meanwhile, soybean generally has a pH of 6.3 (Rui et al., 2019). Therefore, BSG has increased the pH of the soybean extracts at the initial stage as observed in this study. This might be an impact of the instability of plant-based water soluble extracts due to the weak bonds thus allowing the BSG derivatives buffered the SYA.

A significant drop in pH was remarkably observed at the 3rd h of fermentation and the amount of pH derivation is shown in Table 1. As mentioned earlier, the targeted pH was achieved in a different time due to the different pattern in pH derivation. In SYA control, the highest pH drop was observed after 4 h of fermentation. The same phenomenon was observed in SYA substituted with BSGP-C and BSGP-P which reached the pH at 4 h of fermentation. However, the control SYA required a longer period to reach the standard pH at 4.5 h fermentation. Different pattern was identified in SYA prepared with BSGP-PF in which the pH dropped significantly after 3 h of observation, slowly declined after 4 h and continues to decline dramatically at 4.5 h of fermentation. This phenomenon revealed the influence of BSG derivatives in the production of lactic acid due to the growth of lactic acid bacteria during the acidification process. This also showed the impact of BSG derivatives in texture and matrix formation of the SYA during the fermentation.

The decline of the pH during the fermentation shows the growth of LAB. The lower the pH the higher the number of the LAB (Rui et al., 2019). By this, BSG derivatives might have allowed the LAB to grow in SYA fermentation thus lowering the pH. Acidification induces the gelation due to the growth of LAB. BSG might provide sufficient protein to support the growth of LAB. During the fermentation, *S. thermophilus*

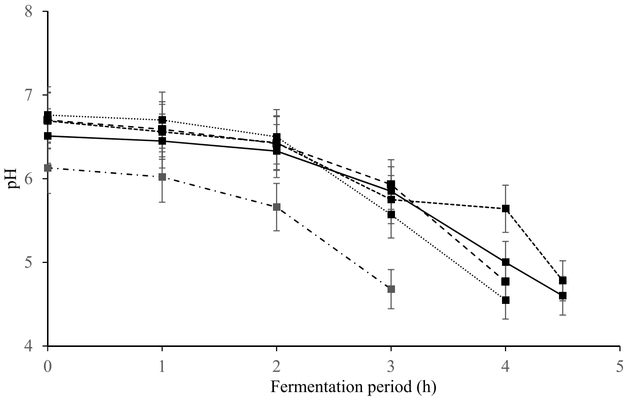


Fig. 1. Fermentation time of soybean-based yogurt alternatives prepared with no BSG derivatives as control (—) and with the addition of 100 g/kg of BSG derivatives (BSGP-C: spent grain protein prepared without protease extraction (····), -PF: with protease and flavorzyme extraction (——), -P: with protease extraction (- - -),; BSGF: spent grain flour (-·-·-)).

Table 1
pH derivation of soy-based yogurt alternatives (SYA) enriched with 100 g/kg spent grains during the fermentation.

Soybean-based yogurt alteratives	$\Delta_{0\text{-}1}$	$\Delta_{1\text{-}2}$	$\Delta_{2\text{-}3}$	$\Delta_{3\text{-}4}$	$\Delta_{4\text{-}4.5}$
Control	0.06	0.12	0.48	0.85	0.4
BSGP-C	0.06	0.2	0.93	1.02	–
BSGP-PF	0.13	0.13	0.68	0.11	0.86
BSGP-P	0.11	0.17	0.49	1.16	–
BSGF	0.11	0.36	0.98	–	–

Note: Control: SYA without spent grain derivatives, BSGP-C: spent grain protein prepared without protease extraction, -PF: with protease and flavorzyme extraction, -P: with protease extraction; BSGF: spent grain flour. The derivatization was calculated based on the average value of pH in Fig. 1.

grows by using free amino acids in the mixture both from WSE and BSG at the initial stage of fermentation (Chandan & O'Rell, 2013). The metabolites (peptides) then will be used by *L. bulgaricus* in addition to the available protein, to grow faster, generating a higher amount of lactic acid thus lowering the pH (Chandan & O'Rell, 2013). This synergistic phenomenon might occur within other strains in the matrix. BSG derivatives might improve the proteolytic action of LAB strain. In the current study, other strains were also present in the system including *Lactobacillus acidophilus,* and *Bifidobacterium lactis*. In the first stage of fermentation (pH > 6.0), the texture stayed stable until 2 h of fermentation while the aggregation of protein particles was started. The gel formation starts when the pH is below 6, and the texture is dramatically formed when the pH between 6 and 5.5. A hardened mixture was dramatically observed at the pH of 5.9 followed by further matrix formation. Consequently, a stronger and more compact structure was generated at the pH below 5.4. At this stage, the springiness and cohesiveness remained stable (Rui et al., 2019). The acidification generates a monodisperse distribution showing a uniform formed structure when the pH reached 5.1 (Rui et al., 2019). Texture formation in SYA might be influenced by the soy protein solubility. Acidification process was observed to reduce the solubility of soy protein thus intensifying the formed structure.

During the network formation, LAB released protons by hydrolytic action thus neutralizing the negative protein complexes. The formed structure was stabilized by Van der Waals attraction, hydrogen bonding and hydrophobic interactions. The properties of BSG protein might also influence the network formation including hydrophobicity level, foaming ability, turbidity properties and stability in addition to the solubility (Wen, Zhang, Duan, Zhang, & Ma, 2019). By this, the amount of available protein from BSG derivatives might have played an important role in the structure formation and stabilization which will be discussed in the next section.

Compared to previous studies, fermentation time depended on the incubation temperature. Lower temperature (30–37 °C) required a higher fermentation time between 9 and 72 h (Cho, Chun, Kim, Kim, & Kim, 2013; Hwang et al., 2021; Kim & Han, 2019; Mei et al., 2016; Patrignani et al., 2018; Rui et al., 2019, ier et al., 2015). Moderate to higher temperatures (40–43 °C) shorten the fermentation process between 6 and 8 h (Park & Lee, 2015; Ye, Ren, Wu, Wang, & Liu, 2013). The specific difference might be due to the pre-treatment, LAB strains and ingredients used. In addition to BSG derivatives, the current study was conducted at incubation temperature 43 °C thus generating a lower fermentation time. A higher incubation temperature needs a shorter fermentation time (Meybodi, Mortazavian, Arab, & Nematollahi, 2020).

3.2. Microstructural characterization by CLSM

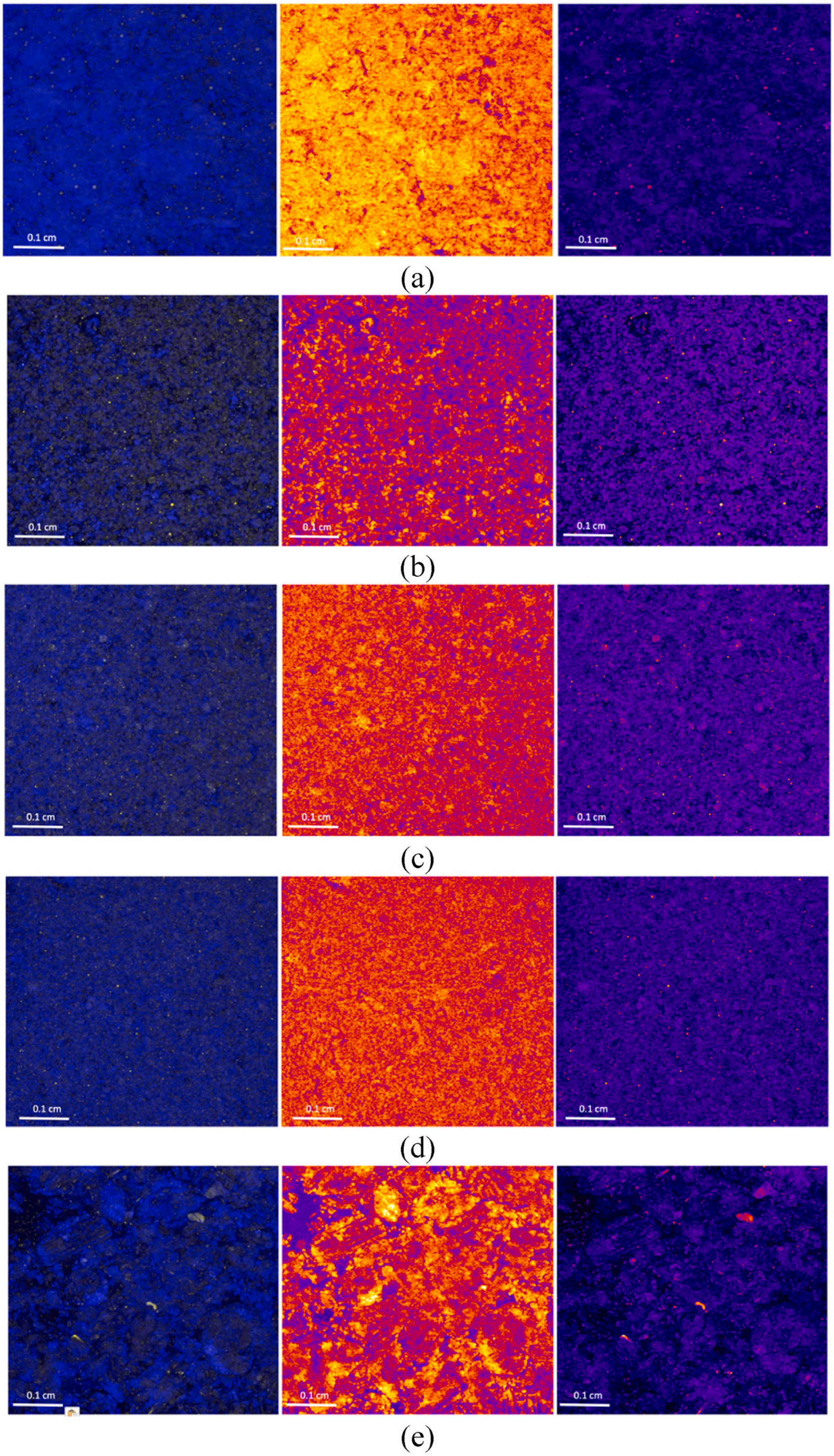
CLSM was conducted in order to characterize the microstructure of fat and the formed network of SYA as an impact of BSG derivatives and the results are presented in Figs. 2 and 3, respectively. SYA control (Fig. 2a) consisted of higher fat particles and less dense fat distribution. However, the addition of BSGPs (Fig. 2b, c, and d) led to a denser fat distribution with a smaller fat particle. The fat globules of SYA control (Fig. 2a) were in between a rough surface (plate-like-appearance). Fat particles of BSGPs-added SYA (Fig. 2b, c, and d) laid in a smooth surface appearance compared to the control. Distributed fat in SYA prepared with BSGP-C (Fig. 2b) were in a less dense matrix while fat globules in SYA prepared with BSGP-P and BSGP-PF (Fig. 2c and d) laid in a denser matrix. Although, the addition of BSGP-P had a smoothest surface matrix appearance compared to all observed groups. Moreover, incorporation of BSGF in SYA (Fig. 2e) generated an irregular and less fat particle distribution as well as irregular formed matrices. Fig. 3 shows that BSG derivatives modified the formed matrices of SYA. Compared to control (3a), BSGPs generated a softer surface appearance (3 b, c and d) while BSGF had irregular matrices and disrupted the network of the SYA (3e).

The network formation of SYA in this study might be aligned with the protein content of BSG derivatives. The higher protein content generated a softer surface appearance. BSGP-C (3 b) tended to form inner space in the matrices of SYA with irregular particle appearance. However, BSGP-P and BSGP-PF (3c and d) generated smoother, denser, and more regular surface appearance of SYA. WSE obtained 30 g/kg of protein while BSGPs ranged from 120 to 300 g/kg of protein content. By this, protein enrichment from BSGPs softened the microstructure of SYA. The ability of BSGF in disrupting the matrix formation of SYA might be related to its structure complexity. BSGF is dominated by insoluble dietary fiber thus disrupting the network formation of food matrices (Naibaho & Korzeniowska, 2021). The same phenomenon has been observed in milk based yogurt in which BSGF disrupted the yogurt matrices (Naibaho et al., 2022b). This might be due to the high amount of dietary fiber thus diminishing the protein mobility in the matrices.

The binding strength in the matrices affects the matrix formation and network distribution (Nguyen, Ong, Kentish, & Gras, 2015). Matrix formation and formed network might be related to the ability of BSG derivatives in binding with WSE during the fermentation. BSGP has emulsifying properties (Wen et al., 2019) thus allowing the formation of SYA matrices. Protein content and protein availability on BSG derivatives plays an important role in the SYA microstructure formation. Therefore, the SYA microstructure might be influenced by hydrogen bonding and hydrophobic interactions between the WSE and BSG derivatives. Final matrices of SYA are stabilized by Van der Waals interactions (Rui et al., 2019). The aggregation of soy protein in SYA is influenced by subunits within β-conglycinin and glycinin (Yang, Fu, & Li, 2012) thus might influence its interaction with protein from BSG derivatives. By this, the difference in protein content and matrix complexity of BSG derivatives influenced the stability of formed microstructure as presented in this study. Furthermore, the fermentation process is observed to lower particle size of fat and protein particles (Rui et al., 2019) thus affecting its homogeneity and distribution. The homogeneity of the matrix distribution in SYA prepared with BSGP-PF and BSGP-P (Fig. 3c and d) might be related to its ability in completing the fermentation process. The tendency of plant-based ingredients in generating a more compact and dense matrix in enriched yogurt has also been observed in previous studies (Nguyen, Ong, Kentish, & Gras, 2014; Qiu, Zhang, Mujumdar, & Chang, 2021; Zhao, Feng, & Mao, 2020). The performance of BSGP-PF and BSGP-P in SYA microstructure might be associated with those enzymes' ability in generating a lower molecular weight of amino acids and peptides during the incubation and protein extraction (Rocha Camargo et al., 2021; Ryan, O'Regan, & FitzGerald, 2020; Yang et al., 2020).

3.3. Chemical surface evaluation by FTIR

FTIR analysis was performed in order to determine the difference in functional groups present in SYA due to the addition of BSG derivatives and the result is presented in Fig. 4. There were some differences in terms of absorbance between those 5 observed groups. In general, the incorporation of BSG derivatives intensified the absorbance spectrum.



(a)

(b)

(c)

(d)

(e)

Fig. 2. Confocal laser scanning micrographs of freeze-dried soybean-based yogurt alternatives without BSG derivative (a) and with BSGP-C (b), BSGP-PF (c), BSGP-P (d) and BSGF (e), stained with Nile red. The images are presented as maximum intensity projections from confocal Z stacks in a fire intensity scale. Overlay image (left), yellow channels (middle): fat phase, blue channels (right): yogurt structure visualized with a laser reflection. (BSGP-C: spent grain protein prepared without protease extraction, -PF: with protease and flavorzyme extraction, -P: with protease extraction; BSGF: spent grain flour). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The lowest absorbance was observed in SYA control and the highest was observed in SYA prepared with BSGP-PF. Interestingly, SYA with the addition of BSGF, BSGP-C, and BSGP-P had almost similar absorbance patterns. This phenomenon might align with the microstructural properties of CLSM as discussed earlier in which the matrix density and fat distribution was most likely similar compared to SYA control and BSGF-added SYA. There were 3 functional groups which had different patterns as is shown in Fig. 4. SYA prepared with BSGP-C had the lowest absorbance in the functional band region of 1000–1100 cm^{-1} (1) which represented the presence of aliphatic ethers and acid functional groups. It had a higher absorbance at a band region of 1600–1800 cm^{-1} (2) which was assigned for aromatic hydrocarbons of lignin, amine I and II,

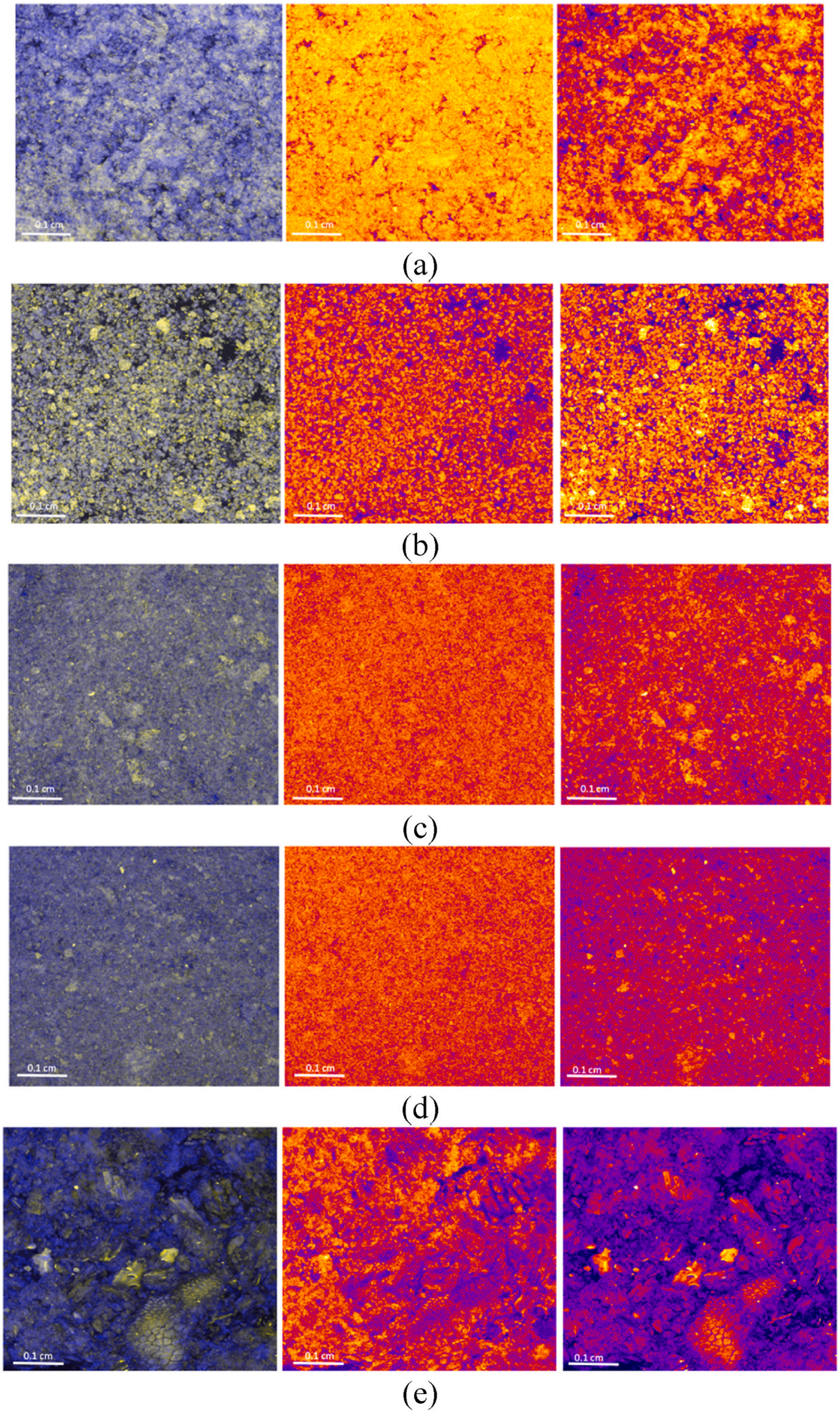


Fig. 3. Confocal laser scanning micrographs of freeze-dried soybean-based yogurt alternatives without BSG derivative (a) and with BSGP-C (b), BSGP-PF (c), BSGP-P (d) and BSGF (e), stained with Rhodamine 123. The images are presented as maximum intensity projections from confocal Z stacks in a fire intensity scale. Overlay image (left), yellow channels (middle): matrix phase, blue channels (right): visualized with a laser reflection. (BSGP-C: spent grain protein prepared without protease extraction, -PF: with protease and flavorzyme extraction, -P: with protease extraction; BSGF: spent grain flour). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fatty acids and fatty acids esters. The fluctuation of the absorbance pattern is also observed at the band region of 3400–3900 cm^{-1} (3). This band region is indicative for hydroxyl and amine groups (Brodziak et al., 2021; Patrignani, González-Forte, & del, 2021; Ravindran, Jaiswal, Abu-Ghannam, & Jaiswal, 2018).

The variability in absorbance pattern was linked to the modification of surface chemistry of BSG derivatives-added SYA. As it was mentioned in the previous section that the binding strength between fat-protein and matrices affected the formed network distribution (Nguyen et al., 2015). This result confirmed the modification in the microstructure characteristic of SYA due to the addition of BSG derivatives. Matrix formation of yogurt is affected by the side chain of the added-ingredients molecules and structural features of the hydrocolloid backbone (Huang et al., 2019).

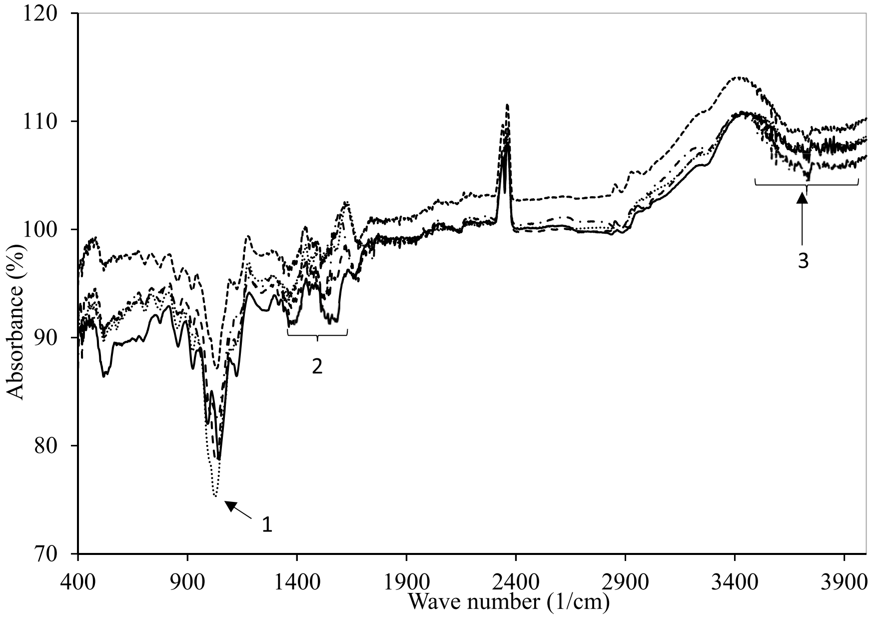


Fig. 4. FTIR spectrum of freeze-dried soybean-based yogurt-alternatives without BSG derivatives as control (—) and with 100 g/kg different BSG derivatives (BSGP-C: spent grain protein prepared without protease extraction (····), -PF: with protease and flavorzyme extraction (——), -P: with protease extraction (- - -); BSGF: spent grain flour (-····-)).

3.4. Evaluation of rheological behavior during the storage

The relation between shear rate and shear stress during the storage is presented in Fig. 5. In general, SYA prepared with the addition of BSG derivatives had a higher shear stress both at the initial period and after 14 days of storage. The lower shear stress was observed at SYA control while the highest shear stress was at BSGF addition. As reported previously, shear stress defines the required energy to break or destroy the matrices of the yogurt (Vénica, Spotti, Pavón, Molli, & Perotti, 2020). By this, the addition of BSG derivatives might have intensified the strength of SYA matrix. The addition of BSGPs, regardless of the enzymatic treatment, had almost similar shear stress at the initial storage (Fig. 5a). The impact of BSG derivatives on matrix strength might be aligned with the complexity of the BSG derivatives. After 14 days of storage (Fig. 5b), SYA control had similar gel strength as it did on the first day. Meanwhile, shear stress of SYA prepared with BSG derivatives increased after 14 days of refrigerated storage. This phenomenon showed the ability of BSG derivatives to increase the gel strength of the SYA which might depend on the matrix complexity of BSG derivatives. Lower shear stress showed a softer texture of the yogurt (Yang et al., 2012). The impact of BSG addition in SYA might also be aligned with its ability in reducing the syneresis level as will be discussed in the next section.

The viscosity of SYA is presented in Fig. 6. The obtained curves were fitted to Power low to obtain certain parameter values including consistency index (k), flow behavior index (n) and apparent viscosity (η50). In general, the viscosity decreased with increasing shear rate showing a shear-thinning behavior. At the initial storage (Fig. 6a), the viscosity behavior of obtained SYA had a similar trend except with the addition of BSG flour. However, the trend changed after 14 days of storage. SYA with BSG incorporation tended to have higher viscosity flow compared to the control SYA. This phenomenon is aligned with the trend in shear stress as discussed previously.

Flow behavior values are shown in Table 2. In general, there was no significant difference ($p > 0.05$) in k, n, and η50 in all groups at the initial storage (first day). The trend changed depending on the BSG derivatives after 14 days of the storage. After 14 days, the k value increased significantly ($p < 0.05$) SYA prepared with BSGP-P and BSGF. However, it was insignificant ($p > 0.05$) in SYA control and SYA prepared with BSGP-C and BSGP-PF. There was no significant difference ($p > 0.05$) in n value after 14 days of storage except SYA prepared with BSGP-C in which it decreased. The decrease in n value is an impact of increase in molecular chains length and cross-linking of protein micelles (Yang et al., 2012). Although it was insignificant ($p > 0.05$) on the first day, η50 significantly increased in certain groups after 14 days including BSGP-PF, BSGP-P and BSGF addition in SYA. BSG derivatives preserved the consistency of SYA during the storage. The difference in k and η50 value is related to hydrolysis level of soybean protein (Yang et al., 2012). By this, the hydrolysis level of soybean protein during the fermentation process might be at the same level. During the storage, protein from BSG derivatives might be available for hydrolysis thus allowing the increase in k and η50 value after 14 days of storage. Consequently, it influenced the viscosity and matrix strength as mentioned earlier.

Flow behavior of SYA is influenced by protein micelle networks due to its ability to strengthen the cross-linking matrices of SYA (Yang et al., 2012). In the current study, BSGPs and BSGF were utilized in SYA. The addition of BSGPs logically provided the sufficient protein thus influencing the network formation and flow behavior. The addition of BSGF provided dietary fiber, fat and protein which might have low availability due to matrix complexity. Initially, the addition of BSG derivatives generated an almost similar flow behavior trend at the first day (Fig. 6a; Table 2). The influence of 100 g/kg BSGF in milk based yogurt also generated almost similar flow behavior with the control (Naibaho et al., 2022b).

3.5. Syneresis

The impact of the enrichment of BSG derivatives on the syneresis level of SYA is presented in Table 2. The study revealed that the addition of BSG derivatives significantly ($p < 0.05$) lowered the syneresis level compared to the control regardless of the type of BSG. The syneresis ranged between 270 and 340 g/kg sample while the control had a range between 430 and 510 g/kg sample. The 100 g/kg sample of BSGPs and BSGF had no significant effect ($p > 0.05$) on the syneresis level of produced SYA. After 14 days of refrigerated storage, a significant ($p < 0.05$) decrease in syneresis of SYA control was observed while BSG derivatives addition remained stable, except BSGP-C. Although SYA control had a decrease in syneresis level, it had a higher level than BSG-added SYA. BSG derivatives were able to bind water in the matrix thus preserving

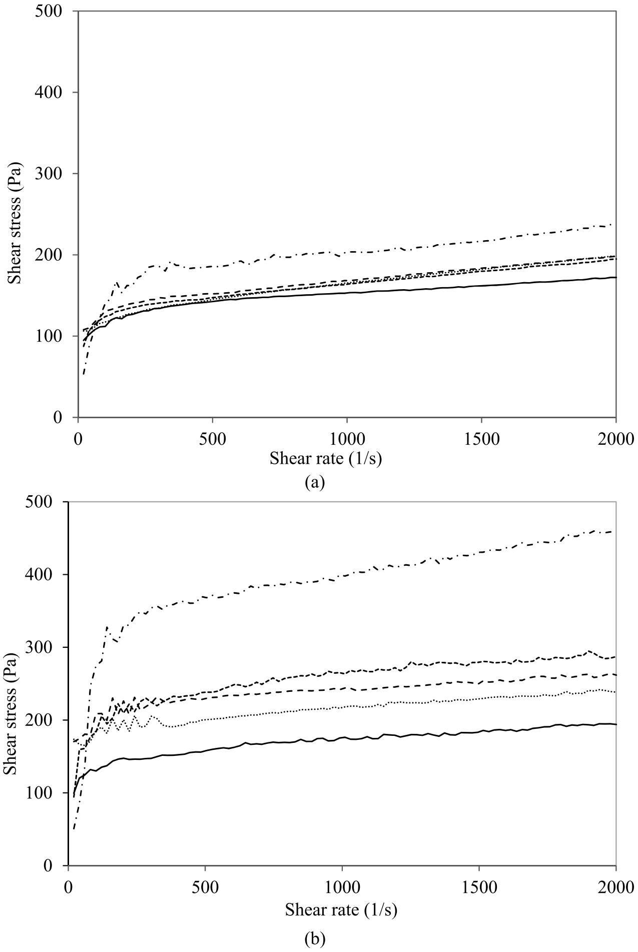


Fig. 5. Rheological behavior: relation between shear stress and shear rate of SYA prepared without BSG derivatives (—) and with 100 g/kg different BSG derivatives during the storage (a. 1 day of storage, b. 14 days of storage). (BSGP-C: spent grain protein prepared without protease extraction (····), -PF: with protease and flavorzyme extraction (—), -P: with protease extraction (- - -); BSGF: spent grain flour (-·-·-)).

the consistency and flow behavior as discussed in the previous section. Compared to previous studies, soy-based yogurt has syneresis levels ranging between 330 and 490 g/kg (Mei et al., 2016; Park & Lee, 2015; Rinaldoni, Campderrós, & Pérez Padilla, 2012). The ability of BSG in lowering the syneresis of yogurt also has been identified previously (Naibaho et al., 2022b; 2022c; 2022d). This phenomenon might be aligned with the ability of BSG derivatives to intensify the strength of formed matrices. For instance, BSGP-C had a higher syneresis than other BSG-added SYA. BSGP-C might have weak bonds in the matrix. It was observed in chemical surface characteristics by FTIR (Section 3.3) that this extract contained different transmittance functional groups.

3.6. *The acidity: pH and lactic acid production*

The pH and lactic acid productions on SYA were assessed during the storage and the results are presented in Table 2. In general, the pH of SYA significantly ($p < 0.05$) increased after 14 days of refrigerated storage in all observed groups. SYA control had a higher pH range compared to that with the BSG derivatives addition which ranged 5.2–5.3 and 4.9–5.2 respectively. SYA prepared with BSG derivatives had a significantly ($p < 0.05$) higher level of acidity compared to control. BSG derivatives enhanced the acid production almost up to 3 times higher than the control. The control had a range of 3.8–4.4 g/kg sample of lactic acids while it was 6–11 g/kg sample in SYA substituted with BSG derivatives. During the storage, the acidity remained stable in all observed groups. The fluctuation of pH and lactic acid during the storage might be due to the fluctuation in the survival of LAB, although the amount of LAB was not included in the current study. In addition, the possibility of BSGF and BSGPs in buffering the yogurt matrix has been shown at the initial pH before the fermentation (Fig. 1) although the pH has been adjusted to a range of 4.3–4.7 due to fermentation. The amount of LAB might fluctuate during fermentation thus the amount of produced lactic acid. The rise in pH might be due to the decrease in the survival of LAB after 14 days of storage. In other words, incorporation of BSG derivatives both BSGPF and BSGPs might perform a higher survival rate of LAB in SYA during the storage period.

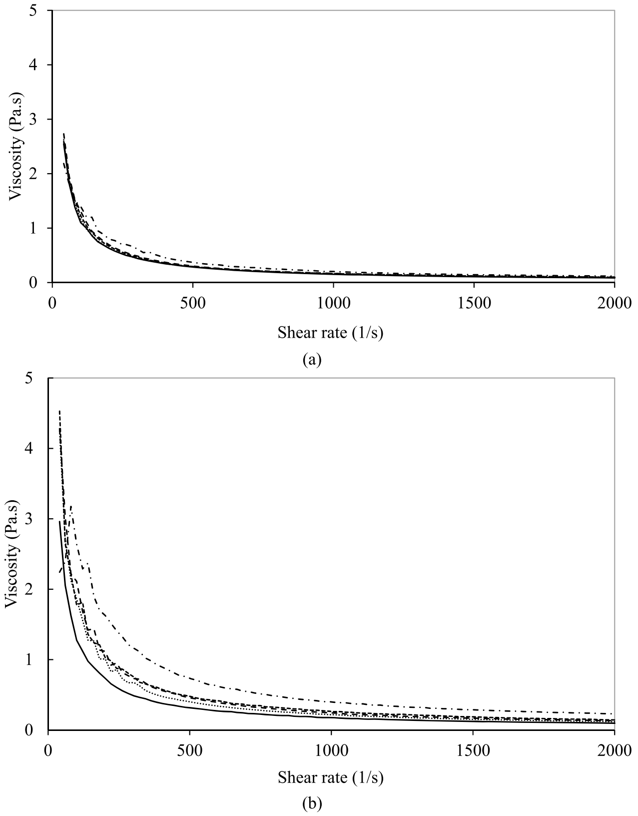


Fig. 6. Rheological behavior: relation between viscosity and shear rate of SYA prepared without BSG derivatives (—) and with 100 g/kg different BSG derivatives during the storage (a. 1 day of storage, b. 14 days of storage). (BSGP-C: spent grain protein prepared without protease extraction (····), -PF: with protease and flavorzyme extraction (—), -P: with protease extraction (- - -); BSGF: spent grain flour (-·-·-)).

The enhancement of lactic acid production in SYA has been reported previously. The addition of inulin and germinated soy bean extracts improves lactic acid up to 6.8 g/kg sample (Cho et al., 2013; Rinaldoni et al., 2012), incorporation of kimchi as a source of LAB strain combined with D-allulose increases the production of lactic acid to 12 g/kg sample (Kim & Han, 2019), and the addition of apple juice increases it up to 14 g/kg sample (İçier et al., 2015). The ability in enhancing the lactic acid production might be influenced by LAB strain and the ingredients used. Important to note that BSG derivatives improved the acid production to the same amount as that in milk based yogurt which ranges between 6 and 9 g/kg sample (Gürbüz, Erkaya-Kotan, & Şengül, 2021; Mehrinejad Choobari, Sari, & Daraei, 2021). Three different BSGPs in the current study generated SYA with a higher acidity. As mentioned earlier, the BSGP-C was observed to have a lowest amount of protein content therefore produced a lower amount of acid when it was applied in SYA. By this, the amount of available protein in BSG derivatives influenced the growth of LAB thus the production of acids. In the current study,

Table 2
Physicochemical properties and flow behavior of soy-based yogurt alternatives (SYA) enriched with 100 g/kg spent grain derivatives during 14 days of storage.

Storage period	SYA with BSG derivatives				
	Control (No BSG)	BSGP-C	BSGP-PF	BSG-P	BSGF
pH					
1	5.19 ± 0.01^{b}	4.94 ± 0.01^{f}	5.01 ± 0.01^{d}	5.11 ± 0.01^{c}	4.91 ± 0.01^{g}
14	5.25 ± 0.01^{a}	5.01 ± 0.01^{d}	5.12 ± 0.01^{c}	5.19 ± 0.01^{b}	4.98 ± 0.01^{e}
Acidity, expressed as lactic acid (g/kg sample)					
1	3.8 + 0.04^{f}	7.9 + 0.04^{d}	10.1 + 0.03^{ab}	9.3 + 0.01^{bc}	6.0 + 0.04^{e}
14	4.4 + 0.01^{f}	8.1 + 0.00^{d}	10.7 + 0.02^{a}	8.7 + 0.04^{cd}	6.3 + 0.00^{e}
Syneresis (g/kg sample)					
1	512.3 + 2.69^{a}	289.0 + 0.72^{d}	273.6 + 0.39^{d}	296.4 + 0.08^{d}	328.2 + 1.05^{cd}
14	436.8 + 3.57^{b}	380.2 + 2.21^{bc}	305.2 + 2.67^{d}	334.5 + 0.43^{cd}	343.1 + 0.59^{cd}
Consistency index, k (Pa.s)					
1	66.24 ± 1.47^{de}	44.76 ± 12.51^{e}	54.90 ± 0.10^{de}	59.76 ± 1.90^{de}	80.70 ± 10.05^{cde}
14	70.68 ± 6.72^{cde}	107.94 ± 2.68^{bc}	91.62 ± 16.35^{cd}	141.42 ± 14.21^{ab}	150.95 ± 12.11^{a}
Flow behavior index, n					
1	0.12 ± 0.00^{abc}	0.19 ± 0.04^{a}	0.16 ± 0.00^{ab}	0.15 ± 0.00^{abc}	0.14 ± 0.02^{abc}
14	0.13 ± 0.02^{abc}	0.10 ± 0.00^{bc}	0.16 ± 0.03^{ab}	0.08 ± 0.00^{c}	0.14 ± 0.00^{abc}
η_{50} (apparent viscosity, Pa.s)					
1	0.15 ± 0.00^{e}	0.17 ± 0.00^{de}	0.16 ± 0.00^{de}	0.17 ± 0.00^{de}	0.20 ± 0.00^{cde}
14	0.18 ± 0.01^{de}	0.22 ± 0.00^{bcd}	0.27 ± 0.03^{b}	0.24 ± 0.02^{bc}	0.40 ± 0.02^{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. Control: SYA without spent grain derivatives, BSGP-C: spent grain protein prepared without protamex extraction, -PF: with protamex and flavorzyme extraction, -P: with protease extraction; BSGF: spent grain flour.

protein availability in BSG derivatives might have influenced the initial synergistic growth of *S. thermophilus* and *L. bulgaricus* during the fermentation. It has been reported that *S. thermophilus* consumes the amino acids available in the matrix to grow and generate peptides. This peptide is then used by *L. bulgaricus* to grow (Chandan & O'Rell, 2013) and produce peptides which support the growth of 2 other strains including *Lactobacillus acidophilus,* and *Bifidobacterium lactis*. The fluctuation of pH and acidity during the storage might be mainly caused by survival of *Lactobacillus acidophilus,* and *Bifidobacterium lactis* which need to be confirmed in further studies.

4. Conclusion

In conclusion, BSG derivatives including BSGF and BSGPs potentially to be used as a sustainable food ingredient particularly in soy-based yogurt-alternatives production. BSG derivatives allowed the growth of LAB which was shown by preservation in pH during refrigerated storage as well as the higher amount of acid production. The substitution of BSG derivatives in SYA fermentation fluctuated the fermentation period due to its impact on the growth of LAB thus consequently on the textural formation. BSG derivatives modified the microstructural formation during the fermentation process thus allowing to preserve the flow behavior and consistency during the storage. The limitation of the study is that the influence of BSG affecting the growth of specific species of LAB remains unclear, in addition to the possibility of BSG in altering the sensory acceptability of SYA. Therefore, the evaluation on LAB profile and amount presented in such products is seemingly important to be studied in near future. In addition, biological properties and consumer acceptability also need to be emphasized for further study.

Author contributions

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

Emir Jonuzi: methodology, formal analysis, writing - review and editing.

Nika Butula: methodology, formal analysis, writing - review and editing.

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

Baoru Yang: writing-review and editing, project administration, and funding acquisition.

Declaration of competing interest

Authors declare no conflict of interest.

Data availability

The data has been included in the submission

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114626.

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