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Potential of brewers' spent grain in yogurt fermentation and evaluation of its impact in rheological behaviour, consistency, microstructural properties and acidity profile during the refrigerated storage

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ABSTRACT

Brewers' spent grain (BSG) contains high amounts of dietary fiber, which may regulate the food matrix behaviour. The study aimed to evaluate the impact of spent grain on yogurt. Different levels of BSG were substituted in yogurt fermentation and its impacts on microstructural properties including surface chemical properties and confocal microstructures were studied. The quality of the yogurt was evaluated by determining rheological behavior, syneresis, color, acidity and the amount of lactic acid bacteria (LAB) at 1, 7 and 14 days of storage. The addition of BSG shortened the fermentation time, increased the viscosity and shear stress. FTIR analysis showed that BSG intensifies the band region stretching which describes a modification in a certain functional group. Confocal laser scanning microscopy identified a disruption in the cross-link between fat-protein and yogurt matrix due to the addition of BSG. During the storage, BSG was able to maintain the flow behaviour and stability, which was shown as the change in flow behaviour and syneresis. Furthermore, BSG provided the nutrients for the survival of LAB during the storage and maintained the amount of lactic acid in yogurt during the 14 days of refrigerated storage. The substitution 5%–10% of BSG improved the quality of yogurt as the maximum quality such as acidity, rheological behavior and LAB growth. Even though 15%–20% of BSG generated the same level of acidity and LAB, and had the lowest syneresis, it diminished the flow performance of the yogurt.

1. Introduction

The valorization of BSG in food products has been reviewed and it is reported that the addition of BSG tends to improve the nutritional value of BSG-added food products (Naibaho & Korzeniowska, 2021b). BSG contains biological compounds including phenolic compounds, amino acids, lipid and fatty acids, and dietary fiber which are responsible for the biological activity of BSG including antioxidant activity, antimicrobial activity, DNA protective and antimutagenic properties, anti-inflammatory and colon health maintenance properties (Naibaho & Korzeniowska, 2021b; Nigam, 2017). Therefore, the potential of BSG as a functional food ingredient and a nutraceutical ingredient has been increasingly evaluated.

BSG possesses a high amount of dietary fiber which is dominated by insoluble fiber (IDF) approximately 36–52%; and IDF tends to be responsible for the texture of BSG-added food products (Ačkar et al., 2018; Fărcaş et al., 2015; Föste, Verheyen, Jekle, & Becker, 2020; Heredia-Sandoval et al., 2020; Ktenioudaki et al., 2015). Therefore, BSG hardened the texture of food products such as bread, cookies, and some snacks products (Ačkar et al., 2018; Fărcaş et al., 2015; Heredia-Sandoval et al., 2020; Ktenioudaki et al., 2015; Nocente, Taddei, Galassi, & Gazza, 2019). This phenomenon occurs due to a high-water

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holding capacity (WHC) of BSG (Naibaho & Korzeniowska, 2021a). WHC properties of BSG seems to be a challenge in regulating the consistency and flow behaviour of yogurt products. Furthermore, high amounts of dietary fiber in BSG strengthen the potential of BSG as a prebiotic in yogurt as a functional food (Amorim, Silvério, & Rodrigues, 2019; Xiros & Christakopoulos, 2012) due to the presence of arabinoxylans and b-glucans which support the microbial growth (Lao, Dimoso, Raymond, & Mbega, 2020); valorization of BSG in lactic acid production due to the abundance of lignocellulosic materials has also been observed (Nigam, 2017). Therefore, BSG has a potential to be a suitable ingredient in the yogurt production.

Those properties might be influenced by the particle size as a function of processing behaviour especially in the milk system in yogurt matrix. Preliminary study (unpublished data) showed that different particle sizes of BSG generated different physico-chemical and lactic acid bacterial growth. This might be due to the nutritional availability and different ability in binding oil and water. BSG possesses a high level of water holding capacity as well as oil holding capacity (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021a). By this, BSG could act as an emulsifier in the yogurt matrix. Reduction in particle size of BSG causes depolymerization and debranching in dietary fiber (Reis, Coelho, Coimbra, & Abu-Ghannam, 2015; Severini et al., 2015). Consequently, it allowed the protein release from aleurone cells (Niemi et al., 2013) and increased the solubility of arabinoxylans (Reis et al., 2015; Severini et al., 2015) due to the degradation of cell walls. This phenomenon might modify the network formation in yogurt. It also modified the bulk density of BSG (Naibaho & Korzeniowska, 2021a). In fact, different sources of breweries impacted the constituents and characteristics of BSG including proximate composition, dietary fiber constituents, antioxidant capabilities and polyphenolic composition (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021a).

The addition of prebiotic ingredients in yogurt production has been shown to enhance the viability of probiotics thus improving the quality of yogurt as a functional food (Meybodi, Mortazavian, Arab, & Nematollahi, 2020). Moreover, the nutritional enrichment in yogurt has been reported by the addition of other ingredients such as protein enrichment, quince seed mucilage powder, forsk seed mucilage, plant extracts (Cavalheiro et al., 2020; Choobari, Sari, & Daraei, 2021; Gürbüz, Erkaya-Kotan, & Sengül, 2021; Meybodi et al., 2020; Minh, 2021). From the perspective of the market, the value of yogurt production steadily increased worldwide from 38.7 billion USD (2018) to a prediction approximately at 51.2 billion USD in 2024 (Shahbandeh, 2020). The utilization of BSG in yogurt production will add beneficial market value for industry because BSG is a low-cost material. Besides the nutritional value of BSG, its potential as an ingredient in yogurt production is supported by the abundance of BSG production. Almost 2 billion hectolitres of beer are produced (Conway, 2019) which generates 40 million tons of BSG annually worldwide (Petit et al., 2020). About 8 million tons of BSG are generated in Europe (Petit et al., 2020) and Poland is in the top ten as the highest beer production worldwide and the second place in European countries after Germany (Conway, 2021). Yogurt production in Poland has shown an increase recently (Sas, 2021), thus the utilization of BSG in yogurt production will economically benefit the industry.

Besides the fact that valorization of side streams, incorporating BSG in yogurt could be beneficial for the idea of the transformation from animal-based diet towards plant-based diet. The study aims to evaluate the influence of different levels of BSG addition in yogurt fermentation and its impact on the physico-chemical properties of yogurt. Surface chemistry evaluation and confocal microstructure were evaluated in order to study the microstructural properties of BSG-added yogurt. Moreover, the quality parameters during the refrigerated storage such as rheological behaviour, syneresis, the acidity and the survival of LAB were evaluated in addition to color value. It was expected that BSG could regulate the flow behavior, reduce the syneresis and allow the lactic acid bacteria to grow.

2. Materials and methods

2.1. Materials

The BSG samples were collected from a local brewery, which produces light beer types (comparable to Budweiser light), in Poland. In general, the BSG is generated as follows: the selected barley kernel goes to some steps including steeping, germinating, mashing and filtration. The filtration generates 2 different fractions: liquid and solid fraction. The liquid fraction then goes to further beer production while the solid fraction is called BSG (Wen, Zhang, Duan, Zhang, & Ma, 2019). The collected BSG was treated as described in the previous study (Naibaho et al., 2021). Briefly, BSG were dried using convective drying to reach stable weight (moisture 2–5%), ground in a laboratory mill and sieved through <250 μm sieving. Finally, the BSG samples were packed into the aluminium foil bag and kept in cold temperature (4 °C) for further study.

Pasteurized-homogenized milk (3.2% fat content; 3% protein; 4.7% carbohydrates and 0.1% salt) and microbial culture with composition maltodextrin, *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp *bulgaricus, Lactobacillus acidophilus,* and *Bifidobacterium lactis*, were obtained from the commercial market. Dry microbial substrate (MRS and M-17) and microbial agar were purchased from Merck, Germany, cycloheximide was from Applichem, and staining Nile Red and Rhodamine 123 from Sigma-Aldrich. All chemicals used for analyses were analytical grade.

2.2. Yogurt preparation

Yogurt were prepared following the instructions in microbial culture packaging with a slight modification with previous study (Szołtysik et al., 2020). BSG was added into the milk with ratio 0:100, 5:95, 10:90, 15:85 and 20:80 (BSG:milk; *w/w*) and then mixed properly. The mixtures were then pasteurized at 90 °C for 15 min and cooled down to 38–43 °C. The microbial cultures were added at 0.05% of the total mixture (*w/w*) and mixed properly. The mixtures were kept at 43 °C to reach pH between 4.3 and 4.8. The pH was recorded throughout the process. During the pH observation, the mixture was homogenized slowly to diminish the sedimentation of BSG during the fermentation. The homogenization was done periodically every hour for 10 s using a laboratory scale mixer at the lowest speed (260 rounds/min; 4 cm gap). Once the pH range was achieved, the fermentation was ended by homogenizing using a laboratory scale mixer at a higher speed for 10 s (380 rounds/min; 4 cm gap) and cooling down to 15 °C. After that, the yogurt was stored at 4 °C for 18 h before the analyses. Some samples were separated for freeze-drying for the microstructural evaluation (*Fourier transform infrared spectroscopy* and confocal laser scanning microscopy). The yogurt was stored at 4 °C and the analyses were conducted at 1, 7, and 14 days of storage. The measurements were done at least in duplicate.

2.3. Microstructural analysis

2.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR measurement was conducted using IRSpiritTM, Shimadzu (Shimadzu Europe, GmbH) following the instruction of the instrument. The measurement was observed at 4000 and 400 cm^{-1} and the data was analysed using the eFTIR software.

2.3.2. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was performed in order to evaluate the microstructure of yogurt following methods as described in the previous study (Nguyen, Ong, Kentish, & Gras, 2014, 2015; Ciron, Gee, Kelly, & Auty, 2012; Torres, Amigo Rubio, & Epsin, 2012) with some modification. CLSM analysis was conducted by Leica SP8 MP Confocal Microscope BADD-002030 (Germany). For storage reasons, the yogurt samples were prepared in freeze-dried form and kept at a cool temperature prior to the analysis. For the analysis, the samples were stained with 10 μg/ml in water Nile Red (72485, Sigma-Aldrich) and 10 μg/ml in water Rhodamine 123 (R8004, Sigma-Aldrich). A small amount of the sample (9–30 mg) was suspended in a staining solution at 1:4 ratio (yogurt:staining solution; *w:v*). The mixture was then transferred into a glass slide and covered with a coverslip. The image was captured on a confocal microscope using a 20x (NA 0.75) air objective. In addition to the fluorescence of a given dye, a reflected laser light was collected in order to visualize the volume and the structure of the sample. Nile Red was excited with a 561 nm laser and the reflected light channel was generated with a 488 nm laser while Rhodamine 123 was excited with the 488 nm laser and the reflected light channel was generated with a 638 nm laser. For each sample, the image was scanned in three representative fields of view in the Z axis (10–80 μm thick, 0.68 μm intervals).

2.4. Evaluation of rheological behaviour

Yogurt samples were left at room temperature for 30 min before the measurement. The measurement was carried out using a rheometer Haake RheoStress 6000 rotational, with thermostatic bath, Haake A10, and a UTM Controller (Thermo Electron GmbH, Karlsruhe, Germany). The measurements were conducted at 20 °C in a plate cone (C60/1° Ti L no.222-1868/stainless steel plate TMP60 no.222-1891) geometry system with a gap of 1 mm, in which 1 ml sample was added. The shear rate was recorded from 0 to 2000/s, shear stress and viscosity were recorded at increasing shear rate (Szołtysik et al., 2020). Due to the presence of grainy particles of BSG, with the particle size <250 μm, the yogurt firstly was slowly homogenized to avoid sedimentation prior to the measurement. It was suggested that a bigger particle size might require a bigger gap size (Barnes, 2000). Because the instrument procedures provide 1 mm gap, therefore, homogenization prior to measurement was done to minimize error during the measurement. Homogenization allowed a regular particle distribution in the system.

2.5. Syneresis

The measurement of syneresis of yogurt was carried out as described in the previous study (Bouaziz et al., 2021; Khubber, Chaturvedi, Thakur, Sharma, & Yadav, 2021). A 5 g of the sample was centrifuged for 15 min at 4500 rpm and 10 °C. The supernatant was separated and the syneresis was calculated following the equation:

$$Syneresis\ (\%) = \frac{Weight\ of\ supernatant\ (g)}{Weight\ of\ yogurt\ (g)}\ x\ 100$$

2.6. Color measurement

The color of yogurt was measured by Minolta Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan). Before the measurement, the instrument was calibrated using a white board which is equipped along with the instrument. The color value of yogurt was determined as L*, a* and b* which stands for lightness, redness to greenness, and yellowness to blueness, respectively.

2.7. The analysis of pH and lactic acid

The pH was measured by InoLab pH-meter following the instructions of the instrument. The total lactic acid was assessed by titration method as described by previous study (Szołtysik et al., 2020). Distilled water was added to the yogurt (1:1) and the indicator phenolphthalein was added. The mixture was titrated with 0.25N NaOH. The total acid was calculated following the equation:

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

2.8. The analysis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

Microbial analysis was performed following ISO 7889/IDF 117 2003. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were counted in MRS (deMan, Regosa and Sharpe) and M-17 agar respectively by deep-plate method with several dilutions. The plates were incubated for 48 h at 37 °C and bacterial counts were performed in a log CFU/g sample referred to lactic acid bacteria (LAB) (Szołtysik et al., 2020).

2.9. Statistical analysis

Statistical analysis was performed using Statistica software (version 13.5.0.17). Two-ways analysis of variance (ANOVA) was conducted to evaluate the significance difference at $p < 0.05$ followed by Tukey post-hoc test.

3. Results and discussion

3.1. Fermentation time of BSG-added yogurt

The change in pH during the fermentation process was observed and is shown in Table 1. The range of pH (4.3–4.7) was obtained after 3–4 h of fermentation. In general, the addition of BSG significantly increased the fermentation rate which is shown by a lower pH at the observed period during the fermentation, except at 4th hours, even though there are some significant differences ($p < 0.05$) in pH during the fermentation among the group and observed period. During the observation, the highest derivation of pH occurred between the second and third hour of fermentation. At this stage, it was observed that the texture formation started to occur and less sedimentation was formed. This phenomenon might be due to the sinergic growth of *S. thermophilus* and *L. bulgaricus*. Initially, pH decreased due to the growth of *S. thermophilus* by using free amino acids in the mixture, providing peptides for *L. bulgaricus* growth. As the pH decreased, *L. bulgaricus* grew faster and produced a higher amount of lactic acid thus significantly lowering the pH (Chandan & O'Rell, 2013). In this study, the presence of *L. acidophilus* and *B. lactis* from the microbial culture also influence the fermentation time and process. The impact of BSG in the change of pH during the fermentation shows the effect of BSG addition in the rate production of LAB. By this, BSG might contribute to the availability of amino acids for LAB growth. This effect might be due to the potential of BSG as a prebiotic for its high amount of dietary fiber and protein (Amorim et al., 2019; Lao et al., 2020; Wen et al., 2019). BSG consists of arabino-xylooligosaccharides (AXOS) which are categorized as prebiotic nutraceuticals (Amorim et al., 2019; Xiros & Christakopoulos, 2012) and thus might increase the fermentation rate. In comparison to previous studies, the addition of plant-based ingredients in yogurt production tends to increase the fermentation time (Adepoju & Selezneva, 2020; Brodziak et al., 2021; Minh, 2021). For instance, the incorporation of moringa leaf powder, sea buckthorn mousse, and other anthocyanin-rich plants extended the fermentation time up to 6–8 h in order to reach a range of pH between 4.5 and 4.6 (Adepoju & Selezneva, 2020; Brodziak et al., 2021; Minh, 2021). The increase in fermentation rate of yogurt has been reported as an impact of the protein availability (Cavalheiro et al., 2020; Choobari, Sari, & Daraei, 2021; Kokabi et al., 2021). The proteolytic action of added microbes in yogurt production reduces the fermentation time as an impact of the releasing in amino acids thus improving the microbial growth and finally shortened the fermentation process (Cavalheiro et al., 2020). Furthermore, the addition of protein and fat replacer contributed to the shorter fermentation time (Cavalheiro et al., 2020; Choobari, Sari, & Daraei Garmakhany, 2021; Kokabi et al., 2021). By this, the impact of BSG in shortening the fermentation period might be due to the presence of protein and fat from BSG. The milk used in this study was claimed to contain 3.2% and 3% of fat content and protein respectively while BSG

Table 1
The change in pH during the fermentation of BSG-added yogurt.

BSG (%)	pH during the fermentation time								
	0 h	1 h	$\Delta_{(0-1)}$	2 h	$\Delta_{(1-2)}$	3 h	$\Delta_{(2-3)}$	4 h	$\Delta_{(3-4)}$
0	6.23 ± 0.01^{a}	6.06 ± 0.02^{b}	0.17 ± 0.03^{jk}	5.80 ± 0.01^{cd}	0.26 ± 0.01^{hij}	4.88 ± 0.03^{j}	0.92 ± 0.04^{a}	4.30 ± 0.08^{n}	0.58 ± 0.06^{de}
5	6.23 ± 0.01^{a}	5.86 ± 0.01^{c}	0.37 ± 0.00^{g}	5.53 ± 0.00^{gh}	0.33 ± 0.01^{gh}	4.68 ± 0.01^{kl}	0.85 ± 0.01^{ab}	4.20 ± 0.00^{o}	0.48 ± 0.01^{f}
10	6.23 ± 0.01^{a}	5.72 ± 0.00^{de}	0.51 ± 0.01^{ef}	5.45 ± 0.00^{hi}	0.27 ± 0.00^{hi}	4.65 ± 0.01^{l}	0.81 ± 0.01^{b}	4.31 ± 0.01^{n}	0.34 ± 0.00^{gh}
15	6.23 ± 0.01^{a}	5.63 ± 0.00^{ef}	0.60 ± 0.01^{de}	5.43 ± 0.01^{i}	0.20 ± 0.01^{ij}	4.63 ± 0.01^{l}	0.81 ± 0.01^{b}	4.31 ± 0.01^{n}	0.32 ± 0.01^{gh}
20	6.23 ± 0.01^{a}	5.56 ± 0.02^{fg}	0.67 ± 0.03^{cd}	5.46 ± 0.00^{hi}	0.10 ± 0.02^{k}	4.77 ± 0.00^{k}	0.69 ± 0.00^{c}	4.46 ± 0.05^{m}	0.32 ± 0.05^{gh}

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

had been reported for its fat content at 9–13% and 22–30% protein content (Naibaho & Korzeniowska, 2021a). By this, the substitution of milk with several levels of BSG will improve the protein content to a range of 3.9%–8.4%. Fat content of BSG will replace the milk fat and increase the amount of fat content to a range of 3.49%–5.16%. In other words, the substitution of fat composition from BSG might be responsible as a fat and protein replacer which modified the fermentation time and improved the rheological behaviour of the yogurt. This phenomenon can be also influenced by the homogenous distribution in food matrices. The main proteins in BSG are hordein, glutelin, globulin and albumin, and the main amino acids in BSG are glutamine, proline, and leucine (Connolly, Piggott, & FitzGerald, 2013; Wen et al., 2019); lipid contents in BSG are including propionic, acetic, butyric acids, palmitic, linoleic, oleic, stearic acid, and tocotrienols (Bohnsack, Ternes, Büsing, & Drotleff, 2011; Fărcaş et al., 2015; Parekh, Khanvilkar, & Naik, 2017; Patel, Mikes, Bühler, & Matsakas, 2018; Tan, Mok, Lee, Kim, & Chen, 2019; Teixeira et al., 2020). Several lipid compositions from BSG have been identified for its ability as emulsifier, stabilizer, lubricants, food textural, and softening agents (Parekh et al., 2017) as well as emulsifying properties of protein from BSG (Wen et al., 2019) thus influencing the textural formation during the pH derivatization.

3.2. The impact of BSG in microstructural properties of yogurt

3.2.1. Fourier transform infrared spectroscopy

The FTIR spectrum of freeze-dried BSG-added yogurt aims to evaluate the surface chemistry of the samples, as is shown in Fig. 1. In general, all the yogurt has the same pattern in terms of absorbance and slightly different in terms of absorption intensity. As shown in Fig. 1, the intensity absorption of control (0% BSG) showed a higher absorbance thus had a lower intensity of transmittance. There are 8 band regions identified which represent the functional groups in BSG-added yogurt as shown in Table 2.

The band region at 3600-3200 cm^{-1} is due to the hydroxyl stretching which describes the presence of hydroxyl and amine groups. This signal shows the abundance of cellulose and hemicellulose from BSG. band signal at region 3000-2800 cm^{-1} aligned with the C–H asymmetric stretch from CH_2 functional groups. This region describes the structure of polysaccharide compounds in BSG. The observed stretching signal at 1800-1700 cm^{-1} represents the lignin and C=O stretch from fatty acids and its esters; the signal at 1700-1600 cm^{-1} shows the signal of amide I and amide II or to the aromatic hydrocarbons of lignin. The stretching at 1300–1200 cm^{-1} represents amide III or the cleavage of acetyl groups in yogurt. The band region at 1100-1000 cm^{-1} is due to the stretching of C–O–C which shows the functional groups of aliphatic ethers. The band region at 900-800 cm^{-1} indicates the β-linkage of cellulose. Finally, the band region at 800-500 cm^{-1} identifies the presence of α-glycosidic bonds (Brodziak et al., 2021; Nielsen, 2017; Patrignani et al., 2020; Ravindran, Jaiswal, Abu-Ghannam, & Jaiswal, 2018; Zhang, Cao, Yin, &

Table 2
The identified functional groups of BSG-supplemented yogurt by FTIR spectrum.

No	Band region (cm-)	Functional group
1	3600–3200	hydroxyl groups from cellulose and hemicellulose
2	3000–2800	structure of polysaccharide compounds and long fatty acids
3	1800–1700	fatty acids and fatty acid esters
4	1700–1600	aromatic hydrocarbons of lignin; Amide I
5	1300–1200	acetyl groups, amide III
6	1100–1000	aliphatic ethers and acid functional groups
7	900–800	β-linkage of cellulose
8	800–500	α-glycosidic bond

Reference: Nielsen, 2017; Naibaho et al., 2021; Brodziak et al., 2021.

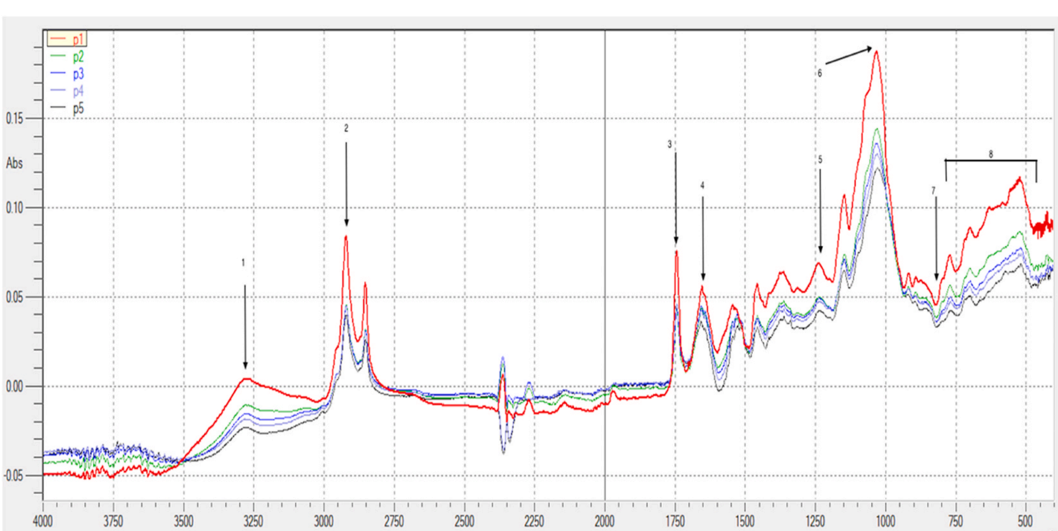

Fig. 1. FTIR spectrum of freeze-dried BSG-added yogurt: P_1. 0% BSG (control); P_2. 5% substitution; P_3. 10% substitution; P_4. 15% substitution; P_5. 20% substitution.

Wang, 2018).

Those bands have been identified previously in BSG and yogurt products (Brodziak et al., 2021; Naibaho et al., 2021). Fig. 1 shows there are some functional groups which were identified to have a higher absorption intensity (lower absorbance) due to the presence of BSG, especially in hydroxyl groups from polysaccharides (1 and 2), fatty acids and its ester (3 and 6), and α-glycosidic bond (8). By this, the addition of BSG impacts the chemical surface of the yogurt which has been observed by stretching band in FTIR analysis. The impact on functional groups might affect the properties of BSG-added yogurt including physical behaviour and biological activity.

3.2.2. Confocal laser scanning microscopy

CLSM was performed to identify the microstructure of the yogurt as presented in Figs. 2 and 3. The staining Nile red and Rhodamine 123 was used to stain fats and protein respectively. The result shows that BSG addition disrupts the cross-link between fat and protein with the matrix in yogurt. As is shown in Fig. 2, the yogurt control (Fig. 2a) has a more regular distribution of fat than that in BSG-added yogurt (Fig. 2b–e). During the pH observation through the fermentation process, the yogurt samples were homogenized for 10 s using a laboratory scale mixer with the lowest speed, thus generating a homogenous yogurt matrix. However, sedimentation formation of BSG was observed which might have influenced the homogeneity and the porosity of fat and protein matrix in the yogurt matrix. BSG entrapped more water thus lowering the local distribution of free water. Higher addition of BSG formed networks which look similar to barley polysaccharides microstructure (Wijngaard, Renzetti, & Arendt, 2007) and the tendency for having the granule-like appearance (Verni et al., 2020). Barley has an unstructured-appearance of protein microstructure surrounded by starch (Wijngaard et al., 2007). Based on light micrographs on barley, the majority of fat seems to be bounded with polysaccharides while amino acids separated in cell walls and aleurone (Nair, Knoblauch, Ullrich, & Baik, 2011). Scanning electron microscopy on BSG revealed that the fiber filaments were the most visible in the microstructure of BSG (Ktenioudaki, Chaurin, Reis, & Gallagher, 2012). Therefore, the network disruption in yogurt might be due to the presence of filament fiber. The higher the amount of BSG, the more irregular the fat matrixes and distribution. The same phenomenon is observed in protein (Fig. 3). Yogurt control has a more homogeneous protein distribution and porosity (Fig. 3a) while the addition of BSG disrupts the protein network. This phenomenon might be due to the ability of BSG to bind high amounts of water and affect the density of structured network thus disrupting the network formation of fat and protein. Furthermore, the substitution of BSG generated a more compact shape microstructure entities of fat and protein matrix. BSG is a complex ingredient which has been reported for diminishing the network formation in baked products (Naibaho & Korzeniowska, 2021b). This phenomenon might be aligned with the ability of BSG as a fat and protein replacer in yogurt products as mentioned previously. Moreover, the homogeneity of the particle size of BSG could influence the matrix of the yogurt. As mentioned in previous section, protein and free fat release from BSG matrix could occur due to the degradation of BSG. The high ability of BSG in binding water also diminished the mobility of protein (Pachekrepapol, Somboonchai, & Krimjai, 2021) thus reducing the homogeneity and density of protein aggregates as observed in this study. The same result has been reported in previous studies: the addition of certain fiber and plant-based extracts tend to increase the cross-link matrix and more compact protein structure (Nguyen, Ong, Kentish, & Gras, 2014; Qiu, Zhang, Mujumdar, & Chang, 2021; Zhao, Feng, & Mao, 2020). The microstructure of fat and protein globules might depend on the binding strength in the matrix (Nguyen et al., 2015). The impact of BSG in regulating the microstructure of BSG-added yogurt seems related to its ability in binding oil and water (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021a). Moreover, the presence of several lipids and proteins from BSG have been identified for its ability as an emulsifying and stabilizing agent (Parekh et al., 2017; Wen et al., 2019). The ability of BSG in binding water has been reported to disrupt the network formation of the food matrix in baked food such as bread and cookies (Naibaho & Korzeniowska, 2021b). However, this specific impact on yogurt will be aligned with flow behaviour and syneresis of yogurt which will be discussed in the next section.

3.3. The evaluation of rheological behaviour

The flow behaviour of the yogurt is shown in Fig. 4 and Fig. 5. As Figs. 4 and 5 showed that shear stress and viscosity of the yogurt are dependent on the shear rate. In general, the addition of BSG increased the shear stress and the viscosity in yogurt. Evaluation of the flow behaviour in general, the addition up to 10% of BSG seems to have the same flow behaviour as in control (BSG 0%). An excessive amount of BSG (15–20%) changes the flow behaviour of the yogurt which might negatively affect the acceptability of the yogurt. This phenomenon is aligned with the microstructure analysis in the previous section that the higher the amount of BSG addition, the disruption in fat-protein-yogurt matrix is higher. During the storage, the flow behaviour fluctuated depending on the amount of BSG substituted. The flow behaviour of the samples was measured after the homogenization process. Shear stress describes the required energy to destroy the structure of the yogurt matrix (Vénica, Spotti, Pavón, Molli, & Perotti, 2020). The decrease in the viscosity as the shear rate increases, shows a shear-thinning behaviour as has been observed in other studies of the yogurt (Pachekrepapol et al., 2021; Vénica et al., 2020). Both viscosity and shear stress fluctuated during the storage: it increased at 7th days and reached the lowest value at 14th days of storage. The increase of flow behaviour (shear stress and viscosity) in the 7th days might be due to the micelles and gel formation during the first week of storage, and this ability is lower in the 14th days. This phenomenon might be also related to the syneresis which increased during the storage period. Due to the increase in syneresis during the storage, the water was released from the matrix which consequently increased the level of dry matter impact on flow behaviour of the yogurt. The higher level of BSG addition, the lower the level of syneresis and led to a higher level of dry matter. The amount of dry matter based on the syneresis evaluation are 46–39%, 52%–69%, 69–80%, 18–98%, and 86–97% at the addition of 0, 5, 10, 15, and 20% of BSG respectively. Therefore, the released water decreased the viscosity during the measurement. However, the addition of 5–10% of BSG maintained the viscosity of the yogurt as the control in the first day of storage (Fig. 5). This phenomenon is related to the WHC of BSG as mentioned previously.

During the fermentation process, the interaction between casein micelles and polysaccharides occurred and formed gelation due to the complexation and interfacial stabilization (Huang et al., 2019, 2021; Khubber et al., 2021). This phenomenon occurred at low pH when the casein and protein milk were acidified at pH below 4.5; casein micelles acted as positively charged and polysaccharides as negatively charged (Huang et al., 2021; Khubber et al., 2021) with an electrostatic interaction. The electrostatic bond formed a dense protein gel structure and aggregated particles thus generating more elasticity rather than viscous flow behaviour (Huang et al., 2021; Khubber et al., 2021; Luo, Liu, & Pang, 2019). The intermolecular entanglement of protein-polysaccharide due to the fermentation process is related to the high hydrodynamic volume, induced a more rigid solid gel behaviour of yogurt and increased the viscosity (Huang et al., 2021; Khubber et al., 2021). By this, chemical composition of BSG might impact acidification process and electrostatic interactions. BSG is a complex material consisting of dietary fiber, amino acids, and fatty acids which might influence its interaction with milk in network formation. Moreover, it might also be related to hydrophobicity level of protein and its ability in foaming, turbidity, stability and solubility (Wen et al., 2019) to interact with hydrophobic casein fractions-like e.g. β-caseins or parts of α-caseins thus having the same interaction as previously reported (Huang et al., 2021). Moreover, small amounts of pectin present in BSG (Langenaeken

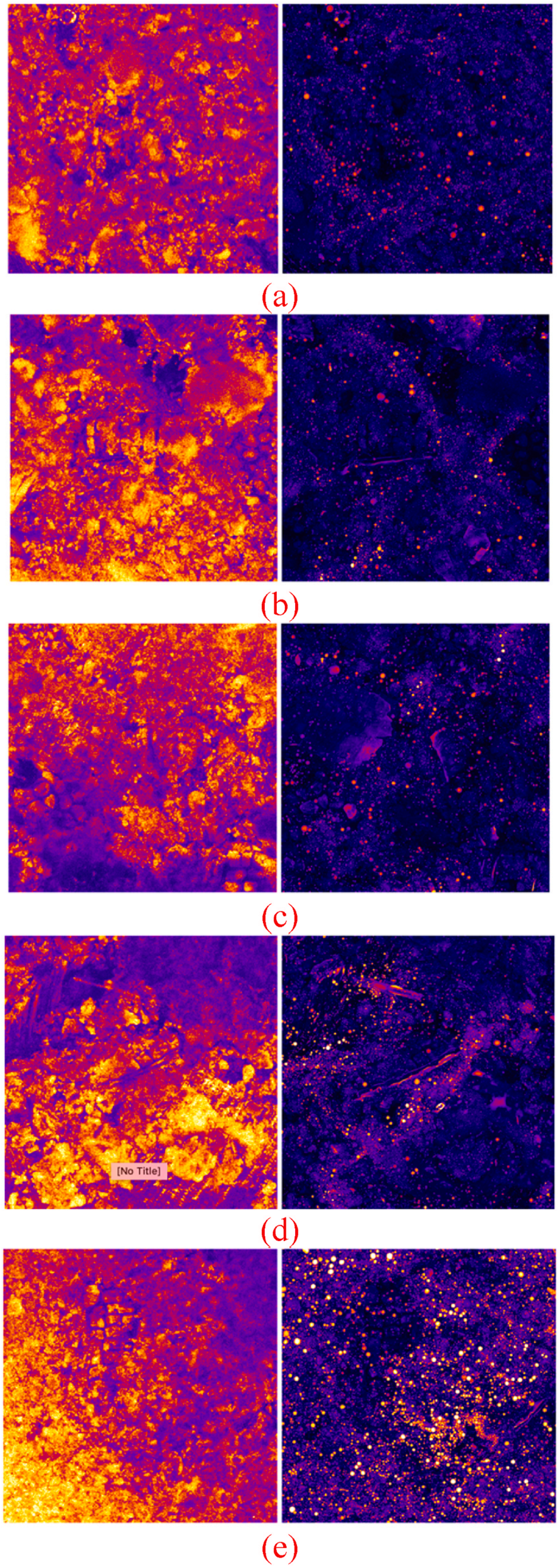

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Fig. 2. Confocal laser scanning micrographs of freeze-dried BSG-added yogurt stained with Nile red. Fat distribution is shown by the silver-yellow a. 0% BSG (control); b. 5%; c. 10%; d. 15%; e. 20% substitution. The images are presented as maximum intensity projections from confocal Z stacks in a fire intensity scale. (left: yellow channels describe formed fat network; right: blue channels describe yogurt structure visualized with a laser reflection). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

et al., 2020) which might contribute to the casein-pectin interaction as reported previously (Khubber et al., 2021). Viscosity of yogurt is also affected by the variation in polysaccharides molecular characteristics such as molecular weight, conformation and charge density (Huang et al., 2021). Moreover, structural features of the hydrocolloid backbone and side chain of the added-ingredients molecules influenced the complexity of the yogurt matrix (Huang et al., 2021).

Interestingly, 14 days storage had a different pattern of flow behaviour (shear rate vs shear stress) compared to 1 and 7 days of storage (Fig. 4c). In comparison to other BSG level addition, 15% BSG had an increase in shear stress in the increase of shear rate. This shows that 15% BSG influenced a stronger structural interaction between protein-fat and polysaccharide that might occur due to the LAB growth which generated metabolites and peptides in different levels (Chandan & O'Rell, 2013). Moreover, an increase in the formed network could occur due to the hydration of macromolecules and stabilization properties of certain ingredients during the refrigerated storage (Ramírez-Sucre & Vélez-Ruiz, 2013). The unstable flow behaviour of BSG-added yogurt might be related to the firm stability of the yogurt which is affected by the fermentation process. A lower fermentation time could diminish the protein network (Pachekrepapol et al., 2021) and generate a lower amount of free fat and protein (Sinamo, Hasan, & Hasanah, 2020b). This could lead to the formation of a weaker gel and irregular protein network (Pachekrepapol et al., 2021).

As mentioned in the previous section, BSG reduces the fermentation process of the yogurt. By this, the fluctuation in flow behaviour is reasonable due to the characteristics of BSG in maintaining fermentation time. Fermentation in yogurt processing, which involves incubation at 40–45 °C to reach the pH at range 4.3–4.7, plays an important role in generating standardized yogurt products (Meybodi et al., 2020). This process is essential for LAB growth and the formation of the texture of the yogurt (Meybodi et al., 2020). Therefore, a shorter fermentation time shows a faster textural formation becomes denser and more viscous which consequently impacts the flow behaviour as is observed in present study. It is suggested that a shorter fermentation should be done with a higher temperature incubation level (Meybodi et al., 2020). A higher temperature will perform the growth of S. thermophilus at 45 °C and its metabolites will synergically improve the growth of L. bulgaricus (Chandan & O'Rell, 2013). As the pH decreased, caseins' micellar structure was destabilized at pH 5.3–5.2 while denaturation and precipitation started to occur at pH 4.7. The acidification is responsible for the gel formation and coagulation (Das, Choudhary, & Thompson-Witrick, 2019). During the acidification process, a higher density and viscosity was generated as a result of network formation. By this time, a higher shear stress and mechanical properties in rheological observation could be expected. Furthermore, BSG has a high WHC which strengthens the mixture of the yogurt. Therefore, the required energy to destroy the mixture (shear stress) and the viscosity become higher. As reported in a previous study, an ingredient with a high WHC in yogurt products could diminish the protein mobility thus increasing the viscosity (Pachekrepapol et al., 2021). The WHC property of BSG also affects the syneresis property in yogurt as will be described in the next section.

3.4. Synereses

Syneresis describes the ability of the matrix to bind water which

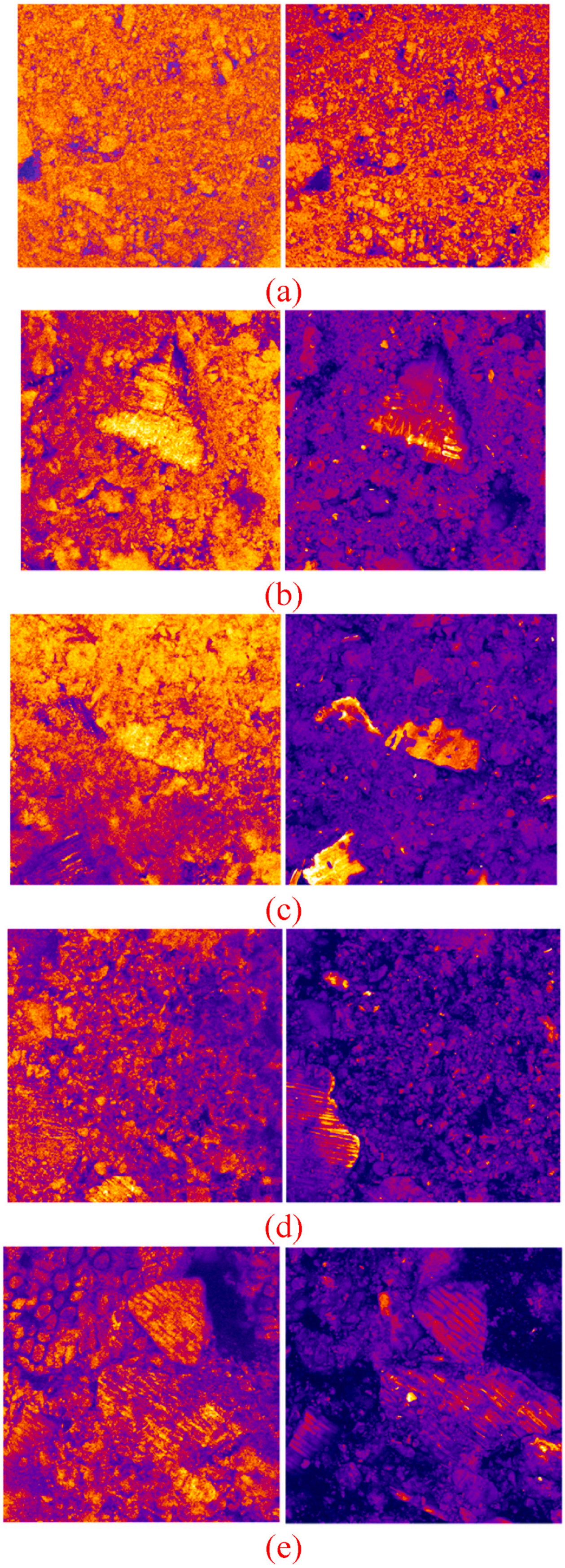

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Fig. 3. Confocal laser scanning micrographs of freeze-dried BSG-added yogurt stained with Rhodamine 123. Protein network is shown by the silver-yellow; a. 0% BSG (control); b. 5%; c. 10%; d. 15%; e. 20% substitution. The images are presented as maximum intensity projections from confocal Z stacks in a fire intensity scale. (left: yellow channels describe formed protein network; right: blue channels describe yogurt structure visualized with a laser reflection). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

describes the amount of water released after centrifugation. As is shown in Table 3, the incorporation of BSG in yogurt significantly ($p < 0.05$) decreased the syneresis level. In general, the higher the amount of BSG the lower the amount of syneresis. The result shows the ability of BSG in binding water thus preserving the consistency of the yogurt and its flow behaviour. However, the syneresis increased during the storage period. This phenomenon shows the ability of BSG in binding water decreased due to the refrigerated storage. Therefore, water was released from the matrix. This might be related to the role of BSG in regulating the flow behavior as mentioned in the previous section. The ability of BSG in binding water has been reported in a previous study (Naibaho & Korzeniowska, 2021a) thus consequently reducing the syneresis in yogurt products. Compared to other studies, BSG-added yogurt in this study had a lower level of syneresis. The incorporation of plant seed mucilage generated a yogurt with syneresis between 70 and 80%, while the addition of plant extract in yogurt generated syneresis in a range of 35–50% (Bouaziz et al., 2021; Choobari et al., 2021). Interestingly, the ability of BSG in preserving the syneresis of the yogurt is higher than that in stabilizer ingredients which generated a syneresis in a range between 39 and 55% (Khubber et al., 2021). This study shows that BSG had a higher ability to maintain the stability of the yogurt. It has been reported that the addition of dietary fiber in yogurt enhanced the stability of the yogurt during the storage from the perspective of syneresis (Bouaziz et al., 2021; Choobari et al., 2021; Khubber et al., 2021). By this, BSG could have acted as a stabilizer agent in yogurt products. Syneresis is an important quality parameter in yogurt products due to its impact on the consumers acceptability (Bouaziz et al., 2021). The ability of BSG in preserving the consistency of yogurt is due to the high amount of dietary fiber. BSG consists of 37.65%–58.2% and 1.3%–9.7% insoluble- (IDF) and soluble dietary fiber (SDF), respectively (Naibaho et al., 2021; Nocente et al., 2019). The main compounds of IDF from BSG are cellulose 28.7%, hemicellulose 17.5%, and lignin 16.9% per 100 g BSG (Sibhatu, Anuradha, Yimam, & Ahmed, 2021).

The ability of BSG in binding high amounts of water has been observed which disrupts the network formation in bread, dough and cookies making (Ktenioudaki et al., 2013; Magabane, 2017; Roth, Döring, Jekle, & Becker, 2016; Steinmacher, Honna, Gasparetto, Anibal, & Grossmann, 2012; Torbica, Škrobot, Janic, Belovic, & Zhang, 2019; Waters, Jacob, Titze, Arendt, & Zannini, 2012). This phenomenon is due to the high amount of dietary fiber which regulates food texture. Such property of BSG is a benefit in yogurt production as is shown in this study. This characteristic of BSG is due to the presence of arabinoxylans. Unextractable arabinoxylans possess a high-WHC which could adsorb water up to tenfold while water extractables arabinoxylans have a high viscosity forming potential (Steiner, Procopio, & Becker, 2015). Therefore, such properties of BSG are beneficial in maintaining the syneresis thus the flow behaviour consistency of the yogurt during the storage, as described previously.

3.5. Color

The impact of BSG substitution in yogurt color and its changes during the storage is shown in Table 3. In general, BSG significantly ($p < 0.05$) lowers the light intensity and increases the red and yellow intensity of the yogurt. Moreover, all the color values such as lightness, redness and yellowness decreased during the refrigerated storage. The change in color intensity occurred depending on the level addition of BSG. The

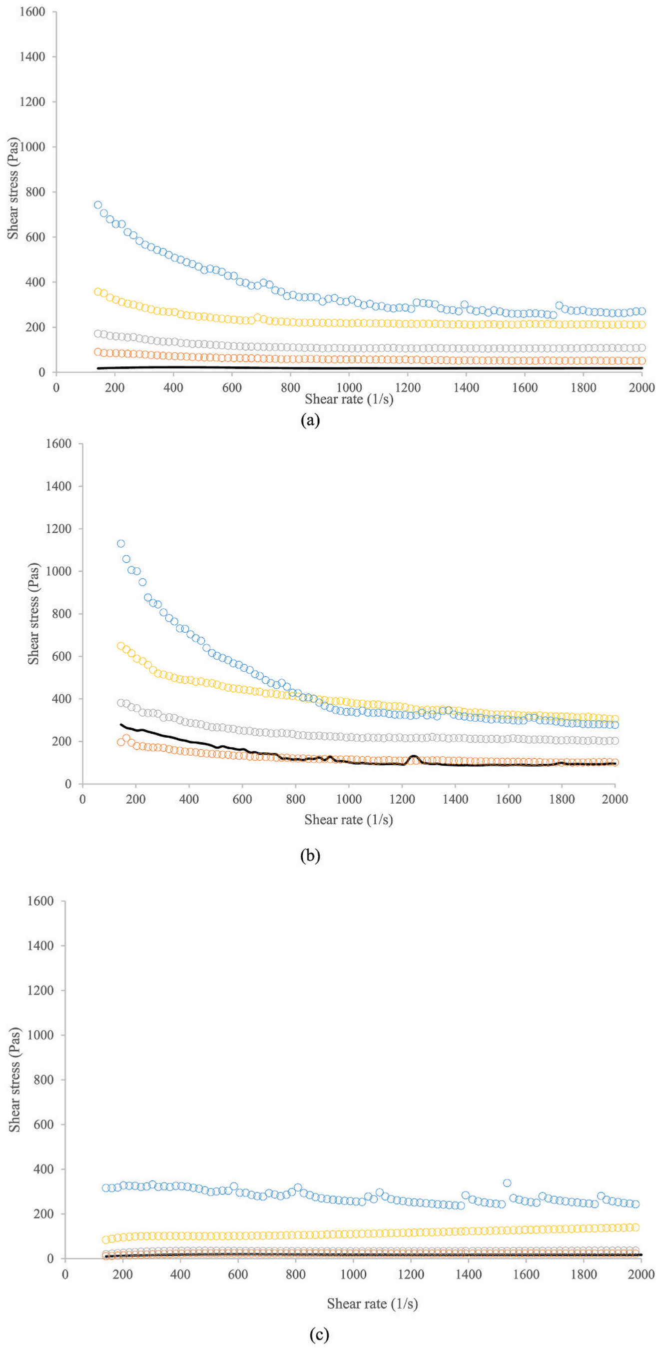

Fig. 4. The relation between shear rate and shear stress of BSG-added yogurt, (–): without addition; (o): 5%; (o): 10%; (o): 15%; (o): 20% substitution, during the storage period: a. 1 day of storage; b. 7 days of storage; c. 14 days of storage.

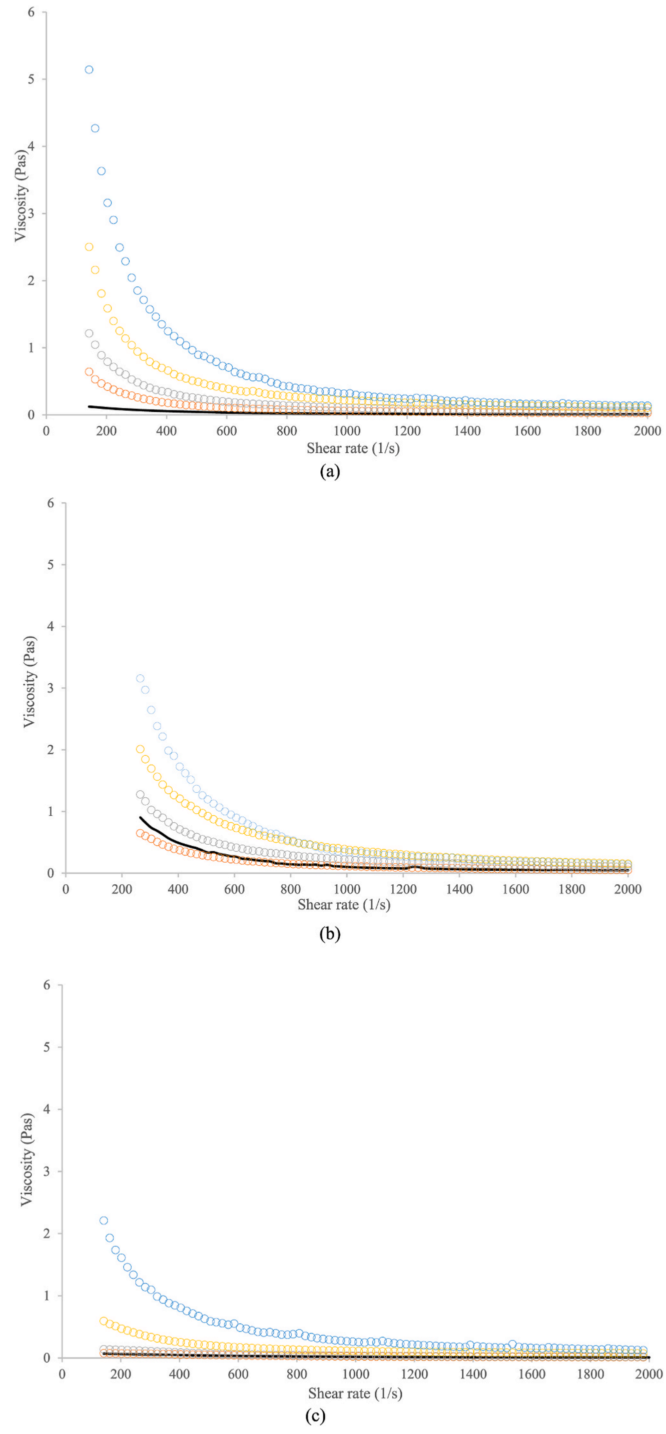

Fig. 5. The viscosity of BSG-added yogurt (–): without addition; (o): 5%; (o): 10%; (o): 15%; (o): 20% substitution, during the storage period: a. 1 day of storage; b. 7 days of storage; c. 14 days of storage.

higher the amount of the addition, the higher the impact on color change. The tendency of BSG in darkening food products such as bread, cookies and pasta has been reported previously (Heredia-Sandoval et al., 2020; Liu, Singh, & Inglett, 2011; Nocente et al., 2019; Thorvaldsson, 2020; Waters et al., 2012). This characteristic is due to the natural brown color of BSG as reported previously (Naibaho & Korzeniowska, 2021a). The darkening effect of BSG is due to the presence of soluble lignin and melanoidin as an impact of Maillard reaction (Patrignani, Brantsen, Awika, & Conforti, 2021) during the drying process of BSG. It is reported that the darkening effect of BSG could reduce the sensory acceptability of food products (Heredia-Sandoval et al., 2020; Liu et al., 2011; Nocente et al., 2019; Thorvaldsson, 2020; Waters et al., 2012). However, the brown color of BSG can be used as a healthy sign of food products for further commercialization as expected by the potential consumers (Combest & Warren, 2019). Furthermore, pre-treatment can be applied in order to increase the lightness of BSG thus darkening effects of BSG can be diminished (Naibaho et al., 2021).

3.6. *The acidity of the yogurt during the storage*

The pH and lactic acids of BSG-added yogurt during the storage period is shown in Table 3. In general, the pH significantly decreased ($p < 0.05$) during the storage in all observed groups. Moreover, the pH of

Table 3
The properties of BSG-added yogurt during the storage period.

Storage period (day)	BSG Addition				
	0%	5%	10%	15%	20%
pH					
1	4.31 ± 0.01^{gh}	4.32 ± 0.02^{gh}	4.52 ± 0.01^{b}	4.51 ± 0.02^{bc}	4.65 ± 0.01^{a}
7	4.22 ± 0.01^{ij}	4.12 ± 0.01^{k}	4.42 ± 0.02^{de}	4.38 ± 0.01^{ef}	4.52 ± 0.03^{b}
14	4.01 ± 0.01^{l}	4.16 ± 0.01^{jk}	4.34 ± 0.01^{fg}	4.26 ± 0.01^{hi}	4.45 ± 0.01^{cd}
Lactic acid (%)					
1	0.71 ± 0.01^{d}	0.58 ± 0.00^{g}	0.67 ± 0.01^{e}	0.71 ± 0.01^{d}	0.85 ± 0.01^{a}
7	0.62 ± 0.00^{f}	0.76 ± 0.00^{c}	0.67 ± 0.00^{e}	0.80 ± 0.00^{b}	0.89 ± 0.00^{a}
14	0.67 ± 0.00^{e}	0.76 ± 0.01^{c}	0.74 ± 0.03^{cd}	0.89 ± 0.01^{a}	0.81 ± 0.01^{b}
Syneresis (%)					
1	53.686 ± 0.61^{bc}	30.932 ± 1.66^{d}	19.997 ± 0.83^{ef}	1.346 ± 0.13^{h}	2.255 ± 0.84^{h}
7	60.304 ± 0.37^{ab}	47.365 ± 1.76^{c}	22.771 ± 0.33^{e}	21.089 ± 0.49^{e}	13.348 ± 0.18^{fg}
14	61.921 ± 3.56^{a}	36.444 ± 3.28^{d}	30.563 ± 2.98^{d}	11.659 ± 0.46^{g}	13.888 ± 1.56^{fg}
L					
1	87.840 ± 0.04^{a}	74.050 ± 0.52^{c}	65.840 ± 0.24^{e}	61.650 ± 0.03^{fg}	57.995 ± 0.06^{ij}
7	80.860 ± 2.35^{b}	69.155 ± 1.07^{d}	64.500 ± 0.61^{ef}	61.125 ± 0.04^{ghi}	56.635 ± 0.12^{j}
14	73.265 ± 0.94^{c}	64.795 ± 0.84^{ef}	61.200 ± 0.01^{gh}	58.050 ± 0.78^{hij}	52.980 ± 0.08^{k}
a*					
1	-1.045 ± 0.02^{j}	2.150 ± 0.07^{g}	3.590 ± 0.06^{de}	4.195 ± 0.04^{bc}	4.640 ± 0.07^{ab}
7	-1.415 ± 0.09^{j}	0.830 ± 0.11^{h}	3.185 ± 0.15^{ef}	4.170 ± 0.14^{bcd}	4.975 ± 0.05^{a}
14	-2.495 ± 0.01^{k}	0.190 ± 0.11^{i}	2.690 ± 0.21^{fg}	3.825 ± 0.35^{cd}	4.385 ± 0.28^{abc}
b*					
1	8.565 ± 0.01^{e}	12.335 ± 1.31^{cd}	14.975 ± 0.18^{abc}	16.010 ± 0.00^{ab}	16.960 ± 0.06^{a}
7	3.080 ± 0.37^{fg}	9.795 ± 1.48^{de}	13.985 ± 1.05^{bc}	16.075 ± 0.05^{ab}	17.145 ± 0.30^{a}
14	0.835 ± 0.06^{g}	5.475 ± 0.67^{f}	9.925 ± 0.19^{de}	13.055 ± 0.91^{c}	13.780 ± 0.35^{bc}

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.

BSG-added yogurt was significantly higher than that in control (0% BSG), except in the 5% of BSG substitution on 7th days of storage. The difference in pH is aligned with the change of the amount of lactic acids during the storage (Table 3). The substitution of BSG increased the amount of lactic acids in yogurt compared to the control group (0% BSG); and during the storage period, the amount of lactic acids significantly differed ($p < 0.05$) among the considered group. Generally, there was a slight increase in lactic acid during the storage period except in 20% BSG substitution and the control group. These results demonstrate that BSG has an influence on pH and lactic acids in BSG-added yogurt. Table 1 shows that the addition of BSG before the fermentation had no effect on pH. However, the derivation of pH during fermentation might influence the release of certain compounds from BSG such as protein and phenolic compounds. It is reported that pH significantly impacts protein solubility (He et al., 2019; Vieira et al., 2014, Vieira, Teixeira, & Ferreira, 2016). During the pasteurization at 90 °C, the disruption of BSG cell walls might occur thus allowing the protein release from the matrix. The pH before fermentation was approximately 6.0 (Table 1). It is reported that protein solubility at pH range 5–6 is low (He et al., 2019; Vieira et al., 2016). Although a higher protein solubility might be reached when pH at 4.3–4.5 (Vieira et al., 2014). Therefore, the fluctuation in pH of yogurt during the storage period might be impacted by the LAB growth due to the nutrients from BSG such as dietary fiber and protein. Current study (Table 4) revealed that the addition of BSG (5–20%) increased the growth of *S. thermophilus*. Although 20% BSG addition generated the same level of *L. bulgaricus*, the addition of 5–15% of BSG enhanced the number of *L. bulgaricus*.

Compared to the control (0% BSG), BSG regulates the yogurt properties differently: BSG increased the pH of yogurt during the storage period and at the same time it increased the amount of lactic acids. Compared to previous studies, the addition of certain ingredients could decrease in pH and lead to an increase in lactic acids (Bouaziz et al., 2021; Brodziak et al., 2021; Choobari et al., 2021; Sinamo, Hasan, & Hasanah, 2020a). Several ingredients such as leaves powder, sea buckthorn mousse and forsk seed mucilage powder, increase the growth of lactic bacteria thus increase the lactose fermentation rate. Therefore, a raise in the amount of lactic acids was observed which leads to a lower pH during the yogurt storage (Bouaziz et al., 2021; Brodziak et al., 2021; Choobari et al., 2021). However, the addition of dietary fiber from certain by-products generated yogurt with a stable pH during the storage (do Espírito Santo et al., 2012) which might be related to the lack of microbial activities. In a previous study, there was only slight change in the presence of LAB during the storage period (do Espírito Santo et al., 2012) while in this study, the fluctuation in the amount of LAB was observed.

In general, the higher amount of lactic acid production is aligned with the decline in pH (Table 3). The decrease in pH described a higher production of lactic acid due to the growth of LAB. The lactic acids in some of the observed groups are aligned with the previous study which reported a range of lactic acids at 0.8–0.9% (Cavalheiro et al., 2020; Choobari et al., 2021; Gürbüz, Erkaya-Kotan, & Şengül, 2021; Minh, 2021). A higher lactic acid is observed in high protein yogurt (Cavalheiro et al., 2020). By this, the increase in lactic acid of BSG-added yogurt might be related to the protein properties of BSG. BSG contains 20–30% of protein (Naibaho & Korzeniowska, 2021a; Wen et al., 2019)

Table 4
Profile of LAB in yogurt during the study, the total amount and the ratio of LAB.

Storage period (day)	BSG Addition				
	0%	5%	10%	15%	20%
S. thermophilus (CFU/mL sample)					
1	7.712 ± 0.21^{f}	8.656 ± 0.16^{bcdef}	8.618 ± 0.19^{bcdef}	9.328 ± 0.25^{bc}	8.545 ± 0.36^{bcdef}
7	8.443 ± 0.47^{cdef}	10.389 ± 0.00^{a}	8.415 ± 0.21^{cdef}	7.815 ± 0.25^{ef}	8.312 ± 0.10^{def}
14	9.425 ± 0.12^{ab}	9.510 ± 0.11^{ab}	9.448 ± 0.20^{ab}	8.859 ± 0.38^{bcd}	8.719 ± 0.16^{bcde}
L. bulgaricus (CFU/mL sample)					
1	5.436 ± 0.26^{de}	5.505 ± 0.04^{de}	6.559 ± 0.06^{abc}	6.386 ± 0.10^{abcd}	5.306 ± 0.34^{e}
7	5.834 ± 0.19^{cde}	7.418 ± 0.23^{a}	5.592 ± 0.28^{cde}	5.563 ± 0.24^{cde}	5.815 ± 0.08^{cde}
14	6.427 ± 0.09^{abcd}	7.319 ± 0.35^{ab}	6.550 ± 0.45^{abc}	6.328 ± 0.37^{bcde}	6.306 ± 0.34^{bcde}
Total of LAB (CFU/mL sample)					
1	13.15 ± 0.48^{e}	14.16 ± 0.20^{de}	15.18 ± 0.25^{cd}	15.71 ± 0.35^{bc}	13.85 ± 0.03^{de}
7	14.28 ± 0.28^{de}	17.81 ± 0.23^{a}	14.01 ± 0.50^{de}	13.38 ± 0.50^{e}	14.13 ± 0.18^{de}
14	15.85 ± 0.03^{bc}	16.83 ± 0.24^{ab}	16.00 ± 0.25^{bc}	15.19 ± 0.75^{cd}	15.03 ± 0.18^{cd}
Ratio of *S. thermophilus* and *L. bulgaricus*					
1	1.42 ± 0.03^{ab}	1.57 ± 0.02^{ab}	1.31 ± 0.02^{b}	1.46 ± 0.02^{ab}	1.62 ± 0.17^{a}
7	1.45 ± 0.13^{ab}	1.40 ± 0.04^{ab}	1.51 ± 0.04^{ab}	1.41 ± 0.01^{ab}	1.43 ± 0.00^{ab}
14	1.47 ± 0.04^{ab}	1.30 ± 0.08^{b}	1.45 ± 0.13^{ab}	1.40 ± 0.02^{ab}	1.39 ± 0.10^{ab}

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.

which might have synergic impact to improve the LAB growth in yogurt. During the fermentation process, certain enzymes are able to degrade the high molecular weight into a lower molecular weight thus improving its availability (Wen et al., 2019). Another possible reason is the synergistic growth of the LAB strain. Initially, *S. thermophilus* grew by using available protein from milk and BSG followed by *L. bulgaricus* which produced higher amounts of lactic acid thus significantly lowering the pH (Chandan & O'Rell, 2013). In this study, other strains including *L. acidophilus* and *B. lactis* from microbial culture were present in the system allowing the synergic production of lactic acid. Furthermore, it was predicted that the isoelectric point of BSG protein is 5 or lower (Vieira et al., 2016) which influenced the significant drop in pH after 2 h of fermentation.

Furthermore, it is reported that lactic acid forming ability in yogurt production is influenced by several factors such as dry matter, protein, phosphate, citrate, lactate and minerals (Gürbüz et al., 2021). Since BSG is a complex material, consisting of dietary fiber, protein, phenolic compounds and some minerals (Lynch, Steffen, & Arendt, 2016; Nigam, 2017). Therefore, there might be the impact of other chemical constituents in pH and lactic acid production during the storage of yogurt which generated a yogurt with a slightly higher pH and at the same time had a higher amount of lactic acids. In addition, the potential of BSG as an ingredient for lactic acids production has been previously reported (Nigam, 2017; Xiros & Christakopoulos, 2012) which might affect the variability of lactic acids in this study.

3.7. The survival of the LABs in BSG-added yogurt during the storage

The survival of LAB, *S. thermophilus* and *L. bulgaricus*, in BSG-added yogurt during the refrigerated storage is shown in Table 4. The result shows that the incorporation of BSG in yogurt production significantly ($p < 0.05$) affects the amount of LAB during the storage period. In general, the number of *S. thermophilus* and *L. bulgaricus* in the control group (0% BSG) increased throughout the observation period. However, the addition of BSG fluctuated the amount of both *S. thermophilus* and *L. bulgaricus.* Interestingly, even though the control had a lower amount of the LAB on the first day, there is no significant difference ($p > 0.05$) at the end of the observation in all observed groups. This phenomenon shows that the LAB growth level in yogurt is unstable due to the presence of BSG depending on the level of the substitution. By this, the addition of BSG in yogurt products had no negative impact on the LAB growth in yogurt during the storage. The fluctuations of LAB growth in yogurt often occurs in the study which employed the commercial cultures of bacteria. This phenomenon occurs because of the presence of several strains with different ability to grow and survive in certain conditions (do Espírito Santo et al., 2012). Another possibility which influences the amount of LAB in this study is the homogeneity of the sample due to the syneresis phenomenon. Certain amount of water was released from the yogurt matrix, called syneresis, which consequently affected the homogeneity of the samples.

The amount of *S. thermophilus* in this study is aligned with the previous study which reported that the addition of plant-based ingredients in yogurt contained approximately 7.0–9.5 log CFU/mL of *S. thermophilus* (Bouaziz et al., 2021; Gürbüz et al., 2021; Szołtysik et al., 2020). However, the amount of *L. bulgaricus* in some groups is lower than in previous studies which reported a range between 5.9 and 5.8 log CFU/mL of *L. bulgaricus* (Bouaziz et al., 2021; Choobari et al., 2021). Compared to previous studies, the amount of LAB usually decreased during the storage (Bouaziz et al., 2021; Choobari et al., 2021; Gürbüz et al., 2021; Nguyen et al., 2014; Szołtysik et al., 2020), while in this study the amount of LAB is stable during the 14 days of storage. By this, the addition of BSG might provide nutrition for LAB growth and survive during the storage. Furthermore, this study shows that the amount of *S. thermophilus* is observed to be higher than that in *L. bulgaricus.* This phenomenon has been observed in previous studies (Bouaziz et al., 2021; Choobari et al., 2021; Gürbüz et al., 2021; Nguyen et al., 2014; Szołtysik et al., 2020). This might be due to the different ability between those both strains to grow and to adapt in the same conditions. As is shown in Table 4, the ratio between *S. thermophilus* and *L. bulgaricus* in general had no significant difference ($p > 0.05$) to each other. This result shows a certain processing tolerance as typical for a commercially used fermentation culture. It is reported that *S. thermophilus* has a higher proteolytic activity than that in *L. bulgaricus*, as well as the susceptibility of the strain to the acidic and cold condition during storage (Nguyen et al., 2014). Therefore, a synergistic effect between S. thermophilus and L. bulgaricus had improved the survival of the total LAB (Table 4) during the storage period. In addition, the synergistic phenomenon might be also influenced by the presence of *L. acidophilus* and *B. lactis* from the microbial culture. The number of LAB at 7 days of storage fluctuated differently in each addition of BSG. This shows its ability in maintaining the growth of LAB during the storage thus generating the same level of LAB at 14 days of storage.

4. Conclusion

The incorporation of BSG in yogurt benefited the production line due to its impact in reducing the fermentation time and at the same time maintained the lactic acid production and supported the LAB growth. BSG modified the microstructural properties of the yogurts showing an impact in disrupting the cross-link between protein, fat and the yogurt matrix. The presence of high dietary fiber in BSG contributed to a lower syneresis, a higher shear stress and maintenance of viscosity at certain levels of addition during the storage period. BSG-added yogurt allowed the LAB growth, increases the amount of lactic acid and increases the pH which can be a benefit for industry to use less sweetener to cover the acidity. Furthermore, the addition of BSG at certain levels strengthened the survival level of LAB during the storage. A maximum substitution of 10% BSG is suggested to be more suitable in yogurt products due to its ability to preserve the flow behaviour and syneresis in addition to other physico-chemical properties. However, the potential change in flavor and biological activities of BSG-added yogurt are important for further study.

Author contributions

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Declaration of competing interest

Authors declare no conflict of interest.

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References

Ačkar, Đ., Jozinović, A., Babić, J., Miličević, B., Panak Balentić, J., & Šubarić, D. (2018). Resolving the problem of poor expansion in corn extrudates enriched with food industry by-products. *Innovative Food Science & Emerging Technologies, 47*, 517–524. https://doi.org/10.1016/j.ifset.2018.05.004

Adepoju, F. O., & Selezneva, I. S. (2020). *Comparative study of yoghurt enriched with Moringa powder in different concentrations*, Article 030001. https://doi.org/10.1063/5.0018035

Amorim, C., Silvério, S. C., & Rodrigues, L. R. (2019). One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. *Food Chemistry, 270*, 86–94. https://doi.org/10.1016/j.foodchem.2018.07.080

Barnes, H. A. (2000). *A handbook of elementary rheology*. University of Wales Institute of Non-Newtonian Fluid Mechanics.

Bohnsack, C., Ternes, W., Büsing, A., & Drotleff, A. M. (2011). Tocotrienol levels in sieving fraction extracts of brewer's spent grain. *European Food Research and Technology, 232*(4), 563–573. https://doi.org/10.1007/s00217-010-1419-z

Bouaziz, M. A., Bchir, B., Chalbi, H., Sebii, H., Karra, S., Smaoui, S., et al. (2021). Techno-functional characterization and biological potential of Agave americana leaves: Impact on yoghurt qualities. *Journal of Food Measurement and Characterization, 15*(1), 309–326. https://doi.org/10.1007/s11694-020-00632-9

Brodziak, A., Król, J., Matwijczuk, A., Czernecki, T., Glibowski, P., Wlazło, Ł., et al. (2021). Effect of sea buckthorn (hippophae rhamnoides L.) mousse on properties of probiotic yoghurt. *Applied Sciences, 11*(2), 545. https://doi.org/10.3390/app11020545

Cavalheiro, F. G., Parra Baptista, D., Domingues Galli, B., Negrão, F., Nogueira Eberlin, M., & Lúcia Gigante, M. (2020). High protein yogurt with addition of Lactobacillus helveticus: Peptide profile and angiotensin-converting enzyme ACE-inhibitory activity. *Food Chemistry, 333*, 127482. https://doi.org/10.1016/j.foodchem.2020.127482

Chandan, R. C., & O'Rell, K. (2013). Principles of yogurt processing. In R. C. Chandan, & A. Kilara (Eds.), *Manufacturing yogurt and fermented milks* (pp. 239–261). John Wiley & Sons. https://doi.org/10.1002/9781118481301.ch11.

Choobari, S. Z. M., Sari, A. A., & Daraei Garmakhany, A. (2021). Effect of *Plantago ovata* Forsk seed mucilage on survivability of *Lactobacillus acidophilus* , physicochemical and sensory attributes of produced low-fat set yoghurt. *Food Sciences and Nutrition, 9*(2), 1040–1049. https://doi.org/10.1002/fsn3.2074

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2012). Modifying the microstructure of low-fat yoghurt by microfluidisation of milk at different pressures to enhance rheological and sensory properties. *Food Chemistry, 130*(3), 510–519. https://doi.org/10.1016/j.foodchem.2011.07.056

Combest, S., & Warren, C. (2019). Perceptions of college students in consuming whole grain foods made with Brewers' Spent Grain. *Food Sciences and Nutrition, 7*(1), 225–237. https://doi.org/10.1002/fsn3.872

Connolly, A., Piggott, C. O., & FitzGerald, R. J. (2013). Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. *International Journal of Food Science and Technology, 48*(8), 1670–1681. https://doi.org/10.1111/ijfs.12137

Conway, J. (2019, August 21). Global beer production 1998-2018. Alcohol Beverages. https://www.statista.com/statistics/270275/worldwide-beer-production/.

Conway, J. (2021, March). European beer production volume by country 2019. https://www.statista.com/statistics/444575/european-beer-production-by-country/.

Das, K., Choudhary, R., & Thompson-Witrick, K. A. (2019). Effects of new technology on the current manufacturing process of yogurt-to increase the overall marketability of yogurt. *Lebensmittel-Wissenschaft und -Technologie, 108*, 69–80. https://doi.org/10.1016/j.lwt.2019.03.058

do Espírito Santo, A. P., Cartolano, N. S., Silva, T. F., Soares, F. A. S. M., Gioielli, L. A., Perego, P., et al. (2012). Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts. *International Journal of Food Microbiology, 154*(3), 135–144. https://doi.org/10.1016/j.ijfoodmicro.2011.12.025

Fărcaş, A. C., Socaci, S. A., Dulf, F. V., Tofană, M., Mudura, E., & Diaconeasa, Z. (2015). Volatile profile, fatty acids composition and total phenolics content of brewers' spent grain by-product with potential use in the development of new functional foods. *Journal of Cereal Science, 64*, 34–42. https://doi.org/10.1016/j.jcs.2015.04.003

Föste, M., Verheyen, C., Jekle, M., & Becker, T. (2020). Fibres of milling and fruit processing by-products in gluten-free bread making: A review of hydration properties, dough formation and quality-improving strategies. *Food Chemistry, 306*, 125451. https://doi.org/10.1016/j.foodchem.2019.125451

Gürbüz, Z., Erkaya-Kotan, T., & Şengül, M. (2021). Evaluation of physicochemical, microbiological, texture and microstructure characteristics of set-style yoghurt supplemented with quince seed mucilage powder as a novel natural stabiliser. *International Dairy Journal, 114*, 104938. https://doi.org/10.1016/j.idairyj.2020.104938

He, Y., Kuhn, D. D., Ogejo, J. A., O'Keefe, S. F., Fraguas, C. F., Wiersema, B. D., et al. (2019). Wet fractionation process to produce high protein and high fiber products from brewer's spent grain. *Food and Bioproducts Processing, 117*, 266–274. https://doi.org/10.1016/j.fbp.2019.07.011

Heredia-Sandoval, N. G., Granados-Nevárez, M. del C., Calderón de la Barca, A. M., Vásquez-Lara, F., Malunga, L. N., Apea-Bah, F. B., et al. (2020). Phenolic acids, antioxidant capacity, and estimated glycemic index of cookies added with brewer's spent grain. *Plant Foods for Human Nutrition, 75*(1), 41–47. https://doi.org/10.1007/s11130-019-00783-1

Huang, T., Tu, Z., Shangguan, X., Sha, X., Wang, H., Zhang, L., et al. (2019). Fish gelatin modifications: A comprehensive review. *Trends in Food Science & Technology, 86*, 260–269. https://doi.org/10.1016/j.tifs.2019.02.048

Huang, T., Tu, Z., Shangguan, X., Wang, H., Zhang, L., & Bansal, N. (2021). Characteristics of fish gelatin-anionic polysaccharide complexes and their applications in yoghurt: Rheology and tribology. *Food Chemistry, 343*, 128413. https://doi.org/10.1016/j.foodchem.2020.128413

Khubber, S., Chaturvedi, K., Thakur, N., Sharma, N., & Yadav, S. K. (2021). Low-methoxyl pectin stabilizes low-fat set yoghurt and improves their physicochemical properties, rheology, microstructure and sensory liking. *Food Hydrocolloids, 111*, 106240. https://doi.org/10.1016/j.foodhyd.2020.106240

Kokabi, S., Soltani, M., Dabirian, S., Kokabian, A., Daraei Garmakhany, A., Jafarzadeh, S., et al. (2021). Incorporation of omega-3 fatty acid-rich grape seed oil in yoghurt: Response surface optimization of physicochemical, textural, and sensory attributes during refrigerated storage. *Food Sciences and Nutrition, 9*(1), 331–344. https://doi.org/10.1002/fsn3.1998

Ktenioudaki, A., Alvarez-Jubete, L., Smyth, T. J., Kilcawley, K., Rai, D. K., & Gallagher, E. (2015). Application of bioprocessing techniques (sourdough fermentation and technological aids) for brewer's spent grain breads. *Food Research International, 73*, 107–116. https://doi.org/10.1016/j.foodres.2015.03.008

Ktenioudaki, A., Chaurin, V., Reis, S. F., & Gallagher, E. (2012). Brewer's spent grain as a functional ingredient for breadsticks: Brewer's spent grain as a functional ingredient for breadsticks. *International Journal of Food Science and Technology, 47*(8), 1765–1771. https://doi.org/10.1111/j.1365-2621.2012.03032.x

Ktenioudaki, A., Crofton, E., Scannell, A. G. M., Hannon, J. A., Kilcawley, K. N., & Gallagher, E. (2013). Sensory properties and aromatic composition of baked snacks containing brewer's spent grain. *Journal of Cereal Science, 57*(3), 384–390. https://doi.org/10.1016/j.jcs.2013.01.009

Langenaeken, N. A., Ieven, P., Hedlund, E. G., Kyomugasho, C., van de Walle, D., Dewettinck, K., et al. (2020). Arabinoxylan, β-glucan and pectin in barley and malt endosperm cell walls: A microstructure study using CLSM and cryo-SEM. *The Plant Journal, 103*(4), 1477–1489. https://doi.org/10.1111/tpj.14816

Lao, E. J., Dimoso, N., Raymond, J., & Mbega, E. R. (2020). The prebiotic potential of brewers' spent grain on livestock's health: A review. *Tropical Animal Health and Production, 52*(2), 461–472. https://doi.org/10.1007/s11250-019-02120-9

Liu, S. X., Singh, M., & Inglett, G. (2011). Effect of incorporation of distillers' dried grain with solubles (DDGS) on quality of cornbread. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 44*(3), 713–718. https://doi.org/10.1016/j.lwt.2010.10.001

Luo, Y., Liu, X., & Pang, Z. (2019). Tribo-rheological properties of acid milk gels with different types of gelatin: Effect of concentration. *Journal of Dairy Science, 102*(9), 7849–7862. https://doi.org/10.3168/jds.2019-16305

Lynch, K. M., Steffen, E. J., & Arendt, E. K. (2016). Brewers' spent grain: A review with an emphasis on food and health: Brewers' spent grain: A review with an emphasis on food and health. *Journal of the Institute of Brewing, 122*(4), 553–568. https://doi.org/10.1002/jib.363

Magabane, I. E. (2017). *Technologies for improving the quality of bread doughs made with barley spent grain and sorghum*. University of Pretoria. https://repository.up.ac.za/handle/2263/65907.

Meybodi, N. M., Mortazavian, A. M., Arab, M., & Nematollahi, A. (2020). Probiotic viability in yoghurt: A review of influential factors. *International Dairy Journal, 109*, 104793. https://doi.org/10.1016/j.idairyj.2020.104793

Minh, N. P. (2021). Physicochemical characteristics, viability of starters, total phenolics and antioxidant activities of functional yoghurt supplemented with extracts from Hylocereus polyrhizus, Hibiscus sabdariffa and Peristrophe bivalvis. *Plant Science Today, 8*(1). https://doi.org/10.14719/pst.2021.8.1.1009

Naibaho, J., & Korzeniowska, M. (2021a). The variability of physico-chemical properties of brewery spent grain from 8 different breweries. *Heliyon, 7*(3), Article e06583. https://doi.org/10.1016/j.heliyon.2021.e06583

Naibaho, J., & Korzeniowska, M. (2021b). Brewers' spent grain in food systems: Processing and final products quality as a function of fiber modification treatment. *Journal of Food Science, 86*(5), 1532–1551. https://doi.org/10.1111/1750-3841.15714

Naibaho, J., Korzeniowska, M., Wojdyło, A., Figiel, A., Yang, B., Laaksonen, O., et al. (2021). The potential of spent barley as a functional food ingredient: Study on the comparison of dietary fiber and bioactivity. *Proceedings, 70*(86), 6. https://doi.org/10.3390/foods_2020-08486

Naibaho, J., Korzeniowska, M., Wojdyło, A., Figiel, A., Yang, B., Laaksonen, O., et al. (2021). Fiber modification of brewers' spent grain by autoclave treatment to

improve its properties as a functional food ingredient. *Lebensmittel-Wissenschaft und -Technologie, 149*, 111877. https://doi.org/10.1016/j.lwt.2021.111877
Nair, S., Knoblauch, M., Ullrich, S., & Baik, B.-K. (2011). Microstructure of hard and soft kernels of barley. *Journal of Cereal Science, 54*(3), 354–362. https://doi.org/10.1016/j.jcs.2011.06.014
Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2014). The effect of fermentation temperature on the microstructure, physicochemical and rheological properties of probiotic buffalo yoghurt. *Food and Bioprocess Technology, 7*(9), 2538–2548. https://doi.org/10.1007/s11947-014-1278-x
Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2015). Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt. *International Dairy Journal, 46*, 78–87. https://doi.org/10.1016/j.idairyj.2014.08.003
Nielsen, S. S. (Ed.). (2017). *Food analysis*. Springer International Publishing. https://doi.org/10.1007/978-3-319-45776-5.
Nigam, P. S. (2017). An overview: Recycling of solid barley waste generated as a by-product in distillery and brewery. *Waste Management, 62*, 255–261. https://doi.org/10.1016/j.wasman.2017.02.018
Nocente, F., Taddei, F., Galassi, E., & Gazza, L. (2019). Upcycling of brewers' spent grain by production of dry pasta with higher nutritional potential. *Lebensmittel-Wissenschaft und -Technologie, 114*, 108421. https://doi.org/10.1016/j.lwt.2019.108421
Pachekrepapol, U., Somboonchai, N., & Krimjai, W. (2021). Physicochemical, rheological, and microbiological properties of lactose-free functional yogurt supplemented with fructooligosaccharides. *Journal of Food Processing and Preservation, 45*(1). https://doi.org/10.1111/jfpp.15017
Parekh, I., Khanvilkar, A., & Naik, A. (2017). Barley-wheat brewers' spent grain: A potential source of antioxidant rich lipids. *Journal of Food Processing and Preservation, 41*(6), Article e13244. https://doi.org/10.1111/jfpp.13244
Patel, A., Mikes, F., Bühler, S., & Matsakas, L. (2018). Valorization of brewers' spent grain for the production of lipids by oleaginous yeast. *Molecules, 23*(12), 3052. https://doi.org/10.3390/molecules23123052
Patrignani, M., Brantsen, J. F., Awika, J. M., & Conforti, P. A. (2021). Application of a novel microwave energy treatment on brewers' spent grain (BSG): Effect on its functionality and chemical characteristics. *Food Chemistry, 346*, 128935. https://doi.org/10.1016/j.foodchem.2020.128935
Patrignani, M., González-Forte, L., & del, S. (2020). Characterisation of melanoidins derived from Brewers' spent grain: New insights into their structure and antioxidant activity. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.14653. ijfs.14653.
Petit, G., Korbel, E., Jury, V., Aider, M., Rousselière, S., Audebrand, L. K., et al. (2020). Environmental evaluation of new brewer's spent grain preservation pathways for further valorization in human nutrition. *ACS Sustainable Chemistry & Engineering, 8*(47), 17335–17344. https://doi.org/10.1021/acssuschemeng.0c04236
Qiu, L., Zhang, M., Mujumdar, A. S., & Chang, L. (2021). Effect of edible rose (Rosa rugosa cv. Plena) flower extract addition on the physicochemical, rheological, functional and sensory properties of set-type yogurt. *Food Bioscience, 43*, 101249. https://doi.org/10.1016/j.fbio.2021.101249
Ramírez-Sucre, M. O., & Vélez-Ruiz, J. F. (2013). Physicochemical, rheological and stability characterization of a caramel flavored yogurt. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 51*(1), 233–241. https://doi.org/10.1016/j.lwt.2012.09.014
Ravindran, R., Jaiswal, S., Abu-Ghannam, N., & Jaiswal, A. K. (2018). A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresource Technology, 248*, 272–279. https://doi.org/10.1016/j.biortech.2017.06.039
Reis, S. F., Coelho, E., Coimbra, M. A., & Abu-Ghannam, N. (2015). Influence of grain particle sizes on the structure of arabinoxylans from brewer's spent grain. *Carbohydrate Polymers, 130*, 222–226. https://doi.org/10.1016/j.carbpol.2015.05.031
Roth, M., Döring, C., Jekle, M., & Becker, T. (2016). Mechanisms behind distiller's grains impact on wheat dough and bread quality. *Food and Bioprocess Technology, 9*(2), 274–284. https://doi.org/10.1007/s11947-015-1620-y
Sas, A. (2021, August 2). Yogurt production in Poland 2000-2020. Yogurt production in Poland from 2000 to 2020. https://www.statista.com/statistics/1140465/poland-yogurt-production/.
Severini, C., Azzollini, D., Jouppila, K., Jussi, L., Derossi, A., & De Pilli, T. (2015). Effect of enzymatic and technological treatments on solubilisation of arabinoxylans from brewer's spent grain. *Journal of Cereal Science, 65*, 162–166. https://doi.org/10.1016/j.jcs.2015.07.006
Shahbandeh, M. (2020, November 4). Global yogurt market value forecast 2018-2024. Forecasted market value of yogurt worldwide from 2018 to 2024. https://www.statista.com/statistics/870893/global-yogurt-market-value-forecast/.
Sibhatu, H. K., Anuradha Jabasingh, S., Yimam, A., & Ahmed, S. (2021). Ferulic acid production from brewery spent grains, an agro-industrial waste. *Lebensmittel-Wissenschaft und -Technologie, 135*, 110009. https://doi.org/10.1016/j.lwt.2020.110009
Sinamo, K. N., Hasan, F., & Hasanah, U. (2020a). Physico-chemical and microbial properties of buffalo milk yoghurt drink. *IOP Conference Series: Earth and Environmental Science, 454*, Article 012119. https://doi.org/10.1088/1755-1315/454/1/012119
Sinamo, K. N., Hasan, F., & Hasanah, U. (2020b). Effect of concentration starter and fermentation time on the quality of yoghurt drink from buffalo milk. *Journal of Physics: Conference Series, 1542*, Article 012070. https://doi.org/10.1088/1742-6596/1542/1/012070
Steiner, J., Procopio, S., & Becker, T. (2015). Brewer's spent grain: Source of value-added polysaccharides for the food industry in reference to the health claims. *European Food Research and Technology, 241*(3), 303–315. https://doi.org/10.1007/s00217-015-2461-7
Steinmacher, N. C., Honna, F. A., Gasparetto, A. V., Anibal, D., & Grossmann, M. V. E. (2012). Bioconversion of brewer's spent grains by reactive extrusion and their application in bread-making. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 46*(2), 542–547. https://doi.org/10.1016/j.lwt.2011.11.011
Szołtysik, M., Kucharska, A. Z., Sokół-Łętowska, A., Dąbrowska, A., Bobak, Ł., & Chrzanowska, J. (2020). The effect of rosa spinosissima fruits extract on lactic acid bacteria growth and other yoghurt parameters. *Foods, 9*(9), 1167. https://doi.org/10.3390/foods9091167
Tan, Y. X., Mok, W. K., Lee, J., Kim, J., & Chen, W. N. (2019). Solid state fermentation of brewers' spent grains for improved nutritional profile using Bacillus subtilis WX-17. *Fermentation, 5*(3), 52. https://doi.org/10.3390/fermentation5030052
Teixeira, M. R., Guarda, E. C., Freitas, E. B., Galinha, C. F., Duque, A. F., & Reis, M. A. M. (2020). Valorization of raw brewers' spent grain through the production of volatile fatty acids. *New Biotech, 57*, 4–10. https://doi.org/10.1016/j.nbt.2020.01.007
Thorvaldsson, M. (2020). *Biotransformation of brewer's spent grain and application as a food ingredient in extruded breakfast cereals*. Chalmers University of Technology. https://hdl.handle.net/20.500.12380/300764.
Torbica, A., Škrobot, D., Janić Hajnal, E., Belović, M., & Zhang, N. (2019). Sensory and physico-chemical properties of wholegrain wheat bread prepared with selected food by-products. *Lebensmittel-Wissenschaft und -Technologie, 114*, 108414. https://doi.org/10.1016/j.lwt.2019.108414
Torres, I. C., Amigo Rubio, J. M., & Ipsen, R. (2012). Using fractal image analysis to characterize microstructure of low-fat stirred yoghurt manufactured with microparticulated whey protein. *Journal of Food Engineering, 109*(4), 721–729. https://doi.org/10.1016/j.jfoodeng.2011.11.016
Vénica, C. I., Spotti, M. J., Pavón, Y. L., Molli, J. S., & Perotti, M. C. (2020). Influence of carrot fibre powder addition on rheological, microstructure and sensory characteristics of stirred-type yogurt. *International Journal of Food Science and Technology, 55*(5), 1916–1923. https://doi.org/10.1111/ijfs.14415
Verni, M., Pontonio, E., Krona, A., Jacob, S., Pinto, D., Rinaldi, F., et al. (2020). Bioprocessing of brewers' spent grain enhances its antioxidant activity: Characterization of phenolic compounds and bioactive peptides. *Frontiers in Microbiology, 11*, 1831. https://doi.org/10.3389/fmicb.2020.01831
Vieira, E., Rocha, M. A. M., Coelho, E., Pinho, O., Saraiva, J. A., Ferreira, I. M., et al. (2014). Valuation of brewer's spent grain using a fully recyclable integrated process for extraction of proteins and arabinoxylans. *Industrial Crops and Products, 52*, 136–143. https://doi.org/10.1016/j.indcrop.2013.10.012
Vieira, E., Teixeira, J., & Ferreira, I. M. (2016). Valorization of brewers' spent grain and spent yeast through protein hydrolysates with antioxidant properties. *European Food Research and Technology, 242*(11), 1975–1984. https://doi.org/10.1007/s00217-016-2696-y
Waters, D. M., Jacob, F., Titze, J., Arendt, E. K., & Zannini, E. (2012). Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *European Food Research and Technology, 235*(5), 767–778. https://doi.org/10.1007/s00217-012-1805-9
Wen, C., Zhang, J., Duan, Y., Zhang, H., & Ma, H. (2019). A mini-review on brewer's spent grain protein: Isolation, physicochemical properties, application of protein, and functional properties of hydrolysates. *Journal of Food Science, 84*(12), 3330–3340. https://doi.org/10.1111/1750-3841.14906
Wijngaard, H. H., Renzetti, S., & Arendt, E. K. (2007). Microstructure of buckwheat and barley during malting observed by confocal scanning laser microscopy and scanning electron microscopy. *Journal of the Institute of Brewing, 113*(1), 34–41. https://doi.org/10.1002/j.2050-0416.2007.tb00253.x
Xiros, C., & Christakopoulos, P. (2012). Biotechnological potential of brewers spent grain and its recent applications. *Waste and Biomass Valorization, 3*(2), 213–232. https://doi.org/10.1007/s12649-012-9108-8
Zhang, H., Cao, X., Yin, M., & Wang, J. (2018). Soluble dietary fiber from Qing Ke (highland barley) brewers spent grain could alter the intestinal cholesterol efflux in Caco-2 cells. *Journal of Functional Foods, 47*, 100–106. https://doi.org/10.1016/j.jff.2018.05.046
Zhao, L., Feng, R., & Mao, X. (2020). Addition of buttermilk powder improved the rheological and storage properties of low-fat yogurt. *Food Sciences and Nutrition, 8*(7), 3061–3069. https://doi.org/10.1002/fsn3.1373