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Research Paper

The roles of brewers' spent grain derivatives in coconut-based yogurt-alternatives: Microstructural characteristic and the evaluation of physico-chemical properties during the storage

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ABSTRACT

Water soluble coconut extract (WSCE) was reported as a suitable matrix for probiotic delivery as yogurt alternatives. The study aimed to evaluate the roles of brewers' spent grain (BSG) derivatives in enhancing the properties of WSCE-based yogurt alternatives. BSG flour (BSGF) and 3 different protein extracts (BSGPs) including protein control (BSGP-C), protamex treatment (BSGP-P), and protamex combined with flavourzyme treatment (BSGP-PF) were incorporated in WSCE-based yogurt alternatives. Confocal laser scanning microscopy showed that BSGPs prepared with protease treatment generated less dense fat distribution and more homogenous globules compared to that in WSCE control yogurt. It also resulted in a softer, denser and more homogenous matrix. The modification in microstructural properties was aligned with differences in several functional groups including α-glycosidic bond and hydroxyl groups from polysaccharides, aliphatic ethers and acid functional groups as well as aromatic hydrocarbons of lignin, amide I, acetyl groups and amide III. BSGF and BSGPs increased the mechanical properties, viscosity and modified flow behaviour properties demonstrating its ability in maintaining textural and gel formation. After 14 days of storage, maintenance in flow behaviour, syneresis and mechanical properties was identified. Furthermore, BSG derivatives enhanced lactic acid production up to 3 folds. In conclusion, BSG derivatives maintained the microstructure and gel formation, improved the properties of WSCE-based yogurt alternatives and preserved its behaviour during 14 days of storage.

1. Introduction

Plant-based yogurt-alternatives has been studied continuously in terms of its health benefits, processing technological issues, consumers' acceptability, and commercialization possibility (Boeck et al., 2021; Ningtyas et al., 2021; M. Yang et al., 2021). High antioxidant capabilities of plant-based yogurt-alternatives prepared from water soluble extracts of bean, soybean, quinoa flour, cashew, and maize products has been reported (Chen et al., 2019; Descalzo et al., 2018; Hwang et al., 2021; Lorusso et al., 2018; Ningtyas et al., 2021; Shori et al., 2022). Yogurt-alternatives from lupin and quinoa flour have been observed for maintaining viscosity, syneresis and textural properties (Hickisch et al., 2016; Lorusso et al., 2018).

The nutritional value of plant-based milk-alternative products is influenced by plant source, processing, and fortification as well as the addition of other ingredients (Mäkinen et al., 2016; Silva et al., 2020). Water soluble coconut extracts (WSCE) have been considered as a suitable matrix in probiotic food development (Rasika et al., 2021). It is reported that most of the yogurt-alternatives studies were conducted on WSCE (coconut milk) (Boeck et al., 2021) and is shown to be capable of maintaining sufficient probiotic levels during the storage (Aydar et al., 2020; Grasso et al., 2020; Rasika et al., 2021). According to sensory

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evaluation, reduction, or masking of the aromatic perception of yogurt-alternatives prepared from WSCE is suggested (Yaakob et al., 2012). Textural properties including viscosity and consistency have also been shown to be important quality parameters for plant-based yogurt alternatives (Greis et al., 2020; Jeske et al., 2018; McClements et al., 2019). A sensory study revealed that thickness and creaminess were considered as the important factor in high acceptability while thinness and wateriness drove to a lower acceptability of plant-based yogurt alternatives (Greis et al., 2020). The physico-chemical properties of WSCE-based yogurt alternatives had been improved by the addition of tapioca starch which was able to increase the sensory acceptability and stability (Pachekrepapol et al., 2021a).

Brewers' spent grain, a byproduct of the brewing industry, has a high level of water holding capacity (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021a) thus potentially regulating the flow behaviour of semi-solid food products and replacing the need for inclusion of e.g. tapioca starch. This capability is due to the high amount of insoluble dietary fiber (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021b), particularly arabinoxylans which possess high capability in binding water (Steiner et al., 2015) and may also play a role in its potential as prebiotic (Amorim et al., 2019; Xiros & Christakopoulos, 2012). BSG also contains high amount of protein which has several biological functions such as antioxidant activities, inhibition and protection against oxidative stress, anti-inflammatory, and immunomodulatory functions, modulates glycaemic response, antithrombotic effects and promotes angiotensin converting enzyme activity (Lynch et al., 2016; Naibaho & Korzeniowska, 2021b; Wen et al., 2019). Therefore, besides regulating the textural properties, BSG has the potential to improve the nutritional value of yogurt-alternatives made from WSCE. Furthermore, aromatic compounds from BSG might be beneficial for masking the unpleasant aroma as mentioned previously.

Our previous study identified the potential of BSG flour in regulating the physical behaviour, enhancing the growth of lactic acid bacteria and preserving the consistency during the storage of milk-based yogurt (Naibaho et al., 2022a). Seemingly important, the current study investigated the ability of BSG in regulating plant-based yogurt-alternatives. The potential of BSG derivatives in plant-based yogurt alternatives, particularly WSCE-based, has never been reported. Due to the growing demand on plant-based yogurt-alternatives, this study aimed to evaluate the potential of different BSG derivatives including BSG flour and its three different protein extracts, as a novel ingredient in the production of yogurt alternatives prepared from WSCE. It was hypothesised that BSG derivatives modified the microstructural-surface characteristics of the prepared yogurt alternative. Consequently, the stability in the flow behaviour, consistency, and viscosity during the refrigerated storage was expected. Furthermore, the production of lactic acids was predicted to be higher due to the survival of lactic acid bacteria during the storage period.

2. Materials and methods

2.1. Materials

The BSG sample was collected from a local brewery in Poland (produces light beer types which are comparable to Budweiser light) and kept at freezing temperature before treatment. Coconut water soluble extract (protein 2%; total fat 17.8%) was purchased from the commercial market as UHT coconut milk. Microbial culture with composition maltodextrin, *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp *bulgaricus, Lactobacillus acidophilus,* and *Bifidobacterium lactis*, was obtained from the commercial market. Staining Nile Red (Cat No 72485) and Rhodamine 123 (Cat No R8004) for confocal analysis were purchased from Sigma-Aldrich. All chemicals used for analyses were analytical grade.

2.2. BSG derivatives preparation

Three different extracts were provided including extract without enzyme treated (control), protamex extracted and protamex/flavourzyme extracted. Protamex and flavourzyme were used in optimising the protein extraction as has been previously described (San Martin et al., 2020). BSG flour (BSGF) was prepared as described in the previous study (Naibaho & Korzeniowska, 2021a). Briefly, BSG was dried using convective drying to obtain a range of 2–5% of moisture content. BSGF was provided at a particle size of 150 μm.

The extraction and preparation of BSGPs were conducted following the procedure described in our previous study (Naibaho et al., 2022b) generating 3 different BSGPs including BSGP-C represented BSGP extracted without protease incubation, BSGP-P represented BSGP prepared with protamex 0.5%, and BSGP-PF represented BSGP extracted with 0.5% protamex followed by 0.1% flavourzyme. Initially, BSG was mixed water with a ratio of 1:10. After the enzymes were added, the mixture was incubated at 50 °C for 3h (pH 8.5), followed by enzymes inactivating at 90 °C. The mixture was cooled down to room temperature and the supernatant was separated by centrifugation. The supernatant was dried by a APV Anhydro A/S LAB S1 spray dryer (Denmark). Evaporation was done with hot air with 160–165 °C of inlet temperature and 82–85 °C outlet temperature. The instrument was operated with an air pressure nozzle at 2 bars and the velocity of the peristaltic pump at 2.5 L/h.

All the prepared samples were packed into an aluminium foil bag, sealed and kept at 10 °C for further studies. BSGF contained 15–25% total protein content (Naibaho & Korzeniowska, 2021a) while protein extracts had protein content at 12.6%, 37.5% and 31.4% respectively for BSGP-C, BSGP-P and BSGP-PF (Naibaho et al., 2022b). BSGP-C contained a lower protein content compared to BSGF because of the low solubility during the protein extraction (Kriisa et al., 2022). Those generated BSGPs had been evaluated for their techno-functionality, antioxidant properties and phenolic composition in our previous study (Naibaho et al., 2022b). Moreover, amino acid composition, protein solubility and sensory analysis of those BSGPs had been investigated in a previous study (Kriisa et al., 2022).

2.3. Yogurt-alternatives preparation

The yogurt samples were prepared following the microbial culture instructions and some modifications were made following previous studies (Naibaho et al., 2022a; Szołtysik et al., 2020). Each of the BSG derivatives was added into WSCE with a ratio 1:9 (*w:w*) and mixed properly, while a control was provided without any addition of BSG derivatives. Therefore, 5 different formulations were obtained. The mixture was heated at 90 °C for 15 min followed by a cooling down to 43 °C. An amount of 0.05% of microbial culture was added and the temperature was kept at 43 °C to reach pH range at 4.3–4.7. During the pH observation, the mixture was homogenised slowly periodically every hour for 10 s using a laboratory scale mixer at the lowest speed (260 rounds/min; 4 cm gap). When the desired pH of 4.3–4.7 was reached, the fermentation was ended by mixing using a laboratory scale mixer followed by cooling down to 15 °C. After that, the yogurt was homogenised again, removed into a cup, closed properly, and kept at 4 °C for 18 h prior to the analysis as day 1. On the first day, freeze-dried samples were prepared due to the storage and distribution reasons for microstructural characterization. Moreover, rheological behaviour, syneresis and the acidity were evaluated on the day 1 and 14 of the storage period.

2.4. Microstructural characterisation

Microstructural evaluation was carried out by CLSM and FTIR in order to evaluate the microstructure of formed network and surface chemical characteristic, respectively. For storage reasons, the samples were freeze dried and kept at refrigerated storage prior to analysis

(Naibaho et al., 2022a).

2.4.1. Fourier transform infrared spectroscopy (FTIR)

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

2.4.2. Confocal laser scanning microscopy (CLSM)

CLSM analysis was performed using a Leica SP8 MP confocal microscope (Leica Microsystems, Germany) following the methods described in a previous study (Naibaho et al., 2022a). The staining solutions (Nile Red and Rhodamine 123) were provided at a concentration of 10 μg/ml in water. An amount of 9–30 mg of the sample was suspended in the staining solution with a ratio 1:4 (sample:staining solution; *w:v*). After that, it was transferred onto a glass slide and covered with a coverslip. The images were presented using a 20x (NA 0.75) air objective. The structure of the sample was visualized using a reflection of laser light in addition to the fluorescence of a given dye. The excitation of Rhodamine 123 and Nile Red was done at 488 and 561 nm laser, respectively. While 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. The maximum intensity projections of scanned volumes (10–80 μm thick, 0.68 μm intervals) were presented using ImageJ/Fiji software in a fire look-up table.

2.5. Evaluation of physico-chemical parameters during the refrigerated storage period

2.5.1. Rheological behaviour

Rheological behaviour analysis was carried out using a rotational Haake RheoStress 6000 rheometer equipped with a thermostatic bath (Haake A10) and a UTM Controller (Thermo Electron GmbH, Karlsruhe, Germany). The measurement was conducted at a constant temperature (20 °C) using a cone/plate (C60/1° Ti L no.222-1868/stainless steel plate TMP60 no.222-1891) with a gap of 1 mm for all samples in the geometry system. 1 mL of sample was added into the plate surface and the measurement was performed at shear rate from 0 to 2000/s. Shear stress and viscosity were recorded as the shear rate increased (Naibaho et al., 2022a; Szołtysik et al., 2020) and the data was presented at shear rate 10 to 2000/s. Power model of Ostwald de Waele was used to fit the flow curves in order to obtain flow behaviour related value, with equation:

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

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

2.5.2. Syneresis level

The syneresis level was measured following the methods as described in previous studies (Bouaziz et al., 2021; Khubber, 2021). A 5 g of the sample was weighed and centrifuged for 15 min at 4500 rpm and a temperature of 10 °C. The sedimentation was weighed and the syneresis was calculated with the equation:

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

2.5.3. pH and lactic acid production

pH analysis was done by InoLab pH-meter with the instrument instruction. The acidity analysis was carried out by the titration method with NaOH 0.25N as described below (Szołtysik et al., 2020). Briefly, distilled water was added to the yogurt with a ratio 1:1 and maximum 3 drops of indicator phenolphthalein was then added. The amount of lactic acid was calculated following the equation:

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

2.5.4. Statistical analysis

Statistical analysis was conducted in two-ways ANOVA (analysis of variance) followed by Tukey post-hoc test for significance difference at 0.95 level. The statistical assessment was done using Statistica software version 13.5.0.17.

3. Results and discussion

3.1. Fermentation time

Fermentation process was observed during the incubation period to achieve the targeted pH range between 4.3 and 4.7 and the result is presented in Fig. 1. In the current study, the pH range was reached at different times depending on the type of BSG derivatives. The addition of BSGF had the same time to reach the pH 4.5–4.7 after 3 h of incubation. However, BSGPs required a longer time (4–4.5 h) regardless the type of the extracts in order to achieve the pH at 4.5–4.7. The differences in time needed to reach the pH range was influenced by the initial pH. BSGF had no impact on the initial pH compared to the pH in control while BSGPs increased the pH of the mixture at the initial stage of incubation. The same phenomenon has been observed previously in the incorporation of BSGF in milk-based yogurt fermentation (Naibaho et al., 2022a). It was shown that BSGF had no significant impact on the initial pH while BSGPs had a range of pH at 6.0–6.5. The significant impact of BSGPs in raising the pH is due to the fact that the extracts were prepared at high pH (8.5) in order to enhance the extraction efficiency. Therefore, the extract was obtained with high pH and impacted the initial pH of the mixture before the incubation.

Fermentation time describes the production of lactic acid in the yogurt due to the growth of lactic acid bacteria (LAB) (Meybodi et al., 2020). By this, the addition of BSGPs appears to have delayed the growth of LAB during the incubation period compared to the control and BSGF addition. This result showed an opposite result compared to our previous experiment; however, it was conducted in milk-based yogurt (Naibaho et al., 2022a). This phenomenon also might be due to the ability of LAB to grow in plant-based milk alternatives. The growth of LAB has been identified in plant-based yogurt alternatives such as in soymilk, lupin based, and cereal-based yogurt alternatives (Coda et al., 2011; Hickisch et al., 2016; İçier et al., 2015; Kim & Han, 2019; Rasika et al., 2021). In general, this study had a shorter incubation period than that in previous reports, which might be depending on the incubation temperature, inoculum types, and ingredients used. WSCE-based yogurt

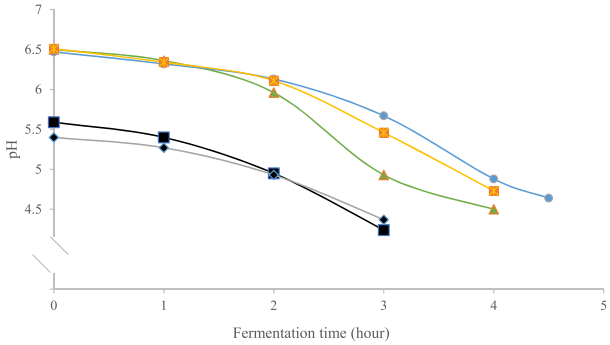

Fig. 1. pH derivation during the fermentation of water-soluble coconut extracts (WSCE) yogurt-alternatives enriched with BSG derivatives: (−): without the addition of BSG derivatives; (—): 10% addition of protein extract without enzymes treated; (—): protamex/flavourzyme-extracted; (—): protamex-extracted; (—): BSG flour. The data is shown as mean of duplicate measurements.

alternatives required 8 h incubation time with temperature 37 °C (Yaakob et al., 2012). Lupin-based yogurt-alternatives required different incubation times depending on the pre-treatment heating temperature (Hickisch et al., 2016). UHT treatment on lupin-based incubation needed 25–35 h while pasteurization required 14–24 h in order to achieve pH at 4.5 (Hickisch et al., 2016).

3.2. Functional groups characterization by FTIR

FTIR spectrum presents the identification of functional groups in the samples and the result is demonstrated in Fig. 2. The results showed that there were 6 observed functional groups which had different transmittance patterns and intensity. The responsible functional groups were identified and presented in Table 1. From those identified groups, the control sample had the lowest transmittance in the group of α-glycosidic bond (1) and hydroxyl groups from polysaccharides (6). This might explain the significant impact of BSG derivatives in polysaccharides bonds in the yogurt alternatives. Sample prepared with BSGP-C had a different transmittance pattern and intensity in the groups of aliphatic ethers and acid functional groups (2) and aromatic hydrocarbons of lignin, amide I, acetyl groups and amide III (3). This phenomenon might be aligned with the difference in the amount and presence of amino acids in the yogurt alternatives due to the addition of BSG derivatives. This also can be due to the presence of soluble dietary fiber and less the amount of protein as an impact of extraction methods.

The identified functional groups at band region of 500–800 cm^{-1} explained the stretching bond due to the presence of α-glycosidic linkages. The band region of 1000–1100 cm^{-1} identified the bond stretching of C–O–C from aliphatic ethers and acid functional groups. The stretching at 1300–1200 cm^{-1} demonstrated amide III or the cleavage of acetyl groups, while the signal at 1700-1600 cm^{-1} showed the presence of amide I and amide II or the aromatic hydrocarbons of lignin. The signal at 1800-1700 cm^{-1} represented the lignin and C═O stretch from fatty acids and its esters. Band stretching at 3000-2800 cm^{-1} is aligned with the C–H asymmetric from CH_2 functional groups. Finally, the band region at 3600-3200 cm^{-1} described the hydroxyl stretching of hydroxyl and amine groups (Brodziak et al., 2021; Naibaho et al., 2021; Patrignani & González-Forte, 2021; Ravindran et al., 2018). The differences in the spectrum pattern and transmittance intensity demonstrated a modification in the matrices of WSCE-based yogurt-alternatives due to the addition of BSG derivatives. The matrix formation of yogurt is influenced by the structural features of the hydrocolloid backbone and side chain of the added ingredients molecules (Huang et al., 2019). Since the BSG derivatives were prepared in different forms, their impact on the microstructural surface of the samples can be observed by the FTIR spectrum. Due to their different functional groups properties as observed in current study, could lead to the variability in the microstructural of yogurt-alternatives as well as related quality parameters including flow behaviour and consistency.

Table 1
Functional groups of WSCE-based yogurt alternatives prepared with BSG derivatives identified by FTIR spectrum.

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

3.3. Microstructural characterization by CLSM

Microstructural evaluation of fat distribution and network formation of yogurt alternatives matrices was conducted, and the results are presented in Fig. 3. The addition of BSG derivatives generated different fat microstructural properties and fat distribution depending on the type of BSG derivatives. As is shown in Fig. 3 (yellow channels), the control sample had more compact and denser fat microstructure (a) while samples containing BSG derivatives had less dense fat network and distribution. The results revealed that BSG derivatives are potentially associated with its ability as a fat replacer in WSCE-based yogurt alternatives. WSCE has been reported for having a high amount of saturated fat (Boeck et al., 2021), therefore, BSG derivatives could improve the nutrition of yogurt alternatives prepared from WSCE. The sample with the addition of BSGP-C (Fig. 3b) had a smaller distributed particle compared to the addition of other BSG derivatives (Fig. 3c, d, e). Remarkably, BSGF addition (e) generated a microstructure with a big particle. This phenomenon might be due to the complex matrices between dietary fiber-protein-fat in BSG flour.

Microstructural appearance of protein and sample matrices is shown

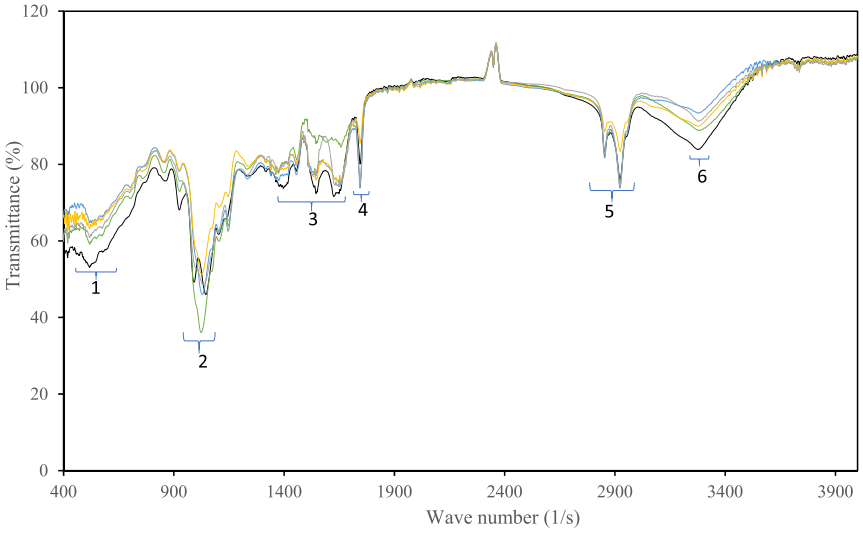

Fig. 2. FTIR spectrum of freeze-dried water-soluble coconut extracts yogurt-alternatives enriched with BSG derivatives: (—): without the addition of BSG derivatives; (—): 10% addition of protein extract without enzymes treated; (—): protamex/flavourzyme-extracted; (—): protamex-extracted; (—): BSG flour.

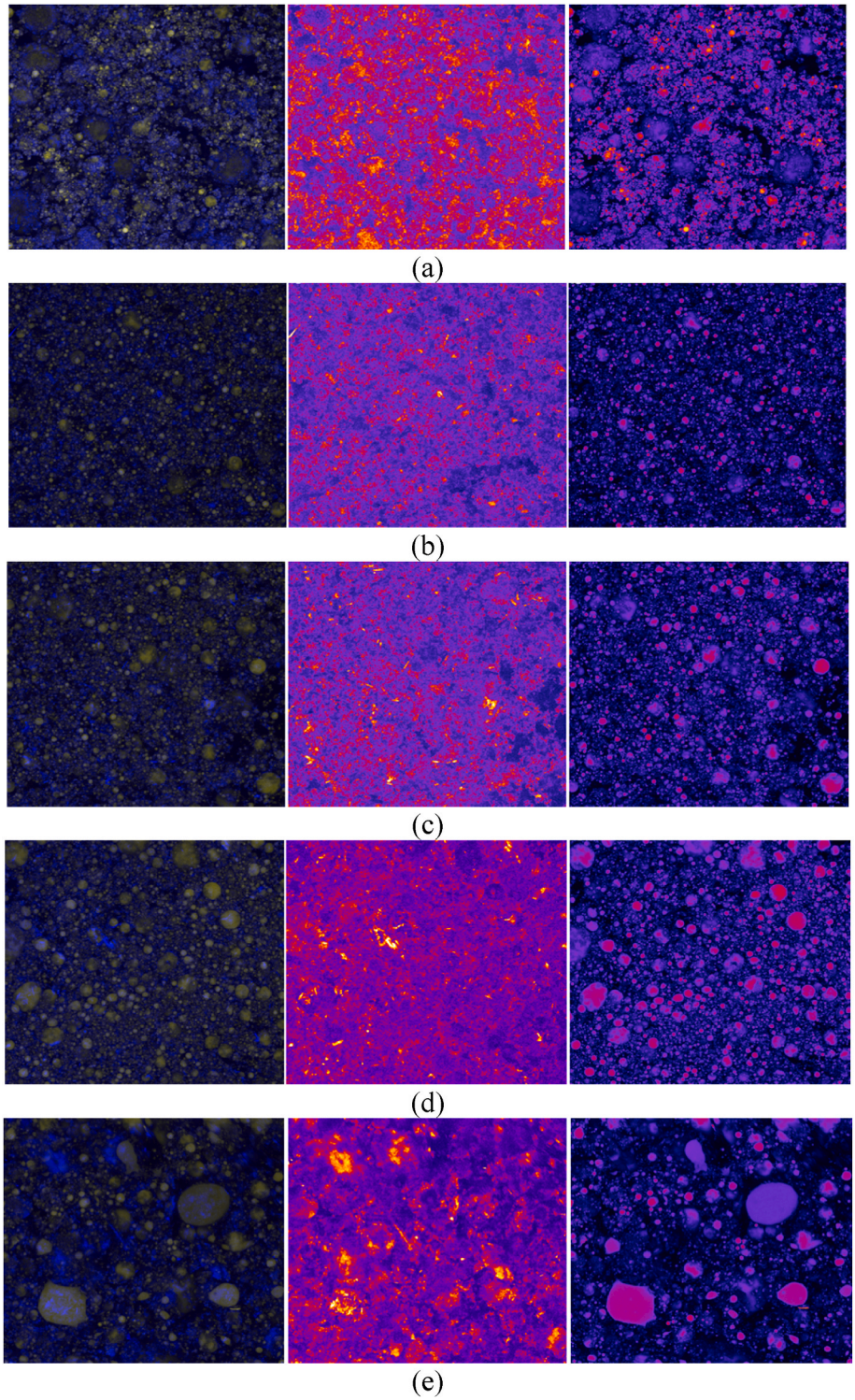

Fig. 3. Confocal laser scanning micrographs of freeze-dried water-soluble coconut extracts yogurt-alternatives enriched with BSG derivatives stained with Nile red: a. control without BSG derivative; b. BSG protein control; c. BSG protein protamex/flavourzyme-treated; d. BSG protein protamex-treated; e. BSG flour. 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in Fig. 4. As has been identified in fat microstructure, the same phenomenon was observed in protein distribution and yogurt alternative matrix structure. However, the addition BSGPs (b, c, and d) generated a denser and more homogenous surface appearance regardless of the extraction methods. WSCE-based yogurt alternatives (a) revealed a more compact surface microstructure and rough appearance, while the incorporation of BSGF generated a surface with a plate-look appearance and less dense microstructure surface. Those differences in protein network formation and distribution can be explained by the different amounts of proteins in the BSG derivatives. Network formation in yogurt matrices is highly influenced by the presence of protein and its amount. The used BSGPs in this study were determined for containing high amounts of available protein. Therefore, it generated a homogenous and denser surface microstructure. WSCE contains a low amount of protein (Boeck et al., 2021) which might be responsible for a rough surface appearance. While BSGF contains protein 20–30% (Naibaho et al., 2021; Naibaho & Korzeniowska, 2021a) and it contains high amounts of dietary fiber (up to 55%) which was reported for its ability to disrupt network formation in food matrices (Naibaho & Korzeniowska, 2021b). BSG has a high ability in binding water (Naibaho et al., 2021; Naibaho &

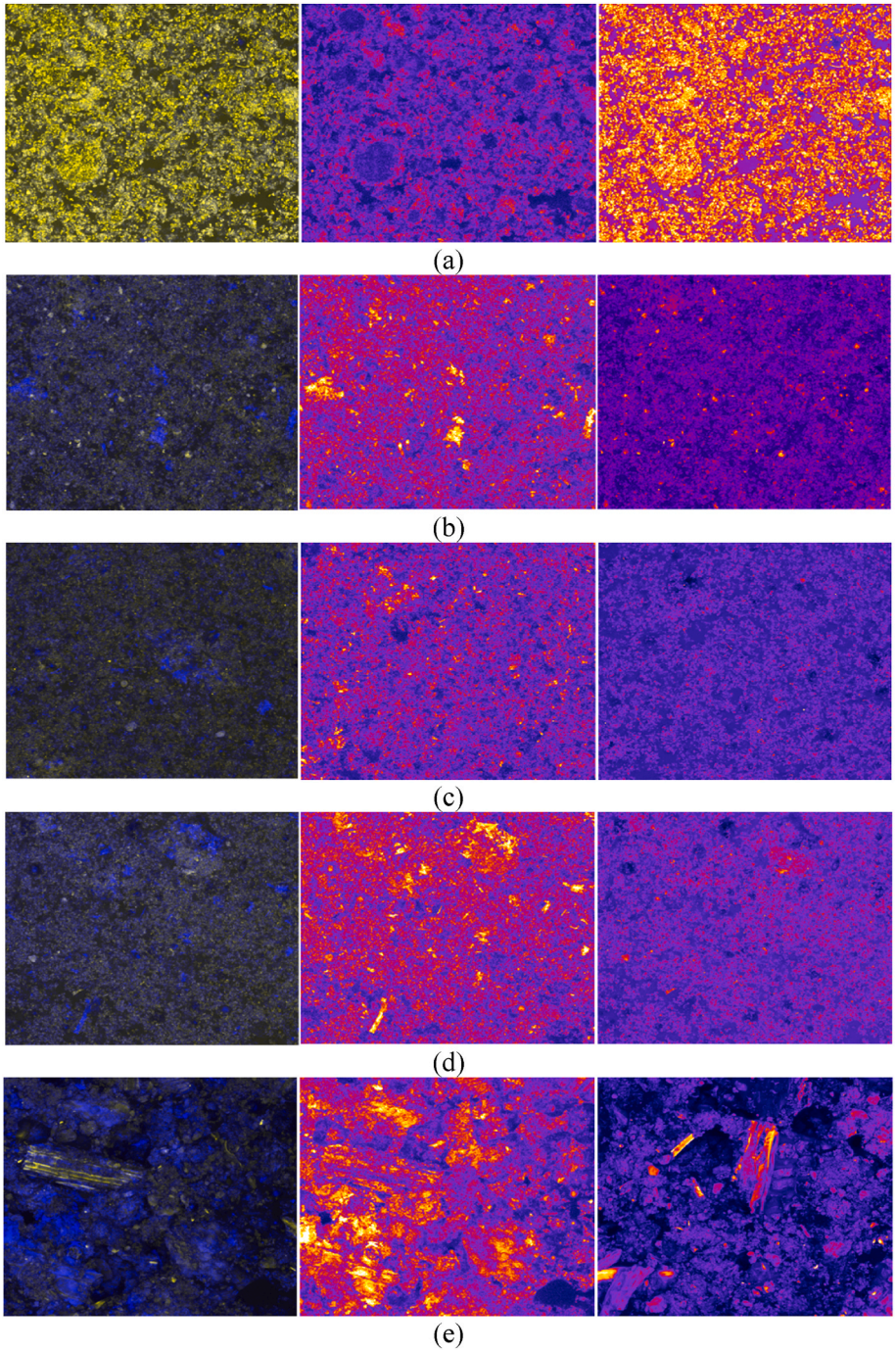

Fig. 4. Confocal laser scanning micrographs of freeze-dri ed water-soluble coconut extracts yogurt-alternatives enriched with BSG derivatives stained with Rhodamine 123: a. control without BSG derivative; b. BSG protein control; c. BSG protein protamex/flavourzyme-treated; d. BSG protein protamex-treated; e. BSG flour. 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): visualized with a laser reflection. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Korzeniowska, 2021a) which could diminish the protein mobility in the yogurt matrix (Pachekrepapol et al., 2021b). Consequently, it reduces the homogeneity and porosity of formed matrices and protein networks as observed in this study (Fig. 4e). This result is aligned with previous reports which discovered that dietary fiber and plant-based addition in yogurt tend to intensify the matrix density and more compact protein structure (Nguyen et al., 2014; Qiu et al., 2021; Zhao et al., 2020).

It has been identified that matrix formation in protein networks is also influenced by binding strength in the matrix (Nguyen et al., 2015). The current study indicates that the different intensity of stretching in certain functional groups were observed as mentioned previously. Extracted protein with protease (protamex and flavourzyme) revealed a functional group with better properties (Fathollahy et al., 2021) which is aligned with this study. Utilisation of protamex and flavourzyme has been reported for its ability to reduce the molecular weight and increase the protein decomposition thus enhancing the amount of amino acids and peptides (Rocha Camargo et al., 2021; Ryan et al., 2020; J. Yang et al., 2020). The higher bioavailability of peptides and amino acids during the fermentation allows a better performance in microstructural surface of the yogurt-alternatives as observed in this study.

3.4. Evaluation of rheological behaviour during the storage

Rheological behaviour of WSCE-based yogurt alternatives during the refrigerated storage is shown in Fig. 5. In general, the shear stress and viscosity of the samples increased during the storage period, regardless of the addition of BSG derivatives. Initially, a slight difference in the shear stress was observed between the samples (Fig. 5a). The addition of

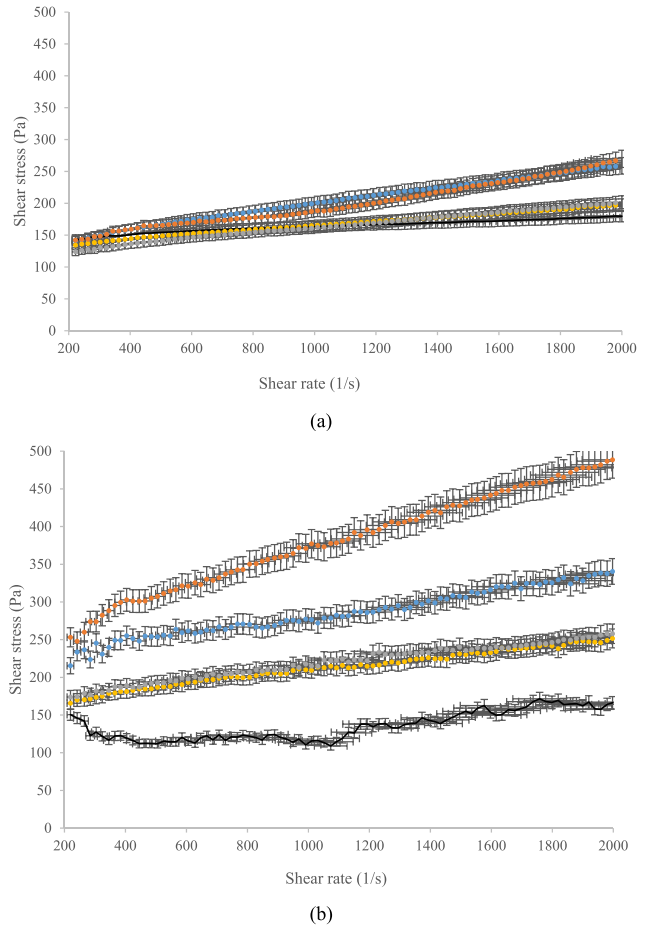

Fig. 5. The relation between shear rate and shear stress of water-soluble coconut extracts yogurt-alternatives enriched with BSG derivatives: (−): without the addition of BSG derivatives; (●): 10% addition of protein control; (●): protamex/flavourzyme-extracted; (●): protamex-extracted; (●): BSG flour, during the storage: a. 1 day of storage; b. 14 days of storage. The data is shown as mean of triplicate measurements.

BSGP-P and BSGP-PF revealed the same trend with the control while BSGF BSGP-C had a slightly higher pattern. This phenomenon explained the different capability of BSG derivatives in texture formation due to their difference in protein availability. BSGP-P and BSGP-PF provided a higher protein availability during the fermentation compared to BSGP-C while BSGF is dominated by insoluble dietary fiber which probably inhibited the texture formation of the yogurt due to its high ability in binding water. Although the addition of protease-extracted derivatives modified the surface appearance and microstructural characteristics of the samples, it had only negligible changes in the shear stress. The pattern of the shear stress changed significantly after 14 days of storage (Fig. 5b) except for the addition of protease-treated BSG derivatives. Regarding shear stress behaviour, the control sample revealed an unstable behaviour after 14 days of storage. However, BSGF and BSGP-C extended the shear stress dramatically. This phenomenon seems to be related to the presence of dietary fiber which contributed to the textural formation. Dietary fiber absorbed high moisture from the system thus altering the flow behaviour of yogurt-alternatives. Furthermore, the evolution in shear stress during the storage could explain its ability in gel forming as well as in preserving the consistency during the storage period. Initially, after the fermentation process was ended, the formed bond between casein micelles of the milk and amino acids from BSG could be weaker (Pachekrepapol et al., 2021b) and after refrigerated storage occurred due to the hydration of macromolecules and stabilization ability of the added ingredients (Ramírez-Sucre & Vélez-Ruiz, 2013) such as BSG derivatives in this study.

After 14 days of storage (Table 2), the viscosity increased except for the control. This showed the ability of BSG derivatives in preserving the consistency of yogurt-alternatives. BSG derivatives intensified the viscosity of the samples thus maintaining the consistency of WSCE-based yogurt alternatives after 14 days of refrigerated storage. This phenomenon can be explained by several flow behaviour related parameters. Consistency index, flow behaviour index and apparent viscosity were evaluated by fitting the viscosity curves into the Power law and the results are presented in Table 2. The data revealed that the addition of BSGF and BSGP-P had the same trend with the control, in which the increase in flow behaviour index was observed. Meanwhile, BSGP-C and BSGP-PF generated a decrease in flow behaviour index after 14 days of storage. Remarkably, all BSG derivatives significantly ($p<0.05$) increased the consistency index and apparent viscosity after 14 days of storage. This result is aligned with the shear stress curves as mentioned previously, in which the increase in shear stress and viscosity was identified. However, the control sample had a decrease in those values (consistency index and apparent viscosity) which is in agreement with the instability of shear stress (Fig. 5b).

Those observed phenomena emphasised the ability of BSG derivatives to form the semi-solid properties of WSCE-based yogurt-alternatives as well as its ability to preserve the consistency during the storage. Matrix formation in yogurt occurred due to the electrostatic interaction between casein micelles from milk and added ingredients,

Table 2
Physico-chemical properties of water-soluble coconut extracts yogurt-alternatives enriched with BSG derivatives.

Storage period (day)	BSG derivatives				
	No BSG	BSGP-C	BSGP-PF	BSGP-P	BSGF
Consistency index (Pa.s)					
1	83.270 ± 2.39^{a}	29.485 ± 8.84^{e}	35.267 ± 1.44^{de}	50.084 ± 0.86^{cd}	28.558 ± 2.27^{e}
14	65.426 ± 6.47^{abc}	75.086 ± 5.50^{ab}	64.616 ± 2.91^{bc}	59.632 ± 2.13^{bc}	49.615 ± 5.41^{cd}
Flow behaviour index					
1	0.099 ± 0.01^{d}	0.284 ± 0.04^{a}	0.224 ± 0.00^{bc}	0.176 ± 0.00^{c}	0.282 ± 0.01^{ab}
14	0.106 ± 0.00^{d}	0.193 ± 0.01^{c}	0.181 ± 0.00^{c}	0.185 ± 0.00^{c}	0.295 ± 0.02^{a}
Viscosity-50 (Pa.s)					
1	0.165 ± 0.01^{e}	0.201 ± 0,01^{d}	0.162 ± 0.00^{e}	0.166 ± 0.00^{e}	0.189 ± 0.00^{de}
14	0.111 ± 0.00^{f}	0.281 ± 0.00^{b}	0.232 ± 0.02^{c}	0.212 ± 0.01^{cd}	0.373 ± 0.01^{a}
Viscosity-100 (Pa.s)					
1	0.090 ± 0.01^{e}	0.129 ± 0.00^{d}	0.101 ± 0.00^{e}	0.099 ± 0.00^{e}	0.135 ± 0.00^{d}
14	0.083 ± 0.01^{e}	0.171 ± 0.00^{c}	0.218 ± 0.00^{b}	0.126 ± 0.00^{d}	0.244 ± 0.01^{a}
Syneresis (%)					
1	24.815 ± 2.31^{c}	40.186 ± 8.09^{b}	47.907 ± 1.15^{ab}	57.962 ± 8.44^{a}	21.989 ± 0.96^{c}
14	0.248 ± 0.10^{d}	0.203 ± 0.07^{d}	0.633 ± 0.19^{d}	0.124 ± 0.01^{d}	0.000 ± 0.00^{d}
pH					
1	4.305 ± 0.01^{e}	4.535 ± 0.01^{c}	4.765 ± 0.01^{a}	4.705 ± 0.01^{b}	4.405 ± 0.01^{d}
14	4.405 ± 0.01^{d}	4.555 ± 0.01^{c}	4.760 ± 0.00^{a}	4.715 ± 0.01^{b}	4.425 ± 0.01^{d}
Lactic acid (%)					
1	0.380 ± 0.03^{e}	0.626 ± 0.01^{d}	0.756 ± 0.00^{c}	0.895 ± 0.01^{ab}	0.575 ± 0.00^{d}
14	0.334 ± 0.03^{e}	0.623 ± 0.01^{d}	0.868 ± 0.03^{b}	0.943 ± 0.00^{a}	0.576 ± 0.00^{d}

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. The measurement was done at least in duplicate.

which leads to a complexation and interfacial stabilization (Hwang et al., 2021; Khubber, 2021). In the current study, BSG protein might have formed a similar electrostatic interaction with soy protein which fluctuates the viscosity during the storage. Matrix formation also occurred due to the LAB growth during the fermentation (Meybodi et al., 2020). pH derivation occurred during the incubation period. At pH 5.3–5.2, casein is destabilised followed by denaturation and precipitation at pH 4.7 (Das et al., 2019). After that, casein and protein milk were acidified at pH below 4.5 (Khubber, 2021). At the acidification stage, casein micelles from milk (acted as positively charged) formed a binding with an electrostatic interaction with added ingredients (acted as negatively charged) then formed a dense protein gel structure and aggregated particles (Khubber, 2021; Luo et al., 2019). Therefore, the acidification phenomenon is responsible for the coagulation and gel formation (Das et al., 2019). During those steps, BSG derivatives might have acted differently in terms of its rule as negatively charged thus generating different flow behaviour and formed microstructure. It has been observed that molecular characteristics of added ingredients including molecular weight, conformation, structural features of hydrocolloid backbone and side chain, as well as charge density play an important rule in yogurt matrix formation (Huang et al., 2021).

The difference properties of BSG derivatives in the formation of yogurt matrices might be attributed to the fermentation time due to the buffering effect of BSG derivatives. BSGPs had a longer fermentation time compared to that in control and BSGF. A shorter fermentation time could reduce the protein network thus leading to the irregularity of the formed network (Pachekrepapol et al., 2021b). Furthermore, it also could reduce the amount of fat and protein in yogurt (Sinamo et al., 2020). By this, a longer fermentation time might allow the availability of protein in modifying the physical properties of the yogurt (Gursel et al., 2016; Körzendörfer et al., 2019). Furthermore, the higher amount of protein facilitated the production of acid whey which hardened the structure of yogurt (Körzendörfer et al., 2019). In other words, BSG derivatives supported the required protein in texture formation of plant-based yogurt-alternatives in this study.

3.5. Syneresis level

Syneresis value describes the amount of water released from the matrices after centrifugation and the results are presented in Table 2. Initially, the addition of three different BSGPs significantly ($p<0.05$) generated a higher syneresis level compared to that in control and BSGF addition. This phenomenon might be due to a weaker gel formed on the first day of storage and it increased during the storage as mentioned previously. It was identified that formed gel could be weaker because of incomplete network (Pachekrepapol et al., 2021b) and hydration of macromolecules occurred during the storage which led to the formation of a stronger binding within the matrices (Ramírez-Sucre & Vélez-Ruiz, 2013). Yogurt control and BSGF-added had a shorter fermentation time and at the same time had a lower syneresis value. Yogurt control and BSGF-added yogurt had a lower syneresis which might be an impact of high fat content in coconut and dietary fiber in BSG flour (Boeck et al., 2021; Naibaho et al., 2022a). While the addition of BSGPs led to the declining amount of fat content and dietary fiber. Moreover, this might be related to the microstructural properties as discussed in the previous section (Section 3.3). Yogurt alternatives with the addition BSGPs obtained a softer, denser and more homogeneous microstructure appearance compared to those in yogurt control and yogurt with BSGF addition. Another possibility is that BSGPs could contain starch due to the extraction process. The presence of starch could induce retrogradation thus leading to expulsion of water from the gel (Wang et al., 2018).

After 14 days of storage, all the samples had the same syneresis level which decreased dramatically ($p<0.05$) from the initial storage. This can be attributed to the modification of flow behaviour, particularly shear stress at the end of the observation (Fig. 5b). The same phenomenon has been reported previously with the addition of tapioca starch in WSCE-based yogurt-alternatives (Pachekrepapol et al., 2021b). However, the derivation in the syneresis value in this study is more intense. In the current study, it was observed a decline from a range of 21.9–57.96% to reach a lowest syneresis at 0%. While a previous study observed a decline from a range of 19–20% to 15-10% after 14 days of storage (Pachekrepapol et al., 2021a). The same impact of BSG powder in milk-based yogurt has also been identified in our previous study (Naibaho et al., 2022a). Compared to other plant-based yogurt-alternatives studies, chickpea-, soybean- and lupin-based study generated syneresis value at the same level as was identified in this study (Hickisch et al., 2016; Wang et al., 2018). However, pea protein and mung bean protein-based yogurt-alternatives generated a higher syneresis level at 87–88% and 90–91% respectively (M. Yang et al., 2021). The variability in syneresis value is also dependent on ingredient pre-treatments and microbial culture strain (Hickisch et al., 2016; Lorusso et al., 2018).

3.6. The acidity: pH and lactic acid production

The evaluation of pH during the storage is performed in Table 2. In general, WSCE-based yogurt-alternatives had a stable pH during the 14 days of storage, except control in which an increase in pH was observed. The results might demonstrate the survival of LAB during the storage. A higher amount of LAB growth will generate a higher amount of lactic acid thus reducing the pH (Bouaziz et al., 2021; Brodziak et al., 2021). Therefore, a stable pH contributed to the stability of LAB growth. By this, sample control had a lower survival of LAB during the storage while the addition of BSG derivatives might have stabilised the LAB growth during the storage. A decrease in pH was also observed during the storage (Fatima & Hekmat, 2020; Pachekrepapol et al., 2021a) which is aligned with the control in current study. Compared to the previous report, commercial WSCE-based yogurt-alternatives had pH at 4.0 (Grasso et al., 2020) which is lower than in current study at 4.3–4.7.

From the perspective of lactic acid production, yogurt control had a drop in pH, although it was insignificant ($p>0.05$). The addition of BSGP-C and BSGF generated a stable production of lactic acid during the observation period. These phenomena demonstrated the same trend as it was in pH. The decline in lactic acid generated an increase in pH and a stable lactic acid production led to a stable in pH. Interestingly, the incorporation of BSGP-P and BSGP-PF resulted in higher lactic acid production after 14 days of storage. This identified the possibility of a higher growth or a higher survival rate of LAB during the refrigerated storage. Remarkably, the incorporation of BSG derivatives generated a higher amount of lactic acid (1.5–3 times higher) compared to the control. Compared to other plant-based yogurt-alternatives studies, soybean had an amount of 0.4% lactic acid while commercial WSCE-based generated an amount of 0.37% lactic acid (Grasso et al., 2020; Hwang et al., 2021). This result is aligned with current study in control with the lactic acid at a range of 0.33–0.38%. However, BSG derivatives enriched the lactic acid production up to 0.9% which is similar with milk-based yogurt (Giacometti Cavalheiro et al., 2020; Gürbüz et al., 2021; Mehrinejad Choobari et al., 2021). By this, BSG derivatives might have improved the stability, growth and the survival rate of the LAB. The addition of BSGF in milk-base yogurt has been observed for improving the lactic acid production, supporting the LAB growth and preserving the survival rate of the LAB during the refrigerated storage (Naibaho et al., 2022a).

4. Conclusion

Different types of BSG derivatives generated different microstructural characteristics confirming the different ability in network formation and interaction between protein and BSG matrices. Three different types of BSGPs generated a softer, denser and more homogeneous microstructural appearance. While control and BSGF-added yogurt had different formed microstructural characteristics due to the high amount

of fat in coconut extracts and dietary fiber in BSGF. Modification in fat distribution showed the potential of BSG derivatives as fat replacer in WSCE-based yogurt alternatives. It was discovered that BSG derivatives generated less dense fat distribution. The modification of microstructural profile is aligned with findings by FTIR which confirmed the modification in certain functional groups. Rheological behaviour showed that BSGPs and BSGF addition tended to increase the shear stress and viscosity demonstrating its ability in maintaining textural and gelling formation. During the storage, the increase in mechanical properties and viscosity was observed in all the addition of BSG derivatives. However, an instability in mechanical properties and viscosity in the control sample was identified during the storage. Compared to control and BSGF-added yogurt, the addition of BSGPs showed a higher syneresis value due to the less amount of fat and dietary fiber. However, all samples had the same level of syneresis after 14 days of storage. Furthermore, BSG derivatives maintained the amount of lactic acid up to 3 times higher than that in control. BSG derivatives in WSCE-based yogurt alternatives demonstrated the same level of lactic acid production with milk-based yogurt. Further investigation in biological activities, flavour compounds, consumers acceptability and identification of LAB strain is needed.

CRediT authorship contribution statement

Joncer Naibaho: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, preparation, Writing – review & editing, Funding acquisition. **Nika Butula:** Methodology, Formal analysis, Writing – review & editing. **Emir Jonuzi:** Methodology, Formal analysis, Writing – review & editing. **Małgorzata Korzeniowska:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, and, Funding acquisition. **Grzegorz Chodaczek:** Methodology, Formal analysis, Writing – review & editing. **Baoru Yang:** Writing – review & editing, Project administration, and, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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