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Alginate and malva nut gum-based hydrogels incorporated with brewer's spent grain as a source of fibre and antioxidants

[1]Nuchchareonpaiboon, P. and [1,2]*Prabsangob, N.

[1]Department of Product Development, Faculty of Agro-Industry, Kasetsart University, 10900 Bangkok, Thailand
[2]Research Unit on Innovative Technologies for Production and Delivery of Functional Biomolecules, Kasetsart University Research and Development Institute, 10900 Bangkok, Thailand

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Abstract

Hydrogels were prepared using malva nut gum (MVG) and alginate as a structuring agent *via* the ionotropic gelation method. For preparation as a source of fibre and antioxidant, the hydrogels were incorporated with brewer's spent grain (BSG) at varying concentrations (0 - 10%). Then, the characteristics and stability of the beads were evaluated based on heating and the storage pH of the beads. The antioxidant activities of the hydrogels increased proportionally with the BSG content. The incorporation of BSG, especially at high concentration, enhanced the bead stability with the beads showing good stability under acidic pH conditions, perhaps because of molecular interactions between the hydrogel-structuring agents and the chemicals available in BSG, as suggested by the FTIR profiles. Then, the hydrogels incorporated with BSG were introduced to passionfruit juice, and their stability was observed throughout 2 w storage. The most appropriate BSG concentration incorporated into the beads was 7.5%, based on its good stability in the juice model, and the relatively high antioxidant capacity of the beads. Therefore, it could be feasible to use the MVG-alginate-based hydrogels incorporated with BSG for the production of healthy food, due to the presence of fibre and the antioxidant in the beads.

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Introduction

Hydrogel formation is an interesting technique for the encapsulation of bioactive compounds due to its feasible fabrication using food-grade biopolymers of proteins and polysaccharides under mild processing conditions (Zhang *et al*., 2016). Encapsulation using hydrogels can improve stability, enhance bioavailability, and control the release of several substances such as peptides (Zhang *et al*., 2016), phenolic extracts from yerba mate (Córdoba *et al*., 2013), and from olive leaves (Flamminii *et al*., 2020), as well as probiotic microorganisms (Li *et al*., 2023). Alginate is widely used to structure hydrogels because of its safety, efficiency, and simplicity for hydrogel formation. Alginate, anionic polymers naturally present in brown algae, is a linear polymer of D-mannuronic acid and L-guluronic acid joined together with 1,4-glycosidic linkages (Dadwal *et al*., 2021). Gelation of alginate is induced through the interaction between the Na^+ ions of carboxylic acid residues and divalent or polyvalent cations such as Ca^{2+}, Ba^{2+}, and Fe^{3+} from the crosslinking solution, thus resulting in an "egg-box" structural rearrangement (Chan, 2011). Alginate hydrogels could be applied effectively to deliver bioactive compounds, food ingredients, and pharmaceuticals regarded for their ability to control release, mask undesirable flavours, and protect bioactive molecules from stress conditions such as heat, light, enzymes, and oxygen (Fu-Hsuan *et al*., 2016). To enhance the stability and encapsulation efficiency of alginate hydrogels, some biopolymers may be used as a co-structurant such as starch (Córdoba *et al*., 2013), pectin, whey protein (Flamminii *et al*., 2020), and chitosan (Anbinder *et al*., 2011). By using different co-structurants, some properties of the hydrogels were greatly affected including particle size, morphology, and encapsulation efficiency (Flamminii *et al*., 2020).

Malva nut, the seed of *Scaphium scaphigerum* which is a native plant of Southeast Asia, is traditionally used as medicine to relief aphthous ulcers and coughing (Somboonpanyakul *et al.*, 2006), as well as having a laxative effect (Srichamroen and Chavasit, 2011a). MVG consists mainly of

*Corresponding author.
Email: faginrpr@ku.ac.th

polysaccharide (≈80%) with slight amounts of protein and lipid (Srichamroen and Chavasit, 2011b). The major composited polysaccharides of MVG are rhamnose, arabinose, and galactose (1.0:1.1:1.0, respectively), thus making MVG a viscous and hydrophilic polysaccharide (Srichamroen and Chavasit, 2011a). Several functional properties of MVG have been reported including thickening, gelling, and water holding abilities (Srichamroen and Chavasit, 2011a). In addition, MVG has some bioactive properties such as antioxidant capacity (Phlicharoenphon *et al*., 2018), α-amylase inhibition, and a role as dietary fibre to increase stool volume and prolong satiety (Srichamroen and Chavasit, 2011b). Therefore, MVG is an interesting ingredient for functional food with beneficial effects relating to anti-obesity, anti-diabetes type II, and lowering the risk factors for cardiovascular disease. However, thus far, there is no information about applications of MVG as a co-structurant for hydrogel formation.

Brewer's spent grain (BSG), the barley malt residues left from wort manufacturing, is the major by-product from breweries amounting to approximately 200 kg for 1,000 L of beer fermentation (Aliyu and Bala, 2011). BSG is always used for low value purposes, mainly as animal feed. Nevertheless, BSG contains nutritive compounds including protein and fibre (Mussatto *et al*., 2006), as well as phytochemicals with bioactivities, especially phenolics with antioxidant activity (Moreira *et al*., 2013). With its contents of lignocellulosic materials, BSG is a practical source of dietary fibre that positively improve human health (McCarthy *et al.*, 2013). Applications of BSG as a source of protein and fibre have been reported in some foods including bread (Stojceska and Ainsworth, 2008) and frankfurter-type sausage (Özvural *et al*., 2009). In addition, BSG has provided phenolics for juice drinks (McCarthy *et al*., 2013). However, the use of BSG in food production is restricted due to its bitter taste and unpleasant mouthfeel. The encapsulation of bioactive compounds using hydrogels can mask the undesirable properties of the compounds (Francesca *et al*., 2019). Therefore, the present work aimed to expand BSG utilisation in food by studying the effect of BSG encapsulation *via* hydrogel formation.

Nowadays, the consumption of food products with health-promoting effects is of ongoing interest. Food fortified with fibre and antioxidative compounds may promote consumers' health by lowering risk factors associated with non-communicable diseases such as obesity, diabetes type II, and cardiovascular disease. The present work aimed to elucidate the possibility of using MVG as a co-structurant in the preparation of an alginate hydrogel incorporated with BSG to provide beads as a source of fibre and antioxidant. The stability of the hydrogels as affected by heating and pH was also analysed, with the potential for hydrogel application in a food model also elucidated.

Materials and methods

Materials

Malva nut was purchased from a local market (Bangkok, Thailand). The BSG was kindly supplied by Muntons PLC (Stowmarket, UK). The fibre content of the BSG was 12.6% (dry basis). The reagents (DPPH, ABTS, Ferrozine, Trolox, and gallic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals included Folin-Ciocalteu reagent (VMR Chemical, Paris, France), $FeCl_2$ (Alfa Aesar, Lancashire, UK), Na-alginate (KemAus, NSW, Australia), and Na-lactate (PURAC Biochem, Gorinchem, the Netherlands). All reagents were of analytical grade.

Preparation and characterisation of hydrogels

MVG was extracted by soaking the Malva nuts in water (nut-to-water ratio of 1:25, weight basis) overnight at room temperature. Excess water was removed by filtering, while the seed and shell fractions of the nut were manually removed. The residual was ground in a blender (Philips HR 2151, Tokyo, Japan), and kept in a plastic bag at 4°C for less than 2 w. The BSG powder was ground and passed through a sieve (70 mm mesh size).

To determine the optimal hydrogel preparation conditions, the content of incorporated BSG was varied within the range 2.5 - 10%, and the amount of added water was subtracted based on the incorporated BSG level (formula for the hydrogel samples is represented in Table 1). Hydrogel preparation was conducted *via* ionotropic gelation according to Alex *et al*. (2013) with slight modification. First, Na-alginate, MVG, and BSG were mixed thoroughly before introduced into a syringe (2 mm in diameter). The hydrogels were added dropwise into calcium lactate solution (0.05 M) by fixing the falling distance to control the bead size. Hardening of the beads was allowed by gentle mixing for 15 min before washing with acetate buffer (10 mM, pH 5.5). The hydrogels

containing BSG at 2.5, 5, 7.5, and 10% were named as BSG-2.5, BSG-5, BSG-7.5, and BSG-10, respectively, whereas Ct represented the MVG-alginate hydrogels without BSG. The hydrogels were then subjected to several analyses.

Table 1. Formulation of the hydrogels.

Composition (g)	Ct	BSG-2.5	BSG-5	BSG-7.5	BSG-10
MVG	1.4	70.0	70.0	70.0	70.0
Na-alginate	1.5	1.5	1.5	1.5	1.5
BSG powder	-	2.5	5.0	7.5	10.0
Water	28.5	26.0	23.5	21.0	18.5
Total	100.0	100.0	100.0	100.0	100.0

Size and shape

The size of the hydrogels (at least 30 beads for each condition) was measured using a vernier calliper, and the shape of the beads was then elucidated as a sphericity index using Eq. 1 (Chan *et al.*, 2009):

$$\text{Sphericity index} = (\text{Dmax} - \text{Dmin})/(\text{Dmax} + \text{Dmin}) \quad \text{(Eq. 1)}$$

where, D_{max} and D_{min} = largest and smallest diameters, respectively, of the hydrogel beads.

Encapsulation efficiency (EE) and loading capacity

EE was quantified according to Alex *et al.* (2013) with slight modifications. First, the beads were soaked in DI water in the ratio of beads-to-water of 1:5 (w/v) before shaking at 37°C in a water bath (Memmert, Schwabach, Germany) for 6 h, and then allowed to stand at ambient temperature for 24 h, after which the soaking water was separated, and its total phenolic content (TPC) was determined using Folin-Ciocalteu assay according to Javanmardi *et al*. (2003). The EE and loading capacity were calculated using Eqs. 2 and 3, respectively.

$$\text{EE}\ (\%) = \left[\frac{TPC_{int} - TPC_{soln}}{TPC_{int}}\right] \times 100 \quad \text{(Eq. 2)}$$

$$\text{Loading capacity}\left(\text{mg}\frac{\text{GAE}}{\text{g}}\text{bead}\right) = (TPC_{int} - TPC_{soln}) \,/\, \text{Weight of hydrogels} \quad \text{(Eq. 3)}$$

where, TPC_{int} = TPC present in the BSG incorporated in the hydrogels before shaking, and TPC_{soln} = TPC present in the soaking solution after shaking.

Fourier-transform infrared spectrometry (FTIR) study

The structural characteristics of the MVG-alginate beads as affected by BSG incorporation were evaluated using an FTIR technique (Bruker model Tensor 27 spectrometer, Bruker, Ettlingen, Germany) with 16 scans, at 4 cm^{-1} resolution over the wavelength range of 4000 - 500 cm^{-1}.

Antioxidant activity of hydrogels

The antioxidant activity of the hydrogels containing different BSG concentrations was evaluated by DPPH and ABTS assay.

DPPH radical scavenging activity

The DPPH scavenging ability of the hydrogels was determined according to Brand-Williams *et al*. (1995) with slight modifications. Briefly, the different solutions of freeze-dried hydrogels (0.5 g/L) were mixed with DPPH solution dissolved in methanol. Each mixture was incubated at room temperature for 1 h, and the absorbance was read at 515 nm (UV-1900, Shimadzu Co. Ltd., Kyoto, Japan). The DPPH radical scavenging ability of the sample was quantified based on the standard curve of Trolox, and reported as mM Trolox equivalent/g hydrogel.

ABTS radical scavenging activity

The ABTS scavenging ability of the hydrogels was determined according to Re *et al*. (1999). Briefly, the solutions of the freeze-dried hydrogels (0.5 g/L) were mixed with ABTS solution, incubated in the dark at room temperature for 2 h, and the absorbance was read at 734 nm. The ABTS radical scavenging ability of the sample was calculated based on the

standard curve of Trolox, and reported as mM Trolox equivalent/g hydrogel.

Stability of MVG hydrogels incorporated with BSG

The stability of the hydrogels was observed for varying heating times and pH conditions.

Thermal stability

The hydrogels were dispersed in DI water at the ratio of beads-to-water of 1:5 (w/v), before heating at 72°C for various times (1 - 5 min). The stability of the beads was estimated by measuring the relative percentage of TPC decrease and the swelling ratio (SR) as a function of heating time. The SR was quantified as the weight ratio of the beads after and before heating (Kurkuri and Aminabhavi, 2004).

pH stability

The hydrogels were dispersed in buffer solutions at varying pH levels (pH 3.0, 5.0 and 7.0) at the ratio of beads-to-buffer solution of 1:5 (w/v). Then, the stability of the beads was estimated by measuring the SR of the beads every 2 d for 2 w. The relative decrease in the TPC of the beads was evaluated after 7 d storage at the varying pH conditions.

Application of hydrogels in food model

Passion fruit juice (PFJ) was selected as a model to elucidate the possible use of the hydrogels in food products. The juice was prepared by mixing passion fruit pulp (20.00%) with water (79.45%), sugar (0.50%), and salt (0.05%). The PFJ was acidic (pH 3.0). The MVG hydrogels incorporated with BSG at the appropriate concentrations were added at the hydrogel-to-PFJ ratio of 1:5 (w/v). Then, pasteurisation was performed at 72°C for 15 s, before rapid cooling to 4°C. The juice samples were kept in a refrigerator for 2 w. The juice samples were evaluated periodically through the storage period based on the SR and TPC of the hydrogels. Any colour change of the juices was observed by determining the colour parameters of L^* (lightness), a^* (red-green component), and b^* (yellow-blue component) (Hunter Lab, VA, USA). The total colour change (ΔE^*) at different storage times was determined using Eq. 4 (dos Reis *et al*., 2018):

$$\Delta E^* = \sqrt{(\Delta L^*) + (\Delta a^*) + (\Delta b^*)} \quad \text{(Eq. 4)}$$

Statistical analysis

Sample was prepared separately and in duplicate. All parameters were measured in triplicate, and values were reported as mean ± standard deviation. Statistical analysis was performed based on One-way analysis of variance using Duncan's test at the confidence level of 95% (SPSS Inc., IL, USA).

Results and discussion

Characteristics of the MVG hydrogels incorporated with BSG

The effects of BSG incorporation at various concentrations on the characteristics of the MVG-alginate hydrogels are shown in Figure 1. Considering the size and shape, the microbeads with diameters of ≈5.00 mm and a sphericity index (Figure 1d) of nearly zero were observed for the beads incorporated with BSG at all concentrations. Material with a sphericity index less than 0.05 was considered as a spherical particle (Chan, 2011); thus, high sphericity of the hydrogels incorporated with BSG could be expected. For the alginate- and MVG-based hydrogels incorporated with BSG, the EE was ≈47%; the EE values of the hydrogels incorporated with BSG at varying concentrations were not significantly different ($p \geq 0.05$), thus suggesting the effectiveness of the MVG-alginate hydrogels to entrap BSG. The addition of starch was reported to improve the EE of the alginate hydrogels loaded with phenolic extracts (Córdoba *et al*., 2013). Alginate tended to form a porous gel matrix, thereby allowing permeability of the encapsulated compounds, especially for those with a small molecular size (Li *et al.*, 2023). The co-structurant agent could exhibit a filler effect to the voids present in the alginate matrix, thus resulting in an improved EE of the hydrogels (Córdoba *et al*., 2013). In the present work, there was improved loading capacity of the hydrogels with an increase in the BSG content ($p < 0.05$), thus implying the efficacy of BSG to strengthen the alginate-hydrogel matrix. MVG is hydrophilic, so it might interact with hydrophilic compounds effectively (Srichamroen and Chavasit, 2011b). This might enhance binding between MVG and the polar residues available in BSG, such as phenolic compounds (Moreira *et al.*, 2013), thus promoting BSG entrapment in the hydrogel matrix.

The antioxidant activity of the hydrogels

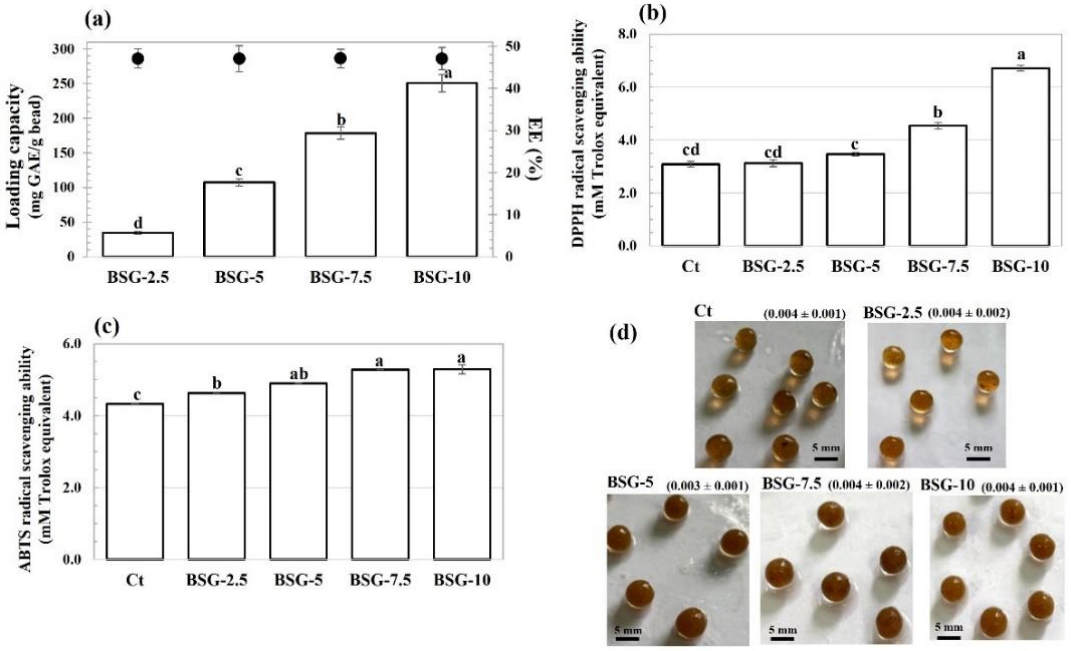

Figure 1. **(a)** Loading capacity (bar) and EE (dot), **(b)** DPPH radical scavenging ability, **(c)** ABTS radical scavenging ability, and **(d)** appearance of the hydrogels (numbers in parenthesis indicate sphericity index of the beads). In **(a)**, **(b)**, and **(c)**, different lowercase letters indicate significantly different means.

incorporated with BSG at different concentrations is shown in Figure 1. The radical scavenging ability of the Ct could have been due to the antioxidative compounds naturally found in MVG, particularly phenolic substances (Phlicharoenphon *et al*., 2018). Interestingly, the increased DPPH radical scavenging ability of the hydrogels was proportional to the BSG concentration, which could be expected due to the greater BSG content incorporated in the beads that previously supported the loading efficiency (Figure 1a). The antioxidant activity of BSG was due to the presence of phytochemicals with antioxidant capacity (McCarty *et al.,* 2013). A positive correlation between the TPC and radical scavenging ability of BSG extract has been reported (Moreira *et al.*, 2013). The phenolic compounds predominantly present in BSG were probably ferulic, *p*-coumaric, and syringic acids, with a minority of sinapic and caffeic acids, as well as isomeric ferulate dehydrodimers and trimers (McCarty *et al*., 2013; Moreira *et al*., 2013). There was a similar trend with the ABTS radical scavenging ability to that of the DPPH radical capability. The hydrogels had slightly higher ABTS antiradical activity with an increased BSG content. However, there was no significant difference in activity between BSG-7.5 and BSG-10 ($p \geq 0.05$). Different capabilities to scavenge DPPH and ABTS radicals might be anticipated in the major mode of actions of the composited phenolics of the BSG. Composited phenolics and the antioxidant activities of the BSG might vary depending on several factors such as the malt varieties and the brewing processes, especially with a kilning temperature (Moreira *et al*., 2013), as well as the soil types and climatic conditions for plant growth (Ikram *et al*., 2019).

The FTIR spectra of the hydrogels incorporated with BSG at various concentrations were used to elucidate the effect of BSG incorporation on the chemical structure of MVG-alginate hydrogels, as shown in Figure 2. Typical bands relating to polysaccharides were evident for all hydrogel samples, and agreed with other works that had studied alginate hydrogels (Dadwal *et al*., 2021). The bands at 2800 - 3000 cm^{-1} indicated stretching C-H bonds of methyl groups (Rafe and Razavi, 2015), and the broad band at around 3000 - 3500 cm^{-1} indicated the presence of the -OH group (Rafe and Razavi, 2015) in all hydrogel samples. The peaks at ≈1590 cm^{-1} indicated asymmetric stretching $-COO^-$ and symmetric stretching of carboxylated salts, probably uronic acids, 1410 cm^{-1} indicated C-N stretching of the primary amide, and at 1030 cm^{-1} indicated vibration of glycosidic C-O-C and C-O-H bonds (Rafe and Razavi, 2015).

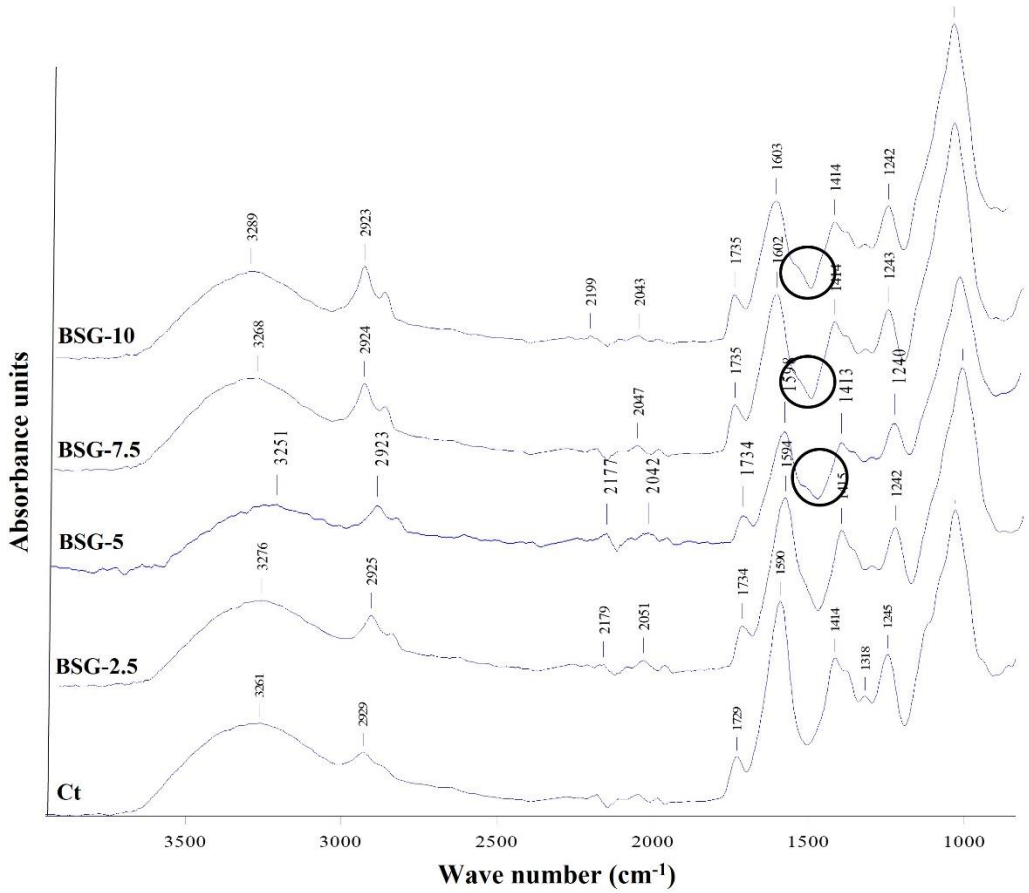

Figure 2. FTIR spectra of hydrogels incorporated with BSG at different concentrations.

The current FTIR pattern of MVG corresponded to the FTIR profiles of guar gum (Ma and Pawlik, 2007) and basil seed gum (Rafe and Razavi, 2015). Interestingly, the peak at ≈1020 - 1050 cm^{-1}, indicating S=O and C-O stretching, could have indicated peptide availability in MVG (Kelly *et al*., 2015). The presence of peptides was also found in the crude polysaccharides extracted from basil seed (Rafe and Razavi, 2015) and raspberry, cherry, and ginseng berry fruits (Kelly *et al*., 2015). Peptides might promote functional properties of the extracted gums in both emulsifying and gelling activities (Rafe and Razavi, 2015). Considering the FTIR profiles of the MVG-alginate hydrogels incorporated with BSG, the emergence of peaks typically relating to phenolics was evident. There were shoulder peaks at ≈1520 cm^{-1} indicating a vibration of C=C (Córdoba *et al*., 2013) for the hydrogels incorporated with BSG at above 5.0% (the circles in Figure 2), thus suggesting interaction between the MVG and phenolic compounds present in BSG when it was introduced at sufficient concentration.

Stability of hydrogels incorporated with BSG

The stability of the beads was observed at varying heating times and pH conditions to determine the feasibility of hydrogel application in food products. Figure 3 depicts the SR and the relative TPC decrease of the hydrogels after heating. BSG-7.5 had the highest stability against heating, suggested by its least deviation of the SR from the baseline as compared to the others at all heating times. Furthermore, there was less change in the TPC for BSG-7.5 and BSG-10, thus suggesting better phenolic entrapability of the beads with an increased BSG concentration.

Figure 4 reveals the storage time dependence on the stability of the hydrogels in different pH environments. Less stability of the beads was observed at a neutral pH as compared to an acidic pH, as indicated by the greatest deviation of the SR from the base line, and the highest TPC loss in the hydrogels stored at pH 7.0 ($p < 0.05$). This behaviour corresponded with other reported results (Zhang *et al*., 2016). The alginate had a slightly negative charge

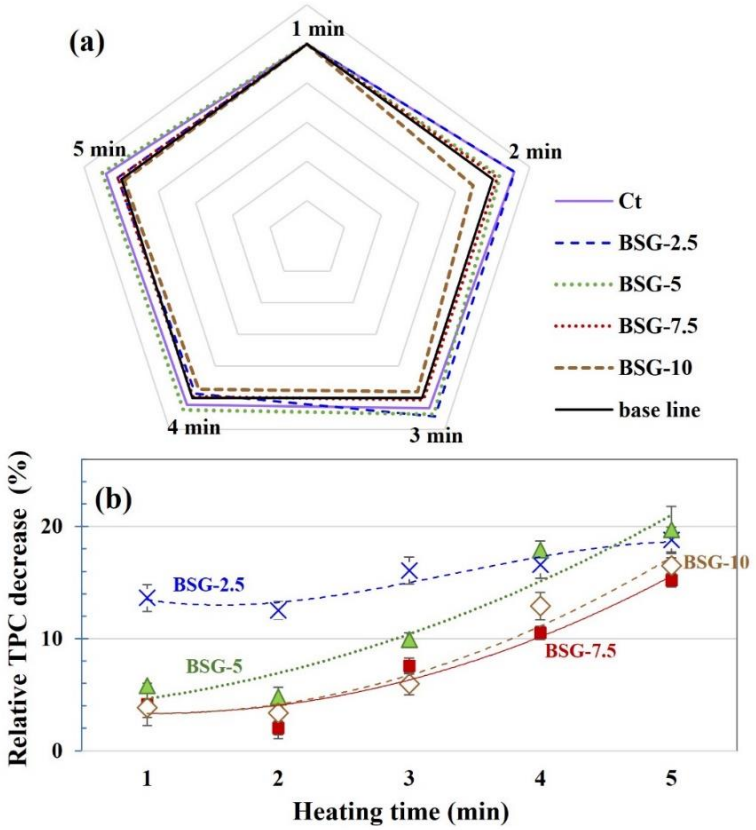

Figure 3. Effect of heating time on **(a)** SR and **(B)** relative TPC decrease of hydrogels incorporated with BSG at different concentrations.

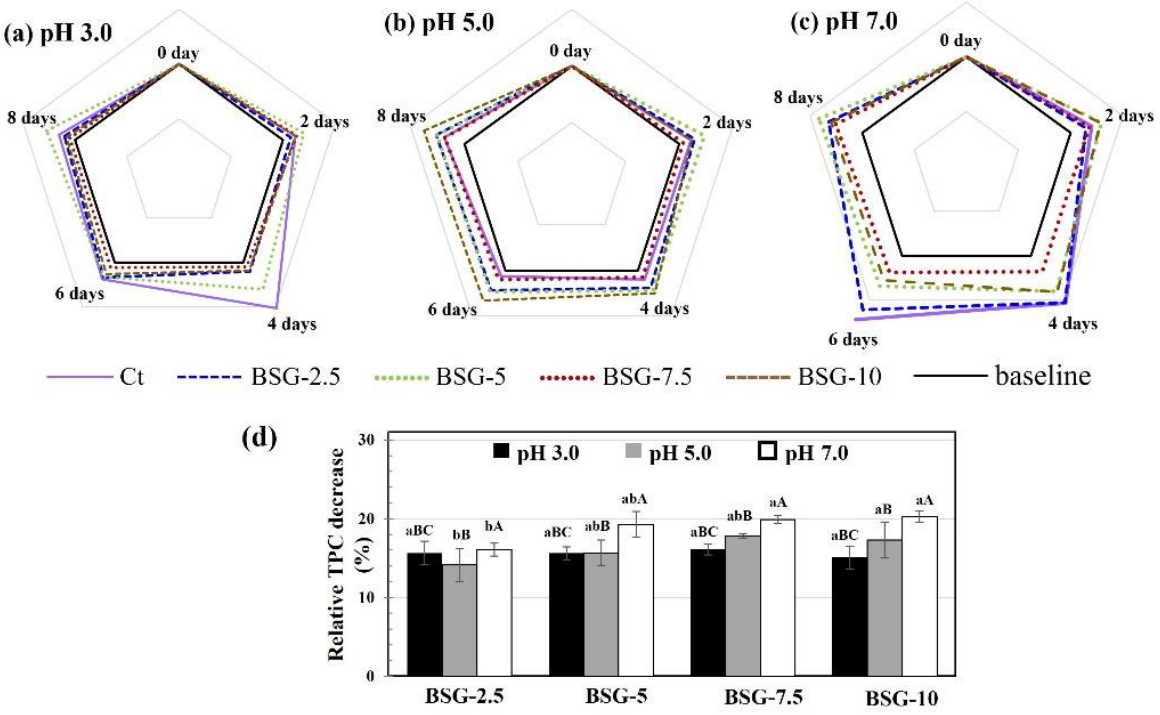

Figure 4. SR of hydrogels stored at pH **(a)** 3.0, **(b)** 5.0, and **(c)** 7.0 for different storage times, and **(d)** relative TPC decrease of hydrogels after 8-day storage at different pH conditions. In **(d)**, different lowercase letters indicate significantly different means as affected by BSG content, and different uppercase letters indicate significantly different means as affected by pH.

at an acidic pH that was greatly increased at a neutral pH: The charges of the alginate molecules at pH 2.0 and 7.0 were -10 and -68 mV, respectively (Zhang *et al*., 2016). Lowering of the net charge of the alginate under acidic conditions could have been due to the partial protonation of the carboxylic residues of guluronic and mannuronic acids possessing the pKa at around pH 3.5 (Lee and Mooney, 2012). A higher net surface charge of alginate at a neutral pH might restrict the interaction between alginate molecules due to the greater repulsive force, thereby lowering the stability of the hydrogel matrix (Zazzali *et al*., 2019). This was in accordance with the current results, in which the collapse of the control beads stored at pH 7.0 was observed after 6 d. Furthermore, at a neutral pH, the phosphate buffer might promote migration of Ca^{2+} present in the alginate hydrogel matrix exchanging with the Na^{+}, thereby resulting in depletion of the microbeads (Bajpai and Sharma, 2004). However, by incorporating BSG, the bead stability could be improved, as indicated by less change in the SR of the hydrogels incorporated with BSG, particularly at an increased BSG concentration. Regarding the effect of acidity, there were similar stability trends for the Ct and the BSG-incorporated hydrogels at both pH 3.0 and 5.0, thus suggesting good acid tolerance of the MVG-alginate hydrogels. However, for these two acidic pH conditions, BSG-7.5 had the least deviation of SR from the base line, thus implying its higher stability. Improvement in hydrogel stability with the presence of BSG, especially at a sufficient concentration, might have been due to the interaction between BSG and the hydrogel structuring agents, which was previously implied by the emergence of new peaks at ≈1520 cm^{-1} in the FTIR profiles.

The presence of macropores was always regarded as a bottleneck for alginate hydrogels that allowed migration of the encapsulated compounds, particularly for the hydrophilic residues, thereby resulting in an inferior EE and the rapid release of the compounds (Li *et al*., 2023). Permeability of water molecules through the alginate membrane could also be expected, thus leading to bead collapse (Li *et al.*, 2021). By using MVG as a co-structurant, the microvoids in the alginate matrix might be occupied, thus resulting in improved bead stability. Starch could also improve the smoothness of the microstructure of the alginate hydrogel, thus resulting in improved stability and EE of the beads (Córdoba *et al.*, 2013). The matrix of alginate hydrogels could also be reinforced by using inulin and chitosan as a co-structurant (Li *et al*., 2021). The FTIR patterns suggested an interaction between BSG and the hydrogel structurants, especially with an increased BSG content. This might further strengthen the hydrogel matrix. The specific interaction between the encapsulated bioactive molecules available in the hydrogel matrix could be a feasible strategy to control desirable properties of the microgel (Zhang *et al*., 2015). By controlling the pH appropriately, interaction between the alginate and encapsulated whey protein could be promoted *via* an electrostatic mechanism, thus resulting in enhanced protein retention ability of the hydrogels (Zhang *et al.*, 2016).

As compared to BSG-7.5, there was less stability for BSG-10, as indicated by its greater SR deviation after heating, with BSG-10 tending to shrink, as indicated by the sub-base line deviation of the SR (Figure 3a). At a sufficient BSG concentration, cross-linking between the chemical composition of BSG (polysaccharides, proteins, and phenolics) and the negative residues of the alginate molecules (hydroxyl and carboxylate groups) could have occurred *via* H-bonding (Hemmati *et al.*, 2015). Excessive binding with BSG might lead to a decreased net charge of the alginate, thereby resulting in bead shrinkage. Reduction in the surface charge caused by protonisation of carboxylic residues of alginate was reported in the extremely acidic conditions (pH 2) of gastric juice that encouraged shrinkage of alginate beads (Rayment *et al.,* 2009). As a result of filler incorporation (such as starch, pectin, and carrageenan) at excessive concentrations, the properties of the alginate hydrogels were reported to be adversely affected as indicated by an inferior EE (Dadwal *et al.*, 2021).

The current results suggested the feasibility of MVG as a co-structurant for the formation of alginate hydrogels incorporated with BSG. The practical BSG content would be 7.5%, as indicated by its good stability against heating and acidic pH conditions. However, BSG-10 had the highest free DPPH radical scavenging ability. Therefore, BSG-7.5 and BSG-10 were selected as representatives for the next study.

Application of hydrogels in a food model

Based on the earlier discussed results, the BSG-incorporated hydrogels showed good stability against acidic pH and heating; thus, PFJ was selected

as a representative model of a pasteurised acidic fruit juice. Passionfruit has several phytochemicals with health promoting effects such as ascorbic acid, carotenes, phenolics, and oligosaccharides (Talcott *et al*., 2003). Figure 5 reveals a storage time dependence on the characteristic of the PFJ added with BSG-7.5 and BSG-10. The SR of the beads slightly increased during storage, with comparable SR values for BSG-7.5 and BSG-10 throughout the 2 w storage, suggesting good stability of the beads in the juice model. There was a colour change in all samples including the control PFJ without hydrogels, whereas the greatest ΔE* value was observed for the PFJ added with BSG-10. The colour change of the PFJ could have been due to the alteration of phytochemicals in the juice. During storage, there was a decrease in the yellowness, probably due to carotenoids deformation (Fernandes *et al*., 2011), whereas degradation of ascorbic acid led to browning of the PFJ (Talcott *et al*., 2003). Based on the phenolic entrapability of the beads, there was a slightly higher phenolic loss for BSG-10 as compared to BSG-7.5 (Figure 5c), thus suggesting superior stability of BSG-7.5, which agreed with the previous result as shown in Figures 3 and 4. Therefore, the reduced ΔE* of the PFJ added with BSG-7.5 as compared to BSG-10 might have been due to the better stability and phenolic entrapability of BSG-7.5. Leakage of BSG from the beads might have promoted some colour change of the juice *via* chemical reactions such as enzymatic browning due to the presence of the phenolics in BSG.

Reduction in the antioxidant activity of the phenolics present in a food product has been reported after digestion (McCarthy *et al*., 2013; Giusti *et al*., 2019), perhaps due to interactions between the phenolics and digestive enzymes (Cilla *et al*., 2009). Considering the alginate hydrogels incorporated with phenolics, the use of polysaccharides as a co-structurant could promote phenolic entrapability, and improve phenolic stability through *in vitro* gastric digestion (Dadwal *et al*., 2021). Therefore, the encapsulation of BSG using MVG-alginate hydrogels might promote tolerance of the phenolics through the human upper gastrointestinal tract, thereby promoting bioavailability of the bioactive compound. Future work is proposed to investigate the phenolic-releasing behaviour and digestive characteristics of the MVG-based hydrogels containing BSG in a stimulated gastrointestinal tract.

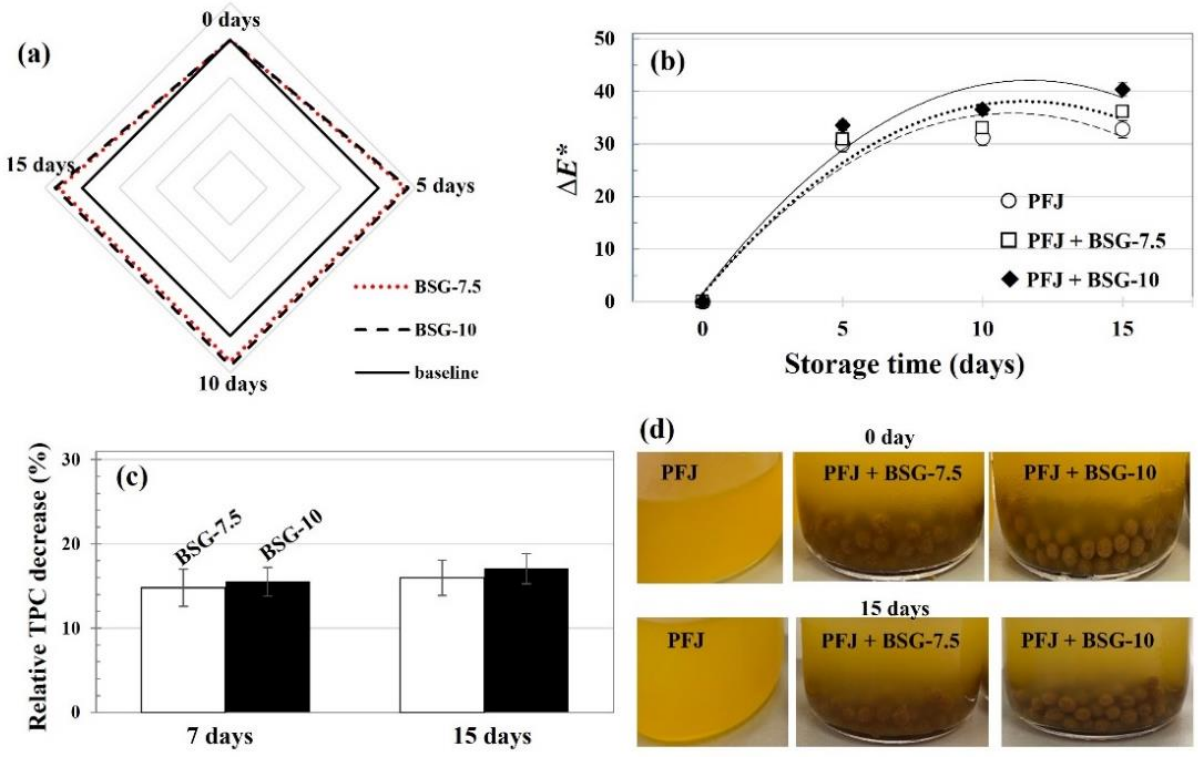

Figure 5. Storage time dependence on **(a)** SR of the hydrogels, **(b)** ΔE* of PFJ, **(c)** relative TPC decrease of the hydrogels, and **(d)** appearance of the PFJ.

Conclusion

The present work successfully prepared alginate-MVG hydrogels incorporated with BSG. The incorporation of BSG at sufficient concentration enhanced the interaction between the chemical components of BSG and the hydrogel structurant, thereby reinforcing the hydrogel matrix. As the BSG content increased, the free radical scavenging ability of the hydrogels improved. The hydrogels showed good stability against heating and acidic conditions. The hydrogels added to pasteurised acidic fruit juice had comparable storage stability with the control juice without hydrogels. The most suitable BSG content for hydrogel preparation was 7.5%. The results suggested the possibility of using MVG and BSG in hydrogel preparation, and that the hydrogels could provide fibre and antioxidant activity, as desirable attributes in healthy food production.

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