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Physicochemical, textural, and microstructural properties of kenaf (*Hibiscus cannabinus* L.) seed tofu as affected by coagulant types and concentrations

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Abstract

Hard tofu was developed from kenaf seed, and the effects of coagulant types and concentrations on the physicochemical, textural, and microstructural properties of the tofu were studied. Glucono delta-lactone (GDL), CH_3COOH, $MgCl_2$, and $CaSO_4$ were the coagulants used at concentrations of 0.25, 0.5, 0.75, and 1.0 g% (w/v). Kenaf seed milky extract was obtained from the seed soaked at 65°C for 2 h 40 min, and ground using 1:6 seed-to-water ratio. The extracted milky extract was cooked to 95°C for 3 min, cooled to 80°C, and then coagulated with appropriate coagulant concentration. The yield, physicochemical, texture, and microstructure of the tofu were examined. The results indicated that the yield and moisture content of the tofu were not significantly affected by coagulant types and concentrations. Coagulant types affected the crude protein, crude fat, and surface colour of the tofu. GDL-coagulated tofu had significantly higher crude protein (35.09 - 39.07 g/100 g), while $MgCl_2$-coagulated tofu had significantly higher crude fat (59.64 - 63.15 g/100 g). The hardness, chewiness, and springiness of the tofu were affected by the coagulant types and concentrations. CH_3COOH-coagulated tofu had significantly higher hardness (2490.1 - 4005.8 g), while $MgCl_2$-coagulated tofu had significantly lower hardness (814.45 - 2009.9 g). Scanning electron microscopy of all the tofu showed a rough-like structure of denser aggregated proteins with large pores, except for the tofu made with 0.25 g% (w/v) which exhibited a pseudo-honey-like structure of compacted network strands. It was concluded that all the coagulants at 0.25 g% (w/v) were suitable to produce kenaf seed tofu.

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Introduction

Tofu is a soybean curd, traditionally produced and consumed in Southeast Asia due to its good nutritional profile and health-promoting potential (Li *et al*., 2011). The texture and microstructure of tofu are most affected by the seed cultivars, coagulant types, concentrations, and processing methods such as heating temperature, stirring rate, coagulation temperature, and moulding degree (Arii and Takenaka, 2014; Xu *et al*., 2016). The types of coagulants used in tofu production are broadly classified into two, acid and salt coagulants. Glucono delta-lactone and acetic acid are the most common acidic coagulants, while calcium sulphate and magnesium chloride are the most common salt coagulants used in tofu production. Acid coagulants have been reported to produce soft tofu (Shih *et al*., 1997), while salt coagulants produce firm or hard tofu (Hsieh *et al*., 2012).

Kenaf (*Hibiscus cannabinus* L.) is a short-day, warm-season herbaceous plant planted as far back as 4000 BC. It belongs to the Malvaceae family, a remarkable plant for horticultural and economic significance (H'ng *et al*., 2009). Kenaf has several local names such as '*mesta*' in India and Bengal, '*rama*' in Nigeria, 'java jute' in Indonesia, 'stockroot' in South Africa, and '*ambari*' in Taiwan (Alexopoulou *et al*., 2013). The kenaf plant is composed of various types of valuable components

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such as stems, leaves, and seeds; the attributes of the components enable abundant product types to be obtained and for sustainable development (Webber III and Bledsoe, 2002). The kenaf stem fibre has been recognised as a good raw material for textiles, wallpaper backing, and furniture (Webber III and Bledsoe, 1993). The whole kenaf plant also has good nutritional profiles and digestibility, and may be used in food and feed productions (Webber III and Bledsoe, 2002). Toxicological studies of aqueous extract of kenaf leaves, and roasted and un-roasted whole kenaf seed meals on experimental rats revealed that kenaf seed is safe for consumption (Agbor *et al*., 2004; Odetola and Eruvbetine, 2012).

Kenaf seed plantation in Malaysia is gaining attention due to the potential economic vision of its stem fibre; the primary focus of kenaf plantation in Malaysia and in the top six commercial kenaf-producing countries, which include India, China, Bangladesh, Myanmar, Thailand, and Nepal (FAO, 2015), is the production of stem fibre. As kenaf seed is one of the secondary products harvested after kenaf plantation, a relatively small amount of the seeds is often used for subsequent plantation. Therefore, the seed needs to be value-added.

Soybean has been historically used for tofu production. Nevertheless, several vegetable seeds have also been used as substitute/alternative to soybean tofu such as lupin seed (Jayasena *et al*., 2010), sesame (Sato, 2017), and peanut (Guo *et al*., 2018), on the basis of their protein content. Likewise, kenaf seed also has the potential to be used for tofu production as it contains 29.8 - 30.5% crude protein with good functionality (Mariod *et al*., 2010). In addition, our previous study has reported the heat coagulation and coagulability properties of kenaf seed protein concentrate (Ibrahim *et al*., 2021). Using kenaf seed for tofu production will serve as a value-addition, functional protein-based food, and a substitute for soybean tofu, especially in countries where kenaf seed is locally cultivated but soybean is not cultivated, such as Malaysia. Differences in coagulants could alter the quality characteristics of tofu such as its texture properties, colour profile, protein, and fat contents. Therefore, the present work aimed to investigate the effects of coagulant types and concentrations on the physicochemical, texture, and microstructure of kenaf seed tofu, and to suggest suitable coagulant(s) and concentration for kenaf seed tofu production.

Materials and methods

Materials

Kenaf seed cultivar KB6 used for tofu production was obtained from the National Tobacco and Kenaf Board, Perlis, Malaysia. All coagulants and reagents used were procured from Sigma-Aldrich, and of analytical grade.

Extraction of kenaf seed milky extract

The kenaf seeds (100 g) were soaked in 500 mL of distilled water at 65°C for 2 h 40 min as reported in our previous study (Ibrahim *et al*., 2021). The soaked seeds were placed in a basket, rinsed thoroughly with distilled water, and excess water was drained off. The seeds were then ground with 600 mL of distilled water using a Waring blender (Model 8011EG, China) at low speed for 3 min. The slurry was squeezed manually with a double layer muslin cloth, and 750 mL of kenaf seed milky extract was then obtained.

Preparation of coagulants

Four different types of coagulant were used namely glucono delta-lactone (GDL), calcium sulphate ($CaSO_4$), magnesium chloride ($MgCl_2$), and glacial acetic acid (CH_3COOH). The coagulants were prepared at four different concentrations (w/v) of 0.25, 0.50, 0.75, and 1.00 g%.

Preparation of kenaf seed tofu

The kenaf seed milky extract (750 mL) was brought to boiling with occasional gentle stirring, and held at 95°C for 3 min as previously described by Ibrahim *et al*. (2021). Then, the cooked milky extract was cooled to 80°C, followed by the addition of coagulant. The coagulum was then transferred to a home-made wooden mould (15 × 15 × 7 cm) lined with cheesecloth, and then pressed with a load of 15 kg for 30 min. Tofu was produced in three different batches. From each batch, two tofu samples were randomly selected for physicochemical and texture profile analyses, making a total of six replicate samples.

Yield and proximate analysis

The yield of the tofu was calculated as the weight of tofu obtained from 100 g of un-soaked seeds, and expressed as g/100 g. The moisture content was determined by drying 2 g of the fresh tofu in an oven at 105°C for 24 h. The crude protein and crude fat were determined on a dry basis by micro Kjeldahl and Soxhlet extraction methods, respectively, following AOAC (2005).

Texture analysis of kenaf seed tofu

The texture profile analysis (TPA) was determined by adapting the method of Gu *et al*. (2016). The analysis was performed with a TA-XT2i Texture Analyzer (Stable Micro System, Godalming, United Kingdom). The tofu samples were cut from the middle portion (10 × 10 × 10 mm). A P36R probe was used, and the setting conditions were pre-calibrated. The pre-test, test, and post-test speeds were set at 1 mm/s each, and 75% deformation force was used. Six replicate tests were conducted for each sample, and the values of hardness, chewiness, springiness, and cohesiveness were obtained automatically.

Colour analysis of kenaf seed tofu

The colour of the tofu samples was measured using a Minolta Chromameter (Model CR-410, Osaka, Japan). The Chromameter was calibrated with white ceramic tile before analysis. The L (lightness), a* (redness), and b* (yellowness) of each tofu were then determined.

Scanning electron microscopy (SEM) analysis

The microstructure of the tofu was observed using SEM (JSM-IT 100 InTouchScope^{TM}, Jeol, Japan) at 100× magnification following the method of Lee and Kuo (2011) with some modifications. The fresh tofu samples were freeze-dried using a freeze dryer (Labconco Freeze Dryer Model Freeze Zone 12, New Jersey) at -45°C for 48 h. The dried tofu samples were cut into 2 × 2 × 1 mm, and fixed onto the SEM slide using double-sided adhesive carbon tape. The tofu samples were then sputter-coated with gold, and the samples viewed at an acceleration voltage of 3.0 kV.

Statistical analysis

Minitab Statistical Software version 17 (Minitab, Inc., State College, Pennsylvania, USA) was used for data analysis, and data were presented as mean ± standard deviation (SD) of six replicate samples drawn from three batches of tofu products. The effects of coagulant types and concentrations were determined by using a two-way analysis of variance (ANOVA), and Tukey's multiple comparisons test was used to ascertain significant differences among the mean at 5% confidence level.

Results and discussion

Yield and proximate composition of kenaf seed tofu

The yield of the kenaf seed tofu ranged from 50.94 - 69.20 g/100 g (Figure 1A). Coagulant types and concentrations had no significant effect on the yield of the kenaf seed tofu except for the tofu made with $CaSO_4$, in which its yields significantly increased as the concentration of $CaSO_4$ increased. During the tofu preparation, visual observation showed that the coagulation of kenaf seed milky extract by $CaSO_4$ was slower as compared to the other coagulants. However, $CaSO_4$ has been observed to have a slower rate of coagulation, which in turn improved tofu yield (Obatolu, 2008).

Similarly, coagulant types and concentrations have no significant effect on the moisture content of the tofu. The moisture content of the tofu ranged from 47.09 – 56.96 g/100 g (Figure 1B). These values were lower than the values of 77.2 – 82.6 g/100 g found for tofu made from soybean (Noh *et al*., 2005), 78.07 – 88.48 g/100 g for peanut tofu (Guo *et al*., 2018), and 82.9 – 84.1 g/100 g for lupin-soybean blend tofu (Jayasena *et al*., 2010). However, the decrease in the tofu yield was reflected in the lower moisture content of the kenaf seed tofu. Likewise, the lower moisture content made the kenaf seed tofu to be denser and coarser, and impeded water retention during moulding.

The protein content of the tofu ranged from 27.85 – 39.07 g/100 g on a dry basis (Figure 1C). These values were lower than the protein content of 54.2 – 64.9 g/100 g dry basis reported for soybean tofu (Obatolu, 2008; Jayasena *et al*., 2010). This variation might have been due to the initial lower protein content of 29.8 - 30.5 g/100 g of kenaf seed (Mariod *et al*., 2010) than values of 35 - 40 g/100 g for soybean seed (Lee and Kuo, 2011). Protein is one of the important components that determine the suitability of a plant seed for tofu production such as lupin seed with a protein content of 32.5 g/100 g (Jayasena *et al*., 2010), and mung bean with a protein content of 23.84 g/100 g (Brishti *et al*., 2017). These plant seeds have been used for tofu preparation. However, the fat content (20.4 - 24.8 g/100 g) of kenaf seed was higher than the fat contents (5.5 and 1.53 g/100 g) of lupin and mung bean seeds, respectively (Jayasena *et al*., 2010; Brishti *et al*., 2017). Fat interference during the coagulation process of the kenaf seed curd might have negatively affected the yield of the tofu, and obstructed protein-water interactions. The coagulant concentrations had no significant effect on the protein content of the tofu. This finding was like the previous study of soybean tofu (Jayasena *et al*., 2014). However, the different types of coagulants had significant effect on protein content. GDL-coagulated tofu had the highest protein content (35.09 - 39.07 g/100 g), followed by $MgCl_2$ (33.35 - 36.65 g/100 g), CH_3COOH (27.85 - 34.72 g/100 g), and $CaSO_4$ (26.88 - 30.13 g/100 g). These values were within the protein content of 32.10 - 39.0 g/100 g reported for soybean tofu by Ndatsu and Olekan (2012). The crude protein content of the kenaf seed tofu might play a vital role in improving the nutritional well-being of many people, and reducing the prevalence of protein-energy malnutrition in most developing countries.

The fat content of the kenaf seed tofu ranged from 48.53 - 65.80 g/100 g on a dry basis (Figure 1D). These values were higher than the fat content (12.30 - 13.70 g/100 g) of soybean tofu (Obatolu, 2008). The higher fat content of kenaf seed tofu was expected as kenaf seed contained higher fat than soybean seed. The coagulant types significantly affected the fat content of the kenaf seed tofu than the concentration. $MgCl_2$-coagulated tofu had significantly higher fat content (59.64 - 63.15 g/100 g) followed by GDL (52.52 - 65.80 g/100 g) and $CaSO_4$ (48.95 - 59.47 g/100 g), and CH_3COOH-coagulated tofu had the lowest fat content of 48.53 - 54.25 g/100 g. These might have been due to the variation in the strength of the coagulants to enhance the fat-binding capacity of the protein network during coagulation. A similar observation has been reported by Obatolu (2008). The significantly higher fat content of the kenaf seed tofu might be advantageous since kenaf seed oil is known to have vital bioactive compounds such as essential omega-6 and omega-9 fatty acids (Chew and Nyam, 2019). These compounds make kenaf seed oil a functional edible oil with health-promoting potentials such as a potent antioxidant (Chan *et al*., 2014), anticancer (Wong *et al*., 2014), and antithrombotic agent (Chew and Nyam, 2019).

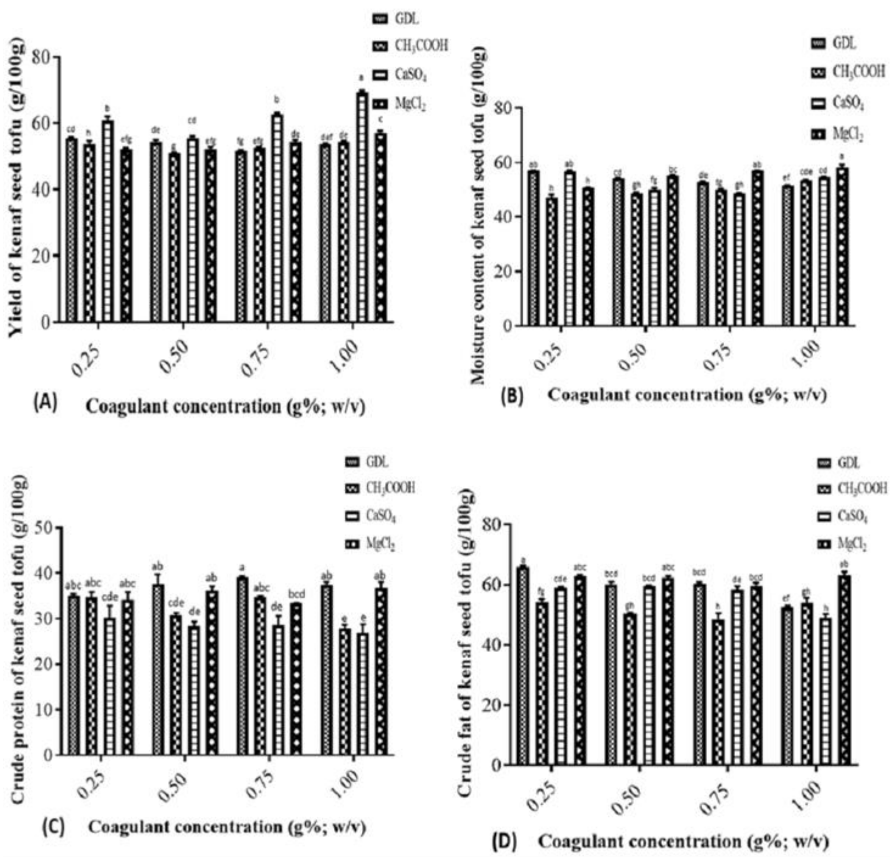

Figure 1. Yield **(A)**, moisture **(B)**, crude protein **(C)**, and crude fat **(D)** of kenaf seed tofu as affected by coagulant types and concentrations. Values are mean of three replicates with error bars indicating ± SD. Error bars smaller than the symbols are not visible.

Colour of kenaf seed tofu

The effects of coagulant types and concentrations on the colour profile of kenaf seed tofu are presented in Figure 2. The lightness (L), yellowness (b*), and redness (a*) of the tofu were significantly affected by the coagulant types. However, the coagulant concentrations had no significant effect on the colour profile of the tofu. The lightness (L) of the tofu indicates the brightness on a scale of 0 to 100. The higher the value means the tofu sample had a bright and lighter colour (Paz *et al*., 2017). The L-values for the tofu varied from 72.49 - 77.09 (Figure 2A). These values were lower than the values 82.7 - 83.3 reported for soybean tofu (Noh *et al*., 2005). This is an indication that soybean tofu had a brighter colour than kenaf seed tofu. Tofu made with GDL and $CaSO_4$ had the highest L-values (76.02 - 76.84 and 73.95 - 77.09, respectively). While $MgCl_2$-coagulated tofu had the lowest L-values of 72.49 - 75.27. The extensive use of GDL and $CaSO_4$ in commercial soybean tofu might have been the positive impact of the coagulants on the tofu colour. Tofu with a creamy or yellowish-white colour is preferred by consumers (Hou and Chang, 2004).

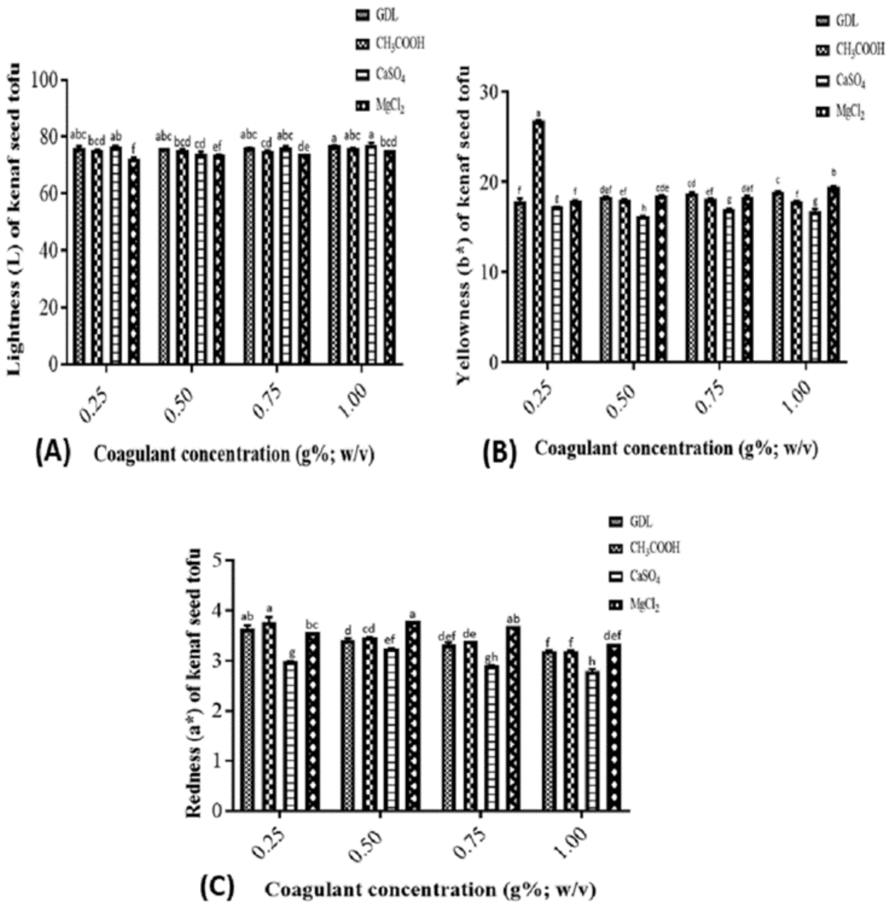

Figure 2. Colour profile of kenaf seed tofu as affected by coagulant types and concentrations. Values are mean of three replicates with error bars indicating ± SD. Error bars smaller than the symbols are not visible.

Tofu with positive a* and b* values indicate redness and yellowness colour, respectively (El-Nimr *et al*., 2010). All the kenaf seed tofu samples had positive a* (2.78 - 3.78) (Figure 2B) and b* (16.25 - 26.80) (Figure 2C) values, which reflected that the kenaf seed tofu was also reddish and yellowish, in addition to a creamy light colour (Figure 3), in comparison to soybean tofu which had a lower range of values for a* (0.3 - 1.6) and b* (9.0 - 12.5) (Noh *et al*., 2005; Jayasena *et al*., 2010). These implied that kenaf seed tofu was less bright in colour than soybean tofu. The redness of the tofu was significantly affected by the coagulant types. The tofu coagulated with $CaSO_4$ had the lowest a*-values (2.78 - 3.24), followed by the tofu made with GDL and CH_3COOH (3.19 - 3.63 and 3.19 - 3.77, respectively). and $MgCl_2$-coagulated tofu had the highest a*-values of 3.33 - 3.78. Correspondingly, the coagulant types affected the yellow colour of the kenaf seed tofu. The increased order of yellowness of the tofu as affected by the type of coagulant was: $CaSO_4$-coagulated tofu (16.25 - 17.31) < GDL-coagulated tofu (17.88 -

18.89) = CH_3COOH-coagulated tofu (17.88 - 26.80) < $MgCl_2$-coagulated tofu (17.94 - 19.47). These concurred with the higher L-values for the tofu coagulated with $CaSO_4$ and GDL, and it was mentioned earlier that the positive influence of these coagulants on the visual appearance of soybean tofu might have been the reason for their common use in tofu production. Nevertheless, the L-values prevailed over the b* and a* values. Hence, all the kenaf seed tofu had the looked-for creamy or yellowish-white colour (Figure 3).

Figure 3. Images of kenaf seed tofu as affected by coagulant types and concentrations.

Texture profile of kenaf seed tofu

The hardness and chewiness of kenaf seed tofu were affected by the coagulant types and concentrations (Figures 4A and 4B, respectively). The tofu coagulated with CH_3COOH had significantly higher hardness (2490.1 - 4005.8 g), followed by GDL and $CaSO_4$-coagulated tofu (1801.7 - 2317 and 1010.2 - 2291.9 g, respectively). While tofu made with $MgCl_2$ had lower hardness (814.45 - 2009.9 g). Previous studies had reported that $MgCl_2$ produced softer tofu compared to the other commonly used coagulants such as $CaSO_4$ (Prabhakaran *et al*., 2006; Jayasena *et al*., 2014). The hardness of $MgCl_2$-coagulated tofu was concentration-dependent. The hardness decreased as the concentration of $MgCl_2$ increased from 0.25 - 1.0 g% (w/v), indicating a significantly higher hardness for the tofu made with 0.25 g% (w/v) $MgCl_2$. However, the hardness of GDL-coagulated tofu increased as the concentration of GDL increased from 0.25 to 0.5 g% (w/v), but further increase in the concentration of GDL above 0.5 g% (w/v) had no significant increase in the hardness of the tofu. Also, an increase in the concentration of $CaSO_4$ from 0.25 to 0.5 g% (w/v) significantly increased the hardness of the tofu, but further increase to 1.0 g% (w/v) significantly decreased the hardness of the tofu. Similarly, the hardness of CH_3COOH-coagulated tofu was significantly higher at the lowest concentration of 0.25 g% (w/v). However, the hardness significantly decreased as the concentration increased to 1.0 g% (w/v). The tofu with higher hardness was found to have significantly lower moisture content, and might have accounted for their hardness. The Pearson's correlation coefficient (r = -0.805) of moisture content and hardness of the kenaf seed tofu indicated significantly strong negative association between the two variables.

The chewiness of the tofu was significantly affected by the types of coagulants (Figure 4B). GDL produced tofu with the highest chewiness (108.4 - 145.57) > CH_3COOH-coagulated tofu (67.8 - 132.25) > $CaSO_4$-coagulated tofu (25.76 - 129.98) > $MgCl_2$-coagulated tofu (21.2 - 51.79). However, coagulant concentrations affected the chewiness of the tofu in a varied way, except for $MgCl_2$, in which the chewiness of the tofu decreased in a concentration-dependent manner. Tofu with higher hardness has been linked with a higher chewiness and lower moisture content. The association of hardness and chewiness of the kenaf seed tofu indicated a significantly moderate positive relationship of r = 0.426.

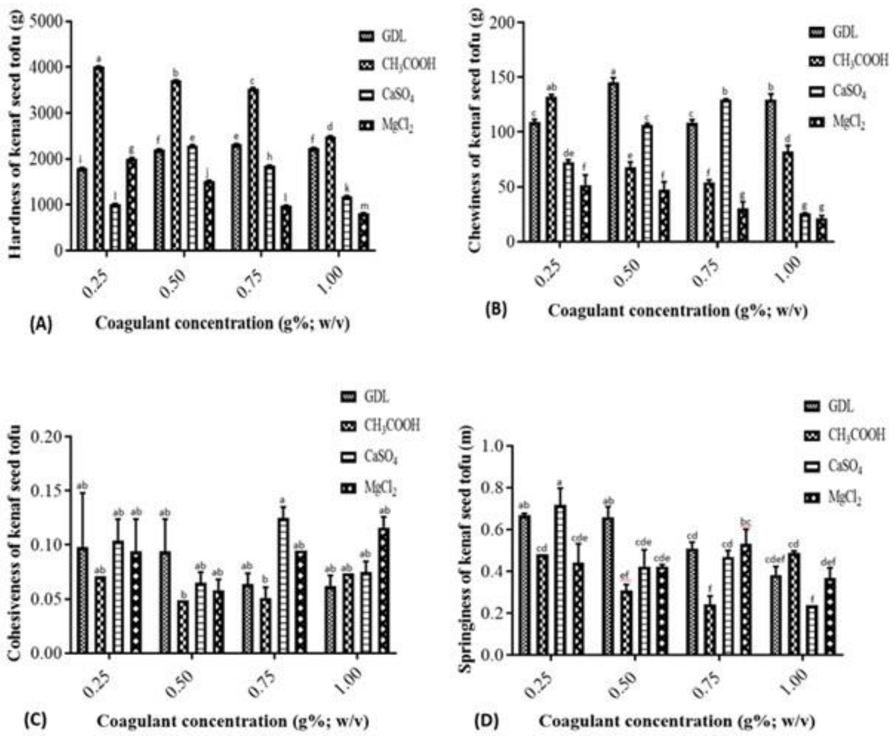

Figure 4. Hardness **(A)**, chewiness **(B)**, cohesiveness **(C)**, and springiness **(D)** of kenaf seed tofu as affected by coagulant types and concentrations. Values are mean of six replicates with error bars indicating ± SD. Error bars smaller than the symbols are not visible.

The cohesiveness of the tofu was not significantly affected by coagulant types and concentrations. The cohesiveness of the tofu ranged from 0.06 - 0.13 (Figure 4C). These values were lower than the cohesiveness values of 0.29 - 0.36 reported for soybean tofu (Noh *et al*., 2005). The low cohesiveness of kenaf seed tofu might have accounted for the coarse and fragile nature of kenaf seed tofu compared to soybean tofu.

The springiness of kenaf seed tofu varied from 0.24 - 0.72 m (Figure 4D). These values were lower than the values of 0.75 - 0.79 m reported for soybean tofu (Noh *et al*., 2005). The tofu made with 0.25 g% (w/v) $CaSO_4$ had the highest springiness of 0.72 m, whereas tofu produced with the highest coagulant concentration of 1.0 g% (w/v) had the least springiness, except for CH_3COOH-coagulated tofu, in which the tofu prepared with 1.0 g% (w/v) had the same springiness as the tofu made with the lowest concentration of 0.25 g% (w/v). In general, the springiness of all the tofu decreased as the coagulant concentration increased.

Scanning electron microscopic structure of kenaf seed tofu

The microscopic images of the freeze-dried kenaf seed tofu are presented in Figure 5. All the tofu showed a coarse-like structure composed of aggregated particulate proteins. There were no variations in the microstructure of the tofu made with the lowest (0.25 g%; w/v) and highest (1.0 g%; w/v) coagulant concentrations. Likewise, the structural networks of the tofu were not different based on coagulant types, except for the tofu made with 0.25 g% (w/v) CH_3COOH, in which an inferior honey-like structure like the microstructural images of most soybean tofu was revealed. GDL-coagulated tofu with 0.25 g% (w/v) showed large, aggregated particulate protein molecules with larger pole sizes (Figure 5A1) compared with the tofu made with the highest concentration of 1.0 g% (w/v) (Figure 5A2). However, the tofu made with 0.25 g% (w/v) CH_3COOH (Figure 5B1) showed interconnected, orderly, denser, and compacted protein strands like structures observed in the case of peanut tofu (Guo *et al*., 2018) and soybean tofu (Lee and Kuo, 2011; Shin *et al*., 2015). This might have been the reason for the significantly higher hardness of the tofu. Moreover, a more orderly and denser tofu structure has been linked to a higher value of its textural properties (Noh *et al*., 2005). However, 1.0 g% (w/v) CH_3COOH-coagulated tofu (Figure 5B2) had a discontinuous network of larger pore size in which the adjacent cell was distinct. This might have accounted for its significantly lower hardness. $CaSO_4$-coagulated tofu (Figures 5C1 and 5C2) had coarse-like structures like that of GDL-coagulated tofu, but with evenly distributed larger pores. The microstructure of 0.25 g% (w/v) $MgCl_2$-coagulated tofu (Figure 5D1) showed a more compacted, denser network of uneven large pores, like the structural image of 1.0 g% (w/v) CH_3COOH-coagulated tofu (Figure 5B2). The microstructural image of 0.25 g% (w/v) $MgCl_2$-coagulated tofu was very distinct from the tofu made with 1.0 g% (w/v) $MgCl_2$ (Figure 5D2), which exhibited discontinuous, loose, and larger cells boundaries. These might have accounted for the significantly lower hardness and chewiness of $MgCl_2$-coagulated tofu at higher concentrations.

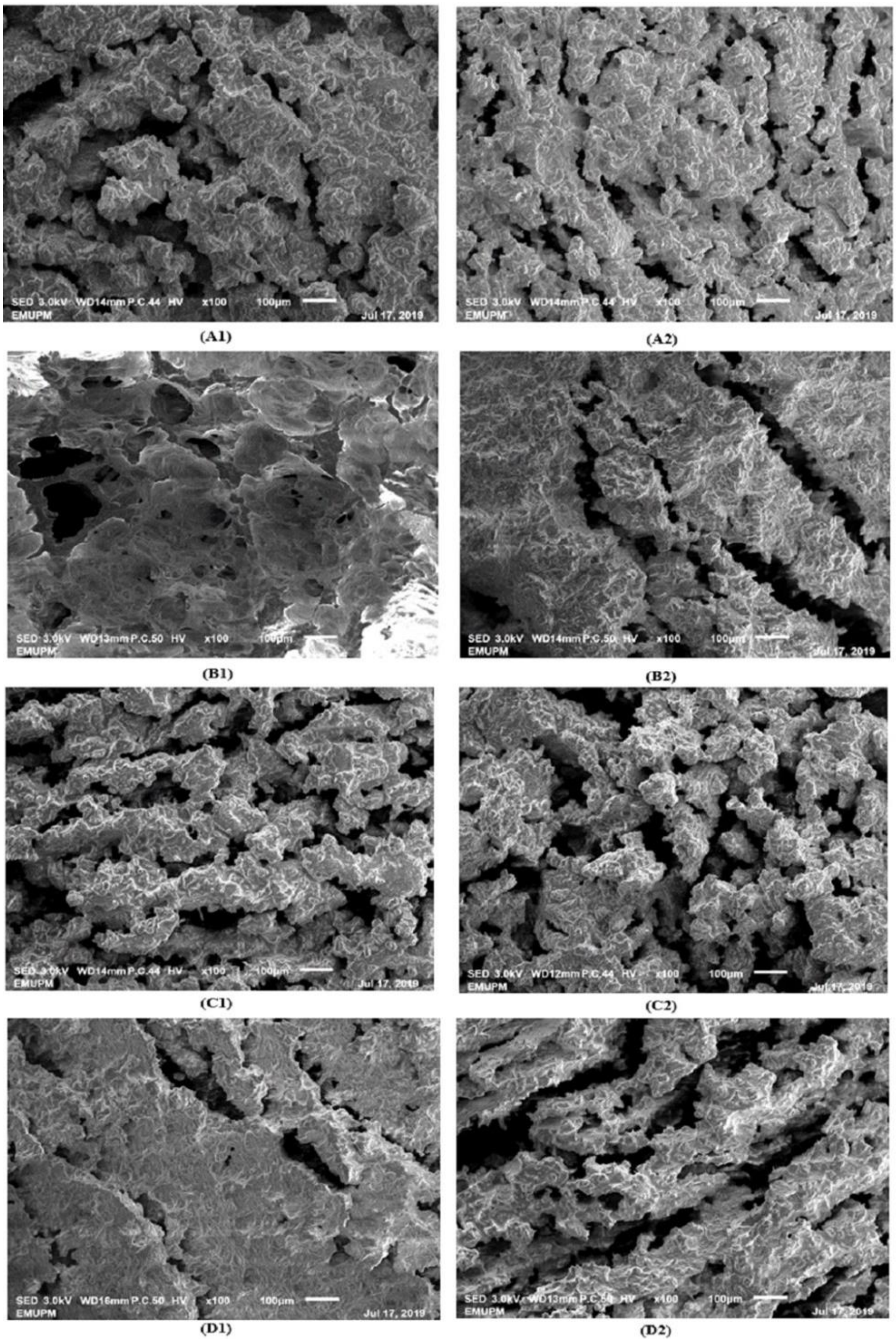

Figure 5. SEM images of kenaf seed tofu prepared with 0.25 and 1.0 g% (w/v) GDL (**A1** and **A2**); 0.25 and 1.0 g% (w/v) CH_3COOH (**B1** and **B2**); 0.25 and 1.0 g% (w/v) $CaSO_4$ (**C1** and **C2**); and 0.25 and 1.0 g% (w/v) $MgCl_2$ (**D1** and **D2**).

Conclusion

In the present work, tofu was produced from kenaf seed. The kenaf seed tofu produced had desirable quality attributes like that of soybean tofu. The different coagulants and their concentrations affected the kenaf seed tofu differently. The types and concentrations of the coagulants had no significant effect on the yield and moisture content of the kenaf seed tofu. However, the crude protein, crude fat, and surface colour of the kenaf seed tofu were significantly affected by the types of coagulant. The hardness, chewiness, and springiness of the kenaf seed tofu were also affected by the coagulant types and their concentrations, but cohesiveness was not affected. CH_3COOH and GDL produced harder kenaf seed tofu and higher chewiness than $CaSO_4$, while $MgCl_2$ at higher concentrations produced kenaf seed tofu with lower hardness and chewiness. The microstructure of CH_3COOH-coagulated kenaf seed tofu at 0.25 g% (w/v) revealed an interconnected and compacted network of strands of proteins, whereas kenaf seed tofu made with the other coagulants had a loose and discontinuous network structure. It can thus be concluded that kenaf seed tofu can be made with either GDL, CH_3COOH, $MgCl_2$, or $CaSO_4$ at a concentration of 0.25 g% (w/v) without significant variation on the final product.

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