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Report on the Use of λ- and κ-Carrageenans Extracted from Seaweeds in Improving Bread Quality

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The increased awareness of food safety issues has highlighted the need for safe food additives. The use of chemical substances in bread making has been correlated with many health problems. This study reported the potential use of λ- and κ-carrageenans, hydrocolloids extracted from the red algae *Halymenia* sp. and *Kappaphycus alvarezii,* respectively, as natural bread improvers. The results showed that the addition of 0.4% λ-carrageenan from *Halymenia* sp. and 0.2% κ-carrageenan from *K. alvarezii* increased bread volume by 30%-50%, improved the texture and structure of bread crumbs, delayed moisture loss by 2%-6%, and maintained crumb elasticity by 5%-15% compared to control bread during a 96-h storage at room temperature. In general, the addition of λ- and κ-carrageenans at lower concentrations increased the acceptance for all sensory parameters in the organoleptic tests compared to the control, but the effects were not statistically significant based on the Tukey test ($p > 0.05$).

Keywords: bread improver; food additive; *Halymenia*; hydrocolloid; *Kappaphycus*; red algae

I. INTRODUCTION

Bread is consumed widely as an alternative foodstuff around the world including in Indonesia. Increasing demand for healthy, high quality bread in large quantities has led to the improvement of technology in the baking process. Food additives are used in bread making to meet the consumers' need for products with desired organoleptic properties and longer shelf life. Several food additives such as potassium bromate (E 924) and calcium propionate, which are used in dough mixture to improve volume, texture and shelf life of the bread, have been banned in Europe, Canada and Indonesia due to the health raised by the Environmental Protection Agency. Unfortunately, not all producers, especially home industry processors, are aware of the consumer health consequences from the use of synthetic food additives. In some developing countries like Indonesia, calcium propionate is still used by many bread producers.

Studies on the use of natural substances for food additives in bread making had been reported by several authors. Rosell *et al.* (2001) stated that κ-carrageenan, a hydrocolloid extracted from seaweeds, could be used as a bread improver to increase dough volume and improve crumb texture. Nayak and Pathak (2016) also reported that carrageenan has the ability to retain water in food products. Natural hydrocolloids were found to improve dough characteristics and extend bread shelf life (Mandala *et al.*, 2007), as well as to improve the texture of bread crumb (Rodge *et al*., 2012). September (2007) and Sciarini *et al.* (2012) found that carrageenan inhibited the growth of spoilage fungi and slowed the staling of bread.

This paper reports the effects of adding λ- and κ-carrageenans extracted from the cultivated seaweeds, *Halymenia* sp. and *Kappaphycus alvarezii,* respectively, on the quality of bread.

II. MATERIALS AND METHODS

A. Research Design

The effect of adding each type of carrageenan (λ and κ) at

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different concentrations (0.2, 0.4, 0.6 and 0.8%) on the quality of bread was tested using a completely randomised design with three replicates. Parameters of bread quality measured were loaf volume, elasticity, moisture content and organoleptic test scores. Analysis of variance was performed at 5% significance level. Data showing significant differences were then further analysed using Tukey's HSD test. All analyses were performed using SPSS statistics software version 21.0.

B. Preparation of λ- and κ-Carrageenans

λ- and κ-carrageenans were extracted from *Halymenia* sp. (harvested from the coastal area in Pantai Pendawa, Bali) and *Kappaphycus alvarezii* (collected from Grupuk, Central Lombok), respectively. The carrageenan extraction process involved the modified hot alkaline method followed by precipitation using isopropyl alcohol as described by Handito (2011). Small pieces of sundried seaweed were boiled in hot water (1 g per 40 mL) at 80-90°C for 1 h, and adjusted to pH 8 using 0.1 M NaOH. The filtrate was collected and an equal volume of 10% NaCl (w/v) was added to a final concentration of 5% (w/v). The solution was then boiled to reduce the volume by 50% and left to settle for 10-15 min. Carrageenan was precipitated by the addition of 2 volumes of isopropyl alcohol and collected by decanting. It was then dried at 40-50°C for 12 h in a cabinet dryer (Memmert, Germany).

C. Bread Making

Basic formula bread was made by mixing 3750 g of wheat flour (basic flour with no additive) in 1725 mL of water with other weighed ingredients: yeast (2% w/w), sucrose (20% w/w), powder milk (5% w/w), margarine (20% w/w), 30 egg yolks and salt (2% w/w). The wheat flour and milk were first mixed in an OX-855 standing mixer (Oxone, Indonesia) at the lowest speed. Yeast diluted in warm water, and egg yolks, were gradually added and agitated slowly to obtain a dough with sandy texture. The dough was divided into 10 portions. λ- and κ-carrageenans were added at various concentrations [0, 0.2, 0.4, 0.6 and 0.8% (w/w)], based on the flour weight and added water, while the dough was kneaded at a slow speed. Margarine was then added and mixed at high speed until the dough became not too sticky. The dough was allowed to ferment in a bowl covered with plastic wrap at room temperature for 30 min. The dough was then punched and divided into dough balls of 70 g. The pieces of dough were wrapped in aluminium foil, proofed for 1 h in an Fx-15S proving chamber (Getra, Indonesia) at 30°C with 85% relative humidity, and baked in an oven at 180°C for 20 min (Mudjajanto & Yulianti, 2004). The bread was then cooled at room temperature and assessed for the loaf volume, crumb structure, staling properties and shelf life, and sensory qualities (including taste, texture, colour, flavour and appearance).

D. Measurement of Loaf Volume

The loaf volume was measured using a modified seed displacement method after the bread was cooled for 1 h. A 100 mL glass beaker was filled with rice grain and shaken vigorously to make the grain settle. The beaker was then overfilled with rice grain and a ruler was used to remove the excess rice grain such that the grain was level with the rim of the beaker. The grain was then weighed and the procedure repeated three times to get the mean grain weight (per 100 mL and give the grain weight per unit volume). A weighed loaf was placed in a beaker which was then filled with rice grain and the excess grain edged off as mentioned above. The grain was weighed, and the difference in grain weight was used to calculate the volume of the loaf after adjusting for weight per unit volume.

E. Observation of Bread Crumb Texture

Slices of bread were cut from the middle part of the loaf for observing the crumb structure. The pieces of bread were placed on a microscope slide, covered with a cover glass, observed under a microscope at a magnification of 400×, and the crumb was photographed to estimate the pore size of the bread slices.

F. Evaluation of Staling Properties and Shelf Life

Bread crumb elasticity and moisture content were measured every 24 h for 4 d upon storage. For determining the bread elasticity, bread pieces having a height of 3 cm (measured from the bottom part) were first placed on a piece of flat glass and pressure was exerted by pressing the bread pieces to half of the original height at 1.5 cm for 1 min using a 50 mL glass beaker. The height of the bread pieces was measured again after pressure was removed for 1 min and expressed as a percentage of the original height to give a measure of the relative elasticity.

Bread crumb moisture content was determined by drying 3-gram portions of finely ground bread crumbs placed in pre-weighed moisture bottles in the oven at 105°C for 4 h. The bottles containing samples were then cooled in a desiccator for 15 min and weighed. This procedure was repeated until the samples attained a constant weight. The change in weight after drying was considered as the amount of moisture lost from the bread crumbs and was referred to as the moisture content of the samples.

$$Moisture\ content = \frac{Weight\ before\ drying - Weight\ after\ drying}{Weight\ before\ drying} \times 100\%$$

Shelf life of the bread samples was determined by observing the physical characteristics. Bread samples were packaged in an oriented polypropylene plastic bag and stored at room temperature for various durations (0, 24, 48, 72 and 96 h), after which they were observed for dryness of the outer crust and the inner crumb. Microbiological observations were also carried out by visually examining the bread for fungal growth after 0, 24, 48, 72 and 96 h of storage at room temperature.

G. Organoleptic Tests

Organoleptic analysis was conducted using the hedonic and scoring tests. The bread samples were cooled for 1–2 h to room temperature before cut into small slices and randomly assigned to the panellist. Twenty-five trained panellists were asked to evaluate each loaf on a given sensory score sheet for the attributes of taste, texture, colour, flavour and appearance of the bread samples. Sensory evaluation was performed on a seven-point hedonic scale.

III. RESULTS AND DISCUSSION

A. Effect of λ- and κ-Carrageenans on Loaf Volume

The carrageenan extracted from *Halymenia* sp. was mostly of the λ-type (Freile-Pelegrin *et al.*, 2011). The results (Figure 1(a)) showed that the addition of λ-carrageenan extracted from *Halymenia* sp. to dough mixture significantly increased the loaf volume compared to the control. The loaf volume was significantly increased with the addition of 0.4% λ-carrageenan, and the highest loaf volume was obtained from dough supplemented with 0.6% λ-carrageenan, while 0.8% λ-carrageenan was less effective in improving the loaf volume (Figure 1(a)). The hydrocolloid extracted from *K. alvarezii*, mainly consisted of κ-carrageenan, significantly enhanced the development of loaf volume at lower concentrations. Addition of 0.2% κ-carrageenan resulted in increased loaf volume, while higher concentration of κ-carrageenan reduced bread volume (Figure 1(b)). Similar results had been reported by Lazaridou *et al.* (2007).

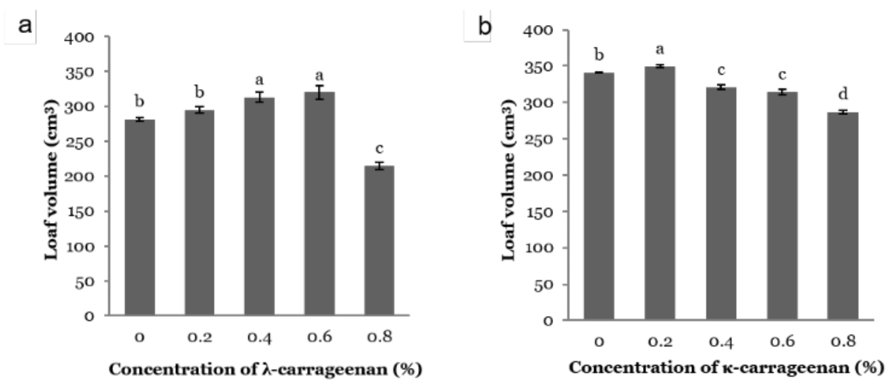

Figure 1. Loaf volume of bread added with (a) 0%-0.8% λ-carrageenan and (b) κ- carrageenan. Data bar shows the mean of three replicates and the error bar indicates standard deviation; treatments with different letters are significantly different ($p < 0.05$).

Development of loaf volume occurs when the structure of dough is sufficiently strong and stable to retain gas formed during fermentation. Although the report on the effect of specific types of carrageenan on the development of loaf volume is not known to the best of our knowledge, Das *et al.* (2013) reported that carrageenan generally has the ability to improve gel network of macromolecules such as gluten, which in turn controls dough elasticity, and hence the gas retention and optimum loaf development. λ-carrageenan is a highly sulphated polysaccharide ester which forms a highly viscous hydrocolloidal solution in water, without forming a gel. The addition of λ-carrageenan at a concentration of 0.8% might have increased the dough viscosity too much for optimal expansion to trap the gas formed by yeast fermentation.

B. Effect of λ- and κ-Carrageenans on Texture, Structure and Pore Size of Bread Crumbs

Visual and microscopic observation on the crumb of bread slices showed that the addition of λ-carrageenan produced crumbs with better texture and structure. Without the addition of λ-carrageenan, crumbs were dry and had big pores of various sizes (Figures 2 and 3). λ-carrageenan is a highly sulphated polysaccharide ester which forms a highly viscous hydrocolloidal solution in water, without forming a gel. The low viscosity of dough without carrageenan might have caused the formation of weak gel network that resulted in suboptimal dough development. Addition of λ-carrageenan gave rise to bread crumbs with small and homogeneous pores that resulted in smooth and moist texture compared to the control. According to Lazaridou *et al.* (2007), the high viscosity of dough improves its ability to maintain the stability of pores during the development of loaf volume and during baking. Observation on slices of bread added with κ-carrageenan showed crumb structure similar to that of the control. However, the treated bread had softer crumb with smaller pores of various sizes (Figures 4 and 5), probably caused by the unique brittle characteristic attributable to κ-carrageenan gel.

Das *et al.* (2013) reported that the addition of hydrocolloids to dough might inhibit the formation of big pores which contribute to the coarse texture of bread crumbs. The increased number of small pores might have contributed to the formation of a homogenous matrix and improved carbon dioxide holding capacity of the crumb during the baking process. We observed that crumbs of bread incorporated with carrageenan tended to have smaller pores.

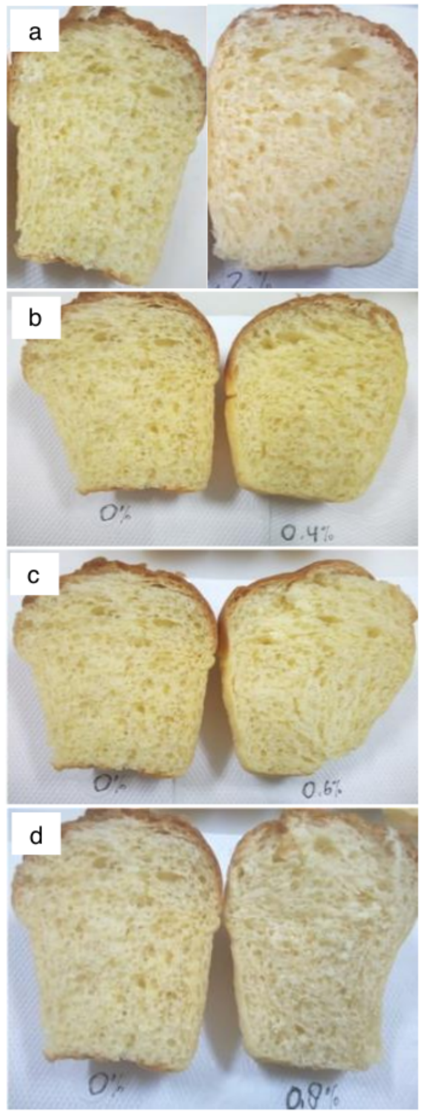

Figure 2. Comparison of the cross section of bread crumb without λ-carrageenan on the left and that of bread added with various concentrations of λ-carrageenan on the right: (a) 0.2%, (b) 0.4%, (c) 0.6% and (d) 0.8%.

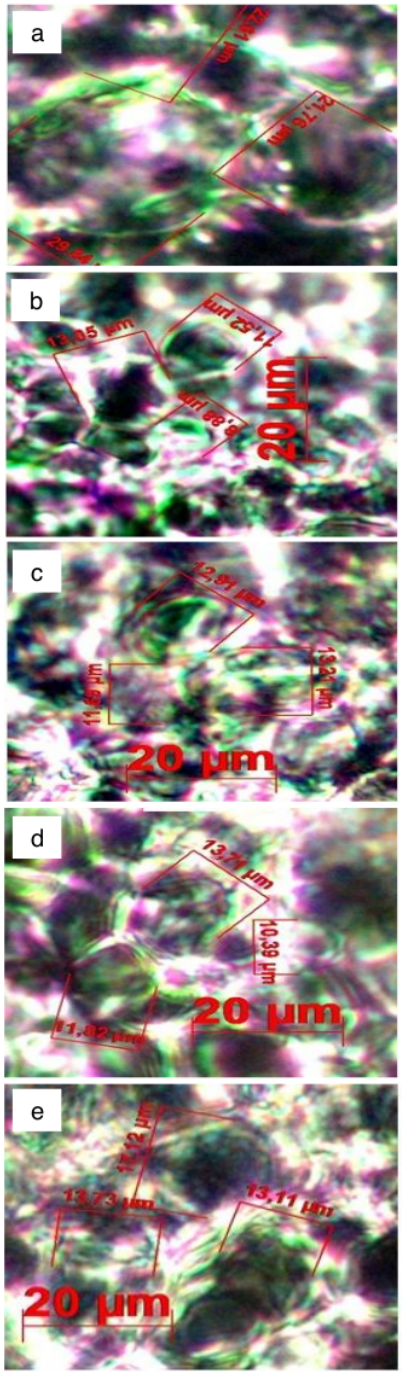

Figure 3. Light microscopy of the crumbs showing larger pores in bread prepared without (a) λ-carrageenan and smaller pores in bread added with various concentrations of λ-carrageenan: (b) 0.2%, (c) 0.4%, (d) 0.6% and (e) 0.8%.

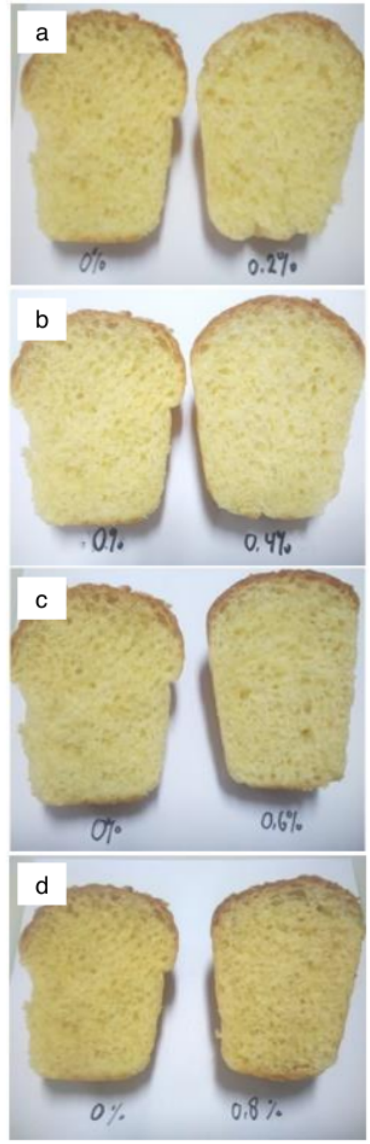

Figure 4. Comparison of the cross section of bread crumb without κ-carrageenan on the left and that of bread added with various concentrations of κ-carrageenan on the right: (a) 0.2%, (b) 0.4%, (c) 0.6% and (d) 0.8%.

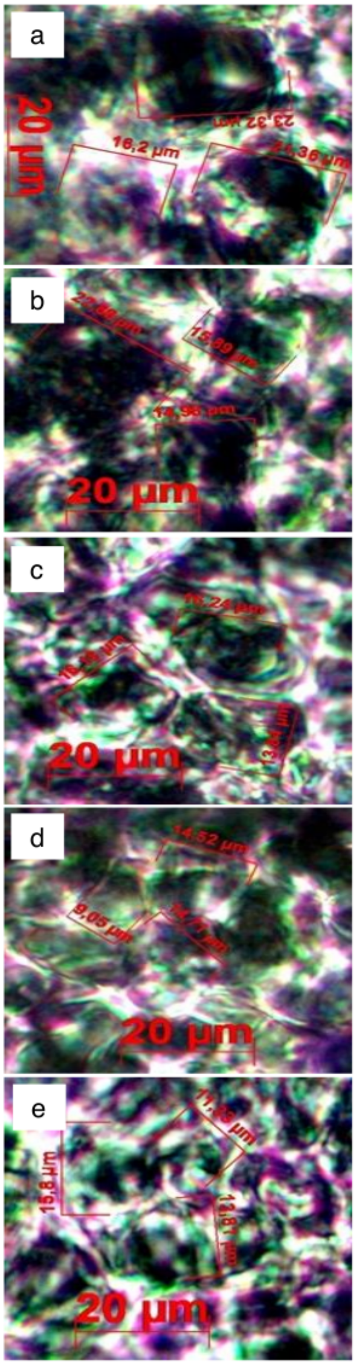

Figure 5. Light microscopy of the crumbs showing larger pores in bread prepared without (a) κ-carrageenan, and smaller pores in bread added with various concentrations of κ-carrageenan: (b) 0.2%, (c) 0.4%, (d) 0.6% and (e) 0.8%.

C. Effect of λ- and κ-Carrageenans on Staling Properties and Shelf Life of Bread

Apart from freshness, which is governed by factors such as reduced elasticity and moisture content that lead to staling, the shelf life of bread is also determined by spoilage caused by microbial growth. Staling of bread is associated with the formation of dry crumb with a leathery texture, and a non-crispy crust. Bread without carrageenan quickly became stale compared to those added with carrageenan, as assessed by touching the bread with hand. Without the addition of carrageenan, staling was observed after 72-96 h as the bread crumbs appeared dry, hard and brittle, showing signs of poor elasticity and significant moisture loss. On the other hand, crumbs of bread added with carrageenan did not show evidence of staling up to 96 h of storage.

Elasticity of the bread prepared in this study reduced during storage at room temperature. In general, the addition of 0.4% λ-carrageenan was the best treatment for maintaining elasticity of the bread during storage (Figure 6(a)). The elasticity of bread containing 0.4% λ-carrageenan was only reduced by less than 10% after 96 h of storage, while that of the control and the bread containing 0.8% λ-carrageenan was reduced by up to 25%. The addition of λ-carrageenan also reduced the amount of moisture lost during 96 h of storage (Figure 6(b)). The rate of moisture loss from bread containing 0.4% λ-carrageenan was 6% less than bread without λ-carrageenan over a period of 96 h, which was in line with the findings reported by Sharadanant and Khan (2003) and Das *et al*. (2013).

Bread containing 0.2% and 0.4% κ-carrageenan had higher elasticity than the control and those incorporated with κ-carrageenan at higher concentrations over a storage period of 96 h (Figure 7(a)). The elasticity of bread added with 0.2% and 0.4% κ-carrageenan decreased relatively gradually by 20% after 96-h storage, while that of the bread added with 0.8% κ-carrageenan declined rapidly after 48 h. Addition of κ-carrageenan also caused the retention of moisture in crumb which maintained the elasticity of bread (Figure 7(b)). Reduction of moisture content enhanced crumb dehydration which led to a reduction of elasticity.

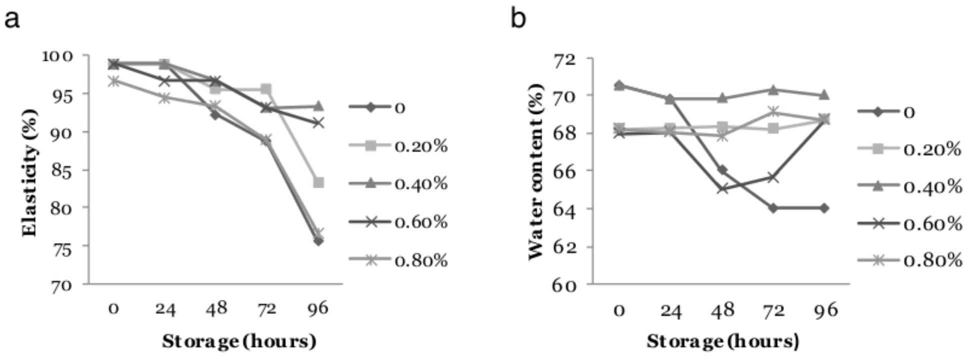

Figure 6. (a) Crumb elasticity and (b) moisture content of bread incorporated with various concentrations of λ-carrageenan measured every 24 h over a storage period of 96 h at room temperature.

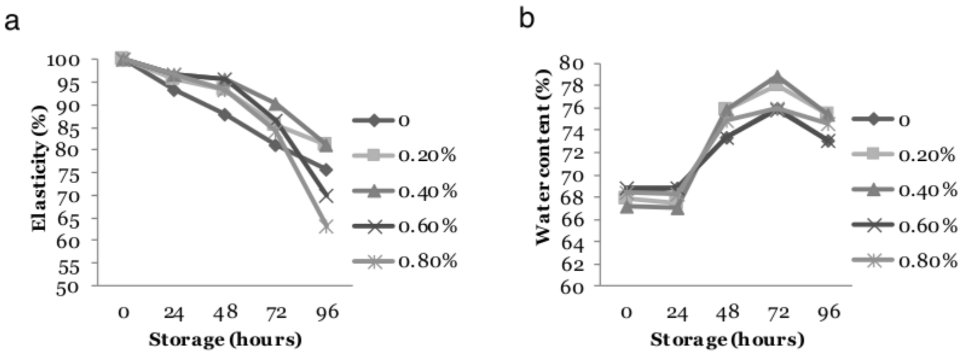

Figure 7. (a) Crumb elasticity and (b) moisture content of bread incorporated with various concentrations of κ-carrageenan measured every 24 h over a storage period of 96 h at room temperature.

During the staling process, moisture is distributed from the crumb to crust. The results demonstrated that λ- and κ-carrageenans have the ability to improve moisture holding capacity of the bread crumbs during 96 h of storage. According to Sciarini *et al.* (2012), hydrocolloids have the capability to slow down moisture movement from the crumb to the crust, and hence the hardening of crust. Bread without carrageenan lost moisture faster than the treated samples, causing the crumbs to lose elasticity quickly. Kohajdová and Karovicová (2008) suggested that hydroxyl groups of hydrocolloids (such as carrageenan) increased the interaction with water molecules through hydrogen bonds. This allowed better retention of water in crumbs, which resulted in less moisture loss and slower loss of elasticity. Similar results had been reported by Ghanbari and Farmani (2013). The decrease in moisture content of the bread accelerated the formation of cross-links network between starch and gluten, which in turn resulted in the crumbs to become hard and lose elasticity (Das *et al.*, 2013).

September (2007) mentioned that carrageenan has the potential to inhibit fungal growth on bread during storage and extend the bread's shelf life. However, in this study, we found no evidence of carrageenan inhibiting the fungal growth on bread since all control and samples showed fungal growth after 96 h of storage.

D. *Effect of λ- and κ-Carrageenans on Sensory Qualities of Bread*

The Tukey tests showed that the addition of both λ- and κ-carrageenans at lower concentrations somewhat improved the acceptability of panellists to the bread (Tables 1 and 2), but the effects were not statistically significant ($p > 0.05$).

Samples treated with 0.6% λ-carrageenan were more tasteful, softer, and easier to chew and swallow compared to the control samples (Table 1). In general, the scores for all sensory attributes were not significantly different between the control and the groups treated with various concentrations of λ-carrageenan, except for the significantly lower scores for texture and appearance of bread treated with 0.8% λ-carrageenan (Table 1). The bread treated with various concentrations of κ-carrageenan was also not significantly different from the control for the scores of all sensory attributes, with the exception that the appearance of bread treated with 0.6% κ-carrageenan was significantly scored lower than the control (Table 2). when we examined the texture and pore size of the bread (Figures 2-5), as the However, we felt a difference in the texture between the treated and untreated samples carrageenan-supplemented bread felt softer than the control. Raman *et al.* (2019) mentioned that carrageenan possibly weakens the starch structure and softens the bread. The non-significant improvement in sensory qualities of the bread treated with carrageenan could probably due to the relatively small sample size of panellists or the conservative nature of Tukey test.

Table 1. Organoleptic evaluation scores for bread added with λ-carrageenan at various concentrations

λ-carrageenan concentration (%)	Taste	Texture	Colour	Flavour	Appearance
0	5.98 abc	5.19 a	4.16 a	6.15 a	6.26 a
0.2	6.24 ab	5.53 a	5.01 a	6.21 a	6.66 a
0.4	5.81 bc	5.31 a	4.38 a	6.10 a	6.45 a
0.6	6.44 a	5.28 a	4.86 a	6.20 a	6.54 a
0.8	5.71 c	4.28 b	3.96 a	5.90 a	5.61 b

Values are means of three replicates. Mean values followed by different letters are significantly different ($p < 0.05$).

Table 2. Organoleptic evaluation scores for bread added with κ-carrageenan at various concentrations

κ-carrageenan concentration (%)	Taste	Texture	Colour	Flavour	Appearance
0	5.92 a	5.62 a	4.43 ab	5.95 a	6.60 a
0.2	6.43 a	5.85 a	4.58 a	6.32 a	6.88 a
0.4	5.58 a	5.58 a	4.31 ab	5.83 a	6.45 ab
0.6	5.52 a	5.23 a	4.17 b	6.00 a	5.77 b
0.8	6.13 a	5.62 a	4.37 ab	6.25 a	6.60 a

Values are means of three replicates. Mean values followed by different letters are significantly different ($p < 0.05$).

IV. CONCLUSION

In general, both λ- and κ-carrageenans improved bread quality at low concentrations. The best treatment was the addition of 0.4% λ-carrageenan which significantly increased loaf volume, and resulted in bread with soft, moist crumb and homogeneous small pores, and delayed loss of moisture and elasticity during a storage period of 96 h, compared to untreated samples. Similarly, the addition of 0.2% κ-carrageenan also enhanced the loaf volume relative to the control but the improvement of other quality parameters was not significant. The addition of λ- and κ-carrageenans to the bread somewhat improved the acceptance for all sensory parameters in the organoleptic tests compared to the control, but the effects were not statistically significant.

V. ACKNOWLEDGEMENTS

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