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Effect of Hydrocolloid on Characteristics of Gluten Free Bread from Rice Flour and Fermented Cassava Flour (*Fercaf*)

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Abstract

Gluten free (GF) bread was made from rice flour and fermented cassava flour. Fermented cassava flour (FERCAF) was produced using a specific design of closed and circulated fermenter, which resulted on a white and neutral aroma flour. However, FERCAF did not have structural component (such as gluten) to provide dough's viscoelasticity and ability to retain gas to hold the volume of bread after baking. Hydrocolloids were added to FERCAF based GF bread to increase water binding of dough. This research aimed to investigate the effect hydrocolloids addition on the characteristics of GF bread made from rice flour and fermented cassava flour (FERCAF). Effect of hydrocolloids to flour ratio (2 %, 3 % and 5 %-wt) and types of hydrocolloid (xanthan gum, agar, and carrageenan) on specific volume of bread, bake loss, bread texture, and microstructure of the bread were investigated. Bread textures were measured using Texture Profile Analyzer (TPA), and microstructure was analysed by SEM. Data experiment showed that addition of hydrocolloids improved GF bread characteristics, specifically increased volume specific, increased porosity, and reduced hardness of GF bread.

Keywords: *gluten-free bread; cassava; fermented cassava flour; Fercaf; hydrocolloids*

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INTRODUCTION

Quality of conventional bread products is strongly influenced by the quality and quantity of gluten, which is composed by glutelin and gliadin, naturally occurring (endogenous) proteins found in wheat. Gluten forms a solid open foam structure common to wheat breadcrumbs (Arendt, et.al. 2008). The viscoelastic thin layer formed by gluten facilitates the formation of bread structures by trapping the gas (gas retention) produced in the fermentation process and the expansion of water vapor when baking. Both processes form a crumb structure that resembles a light but strong foam (sponge). On the other hand, gluten-free starch (gluten free / GF) does not have any structural proteins that can form a strong molecular network and viscoelastic structures.

Without gluten-shaped tissue, GF flour has weak gas and structural retention capabilities due to the lack of viscoelasticity of dough. Consequently, the bread structure made from GF flour usually has a dense, low volume, fragile, and unpleasant structure in the mouth. Hydrocolloids were usually added to the product to increase water binding to produce moisture and soft texture due to the increase of viscoelasticity.

Several studies have also tested the ability of hydrocolloids to improve the quality of GF bread, especially increasing volume, by increasing the dough's ability to retain gas (Anton & Artfield, 2008). BeMiller (2008) stated that several types of hydrocolloids are often used in the laboratory scale manufacture of bread GF such as xanthan gum (XG) and hydroxypropyl methylcellulose (HPMC). Xanthan can form a high viscosity pseudoplastic material, while HPMC can experience thermal reversible gelation, which forms gel in cold water, then turns amorphous with heating, and returns to gel after cooling. *Calle et al.* (2020) investigated the incorporation of hydrocolloids (HPMC, xanthan gum, guar gum) on *Colocasia spp*. cormels flour based GF bread. This formulation had similar quality parameters to the previous GF formulations with enhanced nutritional value such as high protein, high minerals, high fibre content, but with low fat content.

The addition of hydrocolloids HPMC or mixture of HPMC-xanthan gum-guar gum increased moisture content of the GF bread, while also increased hardness compare to the GF bread control (without hydrocolloids). Mohammadi et al. (2014) investigated the effects of Xanthan Gum (XG) and CMC on corn starch-rice flour GF bread quality parameters. The results showed that XG addition on GF bread formulation increased moisture content and the increase in XG concentration effectively reduced hardness and increasing elasticity of GF bread, while CMC addition lead to bigger gas cells which was well corresponding to GF bread porosity appearance. Lazaridou et al. (2007) studied the effect of hydrocolloids CMC, agarose, XG, or beta-glucan on dough rheology and bread quality parameters in rice flour, corn starch, and sodium caseinate GF bread, which also showed that XG addition gave the most improvement of viscoelastic properties and dough strength. Sabanis & Tzia (2011) showed that addition of 1% and 1.5% HPMC, carrageenan, and guar gum increased GF bread loaf volume and resulted in better colour than control GF bread. Liu et al. (2018) studied the effects of hydrocolloids (HPMC, CMC, xanthan gum (XG), and apple pectin) concentration on dough thermo-mechanical properties of potato flour GF steamed bread. This study found that hydrocolloids addition significantly increased the gelatinization temperature and water absorption of potato GF dough.

GF bread has been made from a variety of GF flour. Among the most commonly used types of GF flour are rice flour and cassava starch. Both types of flour are easily digested, white, and have a neutral aroma. Rice flour is classified as hypoallergenic due to its low protein content, especially prolamin (Rossel & Marco, 2008). On the other hand, cassava is a potential source of carbohydrates to support food security in the future, both for direct consumption, components of food products (flour), as well as functional food (starch). Eduardo et al. (2013) investigated the effect of cassava processing (sun-dried, roasted and fermented) on composite cassava-wheat-maize bread quality containing cassava levels from 20 to 40% (w/w) in combination with high-methylated pectin (HM-pectin). This study showed that cassava concentration and processing had significant influenced on GF bread quality. At high cassava concentration, roasted cassava GF bread had a higher volume than sun-dried or fermented cassava GF bread. Pectin concentration had a significant effect on increasing volume of roasted cassava GF bread. Crust colour of roasted cassava GF bread was the most similar to wheat bread.

Based on the Central Bureau of Statistics 2015, cassava productivity in Indonesia reached 23 tonnes/ ha or about four times higher than rice productivity (5.3 tonnes/ ha) (BPS, 2017). In recent years, the Chemical Engineering Department of ITB has developed fermented cassava flour (FERCAF). In general, processing of cassava into FERCAF aim to reduce organic cyanide content, soften the flour texture (by shortening fiber matrix), produce white color, and neutral odor. FERCAF is produced by fermentation of cassava chips using mixture of three microorganism: *Bacillus subtilis, Lactbacillus plantarum, and Aspergilus niger* (Kresnowati, 2015). Cellulolytic activity of *Bacillus subtilis* was used to disrupts cassava cell walls which resulted in softer texture of the flour and produced glutamate to improves odour (Anyogu et al., 2014). Amylolitic activity of *Lactobacillus plantarum* was used to hydrolyse starch while its linamarase activity was utilized to cut cyanogenic glucoside structures (Brauman et al., 1996). β-glucosidase activity of *Aspergillus oryzae* provided single cell protein and improve protein content of the flour (Crewter et al., 1953).

FERCAF production process was conducted in closed circulated reactor at semi-continuous production, enabling large scale production with more hygiene and consistent quality (Kresnowati et al, 2014, Lestari et al., 2019). In addition, the shelf life of packaged FERCAF has been studied and ranged between 3-4 months at ambient temperature (Lestari et.al, 2019) has studied the shelf life of packaged FERCAF in ambient temperature.

During FERCAF production, starch and fiber was not separated. Therefore, FERCAF still contains fiber which differentiate its characteristics and applications with isolated cassava starch (tapioca). Based on starch/fiber composition, FERCAF is similar to wheat flour. However, unlike wheat flour, FERCAF have no gluten which has viscoelastic properties to provide structure for many food products, especially bread, pasta, or noodle. While FERCAF can be very suitable to be used as ingredients for cookies with brittle structure, FERCAF will need additives to build viscoelastic structure necessary for bread, pasta, and noodle. Hydrocolloids were expected to improve FERCAF-Rice Flour GF bread. However, the effect of hydrocolloids on GF bread structure still need to be investigated. The objectives of the research were to investigate the effect of hydrocolloids to flour mass ratio (2 %, 3 %, 5 %-wt of flour) and hydrocolloids types (Xanthan Gum, Agar, and Carrageenan) on bread specific volume, bake loss, bread texture, and microstructure of GF bread.

MATERIALS AND METHODS

Fresh cassava tubers as raw material for fermented cassava flour (FERCAF) were bought from supplier in Bandung, Indonesia. Fermentation was conducted using three microorganisms: *Lactobacillus plantarum* (ITB B188), *Bacillus subtilis* (ITB B128), and *Aspergillus oryzae* (ITB L24), which prepared in the Laboratory of Microbiology and Bioprocess Technology, Department of Chemical Engineering, Institut Teknologi Bandung, Indonesia. Briefly, FERCAF was produced following 3 consecutive stages: inoculum preparation, pre-treatment of cassava chips, followed by cassava chips fermentation according to the method from Kresnowati (2014).

Inoculum preparation

Briefly, mixture of starter culture of microorganisms was made by growing *Bacillus subtilis* and *Aspergilus oryzae* in 20 mL of potato dextrose broth and growing *Lactobacillus plantarum* in 20 mL MRS broth. The culture was incubated at ambient temperature (30 °C) for 48 h until the exponential phase was reached. Starter culture was grown on a mixture of 200 g of cassava per 2 L of sterilized demineralized water and incubated at ambient temperature for 24 h.

Production of Fermented Cassava Flour (FERCAF)

Fermentation were conducted in a circulated reactor following the method from Kresnowati (2014) and Lestari (2019). Briefly, fresh cassava tubers were processed within 24 hours, consisted of peeling, washing, and chipping to approx. 0.1 cm thickness. Fermentation was conducted by soaking 3 kg of cassava chips with 2 L of previously prepared inoculum solution in a circulated fermentation reactor at controlled temperature of 34˚C for 24 hours, followed by the addition of demineralized water to give final volume of 20 L. Next, fermented cassava chips were dried (no washing required) at room temperature, milled, and sieved.

Bread preparation

Bread dough of 200 g was made with the following steps. Firstly, dry mix, consist of flour (130 g), sugar (3 g), salts (0.5 g), and fat (5 g), was mixed with 50 ml water until homogeneous. In a separate container, about 2 g of yeast and sugar was dissolved in 50 ml of warm water for 5 minutes. Next, yeast and sugar mixture were fed into dough mixture, followed by mixing at the lowest speed until homogeneous. Hydrocolloids were added into each dough at varied concentration of 2 %, 3 %, or 5 %-wt. dough, then further mixed until homogeneous. Each dough mixture was put in a baking tray, followed by proofing at room temperature for 1 hour. The increase height of the dough in the baking tray was measured. Afterwards, dough was baked in a preheated oven at 190°C for 30 minutes and then cooled for 2 hours at room temperature.

Bread characterization

Bread specific volume was measured using displacement of millet seeds method according to AACC methods (AACC, 1983). Briefly, a container of a certain volume was filled with millet seeds (which have been washed and dried). Total mass of millet seeds was measured, and bulk density of millet seeds was determined. Bread volume measurement was conducted by loading baked and cooled bread into container, then the millet seeds were loaded to fill the container. Volume of bread was proportional to the difference in total bean mass that can fill the container, before and after the addition of bread. The specific volume was calculated by using the formula in (1).

$$\text{Specific Volume}\ \left({cm^3}/{g}\right) = \frac{V_{\,bread}\,(cm^3)}{m_{\,bread}\,(g)} \qquad (1)$$

Bake loss (%) was calculated by measuring initial dough mass and bread mass after cooling. Bake loss during baking (%) was calculated by using the formula in equation (2).

$$\text{Bake loss}\ (\%) = \frac{m_{dough} - m_{bread}}{m_{dough}} \times 100\% \qquad (2)$$

Texture Profile Analysis bread was measured using TA-XT Texture Analyzer Stable Micro System. First, Bread was cut into four slices from the center using a bread knife to form a uniform size with a thickness of each slice of 12.5 mm. Two slices of bread were stacked to produce a total thickness of 25 mm for each measurement. The probe used in the TPA measurement was the aluminum cylindrical probe with a diameter of 36 mm. Before measurement, the height of the probe was calibrated using 30 mm height setting. The parameters measured were hardness/ firmness (peak force on the first compression) by using the one bite test method. Texture analysis were conducted in duplicate.

Microstructure of baked bread was measured using a low-Vacuum Scanning Electron Microscope (SEM). Each sample was cut into dice sizes, dried, and milled into fine powders. Structural analysis of the samples was performed using Scanning Electron Microscope (Hitachi SU3500) and sputter ions (Hitachi MC1000) at 20 kV and magnification of 60x to 1000x. First, the sample was placed onto the SEM sampler using a special adhesive. Next, the sample was coated with a gold compound using sputter ions. The coating process was carried out with a strong current of 10 mA for 30 seconds to produce a layer of gold coating with 2.5 nm thick.

RESULTS AND DISCUSSIONS

Effect of hydrocolloids to flour ratio and types of hydrocolloids on baking characteristics of FERCAF-Rice Flour GF bread

Specific volume of bread is defined as the volume of bread per unit of dough mass. In this experiment, bread-specific volume was measured before and after baking to investigate GF bread volume development during baking. In general, specific volume of bread increases after baking due to the dough expansion. This dough expansion occurs as gas (e.g. CO_2 produced during fermentation) expanded and water evaporated during baking. Therefore, dough expansion was influenced by the extent of bread fermentation (by yeast) during proofing to produce CO_2, the amount of water evaporated (influenced by baking duration and temperature), and how strong the dough can hold the expansion during baking, which is well corresponded to the dough viscoelasticity.

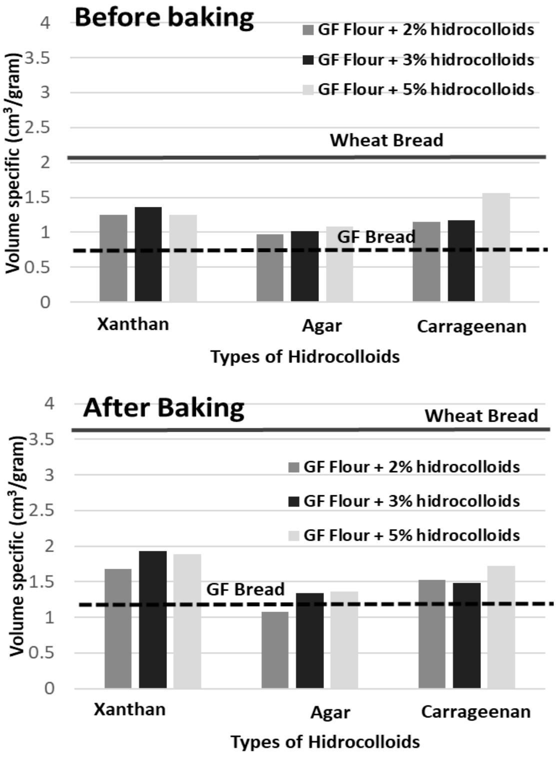

Figure 1. Effect of various hydrocolloids on specific volume of GF bread from FERCAF-Rice flour mix (2:1)

Cuenca et al. (2017) investigated the extent of bread dough fermentation by yeast on several bread dough fermentation of different flours, e.g. wheat and commercial gluten free mixture, by following heat flow using isothermal microcalorimetry. Further, Cuenca (2017) concluded that the amount of CO_2 produced was well correspondent to the extent of fermentation and that wheat dough and commercial gluten free bread mix flour showed similar level of fermentation extent.

Wheat bread had the highest specific volume compare to the GF breads (Figure 1). This may be correlated not only to the higher fermentation rate of wheat dough, but also due to the viscoelastic network of gluten that hold the bread structure. In contrast, GF bread, which contains no gluten or other structural components, had the smallest specific volume. Addition of hydrocolloids as additives to the GF bread were expected to improve water binding properties of flour and provide sufficient viscoelastic properties of dough in order to increase the specific volume of bread.

Data experiments showed that hydrocolloids improved specific volume of FERCAF-Rice Flour GF bread up to 80%, but still lower than wheat bread specific volume. Xanthan gum resulted in the highest increase of GF dough specific volume (1.9 cm^3/g). Specific volume of control wheat bread showed similar value to the result from Crockett (2011). However, FERCAF-Rice flour (2:1) GF bread had lower specific volume than cassava-rice (1:2) flour GF bread (Crockett, 2011). Increase of FERCAF composition in GF flour mixture reduced specific volume.

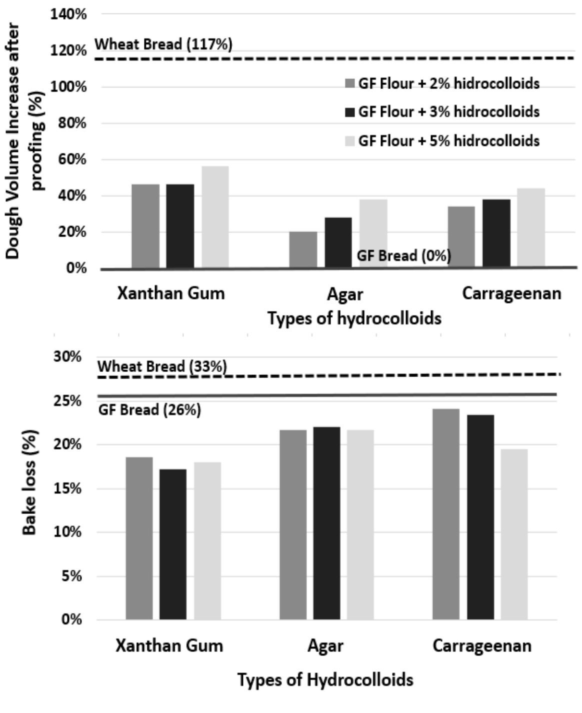

Figure 2. Dough volume increase after proofing and bake loss of GF bread from FERCAF and rice flour mix (2:1) by the addition of various hydrocolloids.

Specific volume of GF bread increased by the increased of hydrocolloid to flour ratio from 2 % to 3 %, which well correspond to the result from Crockett (2011). As comparison, Berk (2017) investigated the effects of partial replacement of rice flour by 10-30% carob bean flour on specific volume of rice – carob bean flour GF cakes, which resulted on cakes specific volume of 1.58 to 1.91 cm^3/g with 20 % carob bean flour gave the best result. The increased of hydrocolloid to flour ratio from 3 % to 5 % showed no significant effect on FERCAF-Rice Flour GF bread specific volume. In general, hydrocolloids have strong water binding ability, which may be responsible to improve viscoelasticity and stronger dough network of FERCAF-Rice Flour GF bread. Furthermore, water that were bound in the dough resulted on a moist and softer texture of FERCAF-Rice flour GF bread. Hydrocolloids also functioned as emulsifiers to assist formation and incorporation of air bubbles during kneading (Seyhun, 2003).

Increase of dough volume may be caused by the production of gas and other volatile matter during proofing (fermentation), while bake loss represents the amount of mass loss from bread dough, such as gas, volatile matter, and water, during baking process. Like specific volume, bake loss may be correlated to dough ability to bind water (water binding capacity) and viscoelastic properties. Dough with a high bake loss value may have a low water binding capacity. Dough volume increased after proofing and bake loss of from FERCAF-Rice Flour GF bread (2:1) by the addition of various hydrocolloids is shown in Figure 2. Bake loss of GF bread was lower than wheat bread, but still higher than GF bread with hydrocolloids. Lower bake loss of GF bread may be corresponded to the higher water binding capacity compared to wheat dough. Addition of hydrocolloids to the GF dough seemed to increase water binding capacity of the dough, thus lowering bake loss. Wheat bread had the highest bake loss, while GF bread with 3 % xanthan gum had the lowest bake loss. Bake loss in GF breads seemed to be inversely correlated to the specific volume. While having the lowest bake loss, GF bread with 3 % xanthan gum had the highest specific volume (Figure 1). Sabanis & Tzia (2011) indicated that addition of hydrocolloids GF bread formulation provided polymeric substances to create viscoelastic properties of gluten and increase the ability of dough to retain gas, furthermore increased specific volume of bread. Liu et al. (2018) also estimated that hydrocolloids may formed higher molecular weight complexes with protein, which reduced protein solubility. Morreale et al. (2018) investigated how the viscosity of hydrocolloids developed structure of GF bread. Experimental data showed that hydration level had significant effect in creating viscoelastic behaviour of GF dough and determining rheological properties of GF bread.

In GF bread, the addition of hydrocolloids decreased bake loss, but increased bread specific volume. Specifically, GF-Xanthan Gum 3 % had the lowest bake loss and had the highest specific volume after baking compare to GF bread control and other GF bread variations. In opposite, although wheat bread had the highest bake loss, it also had the highest specific volume than any other GF bread. This may indicate that wheat bread had different mechanism of volume development than GF-hydrocolloids bread. Bake loss may be well corresponded to water binding capacity of the dough. Therefore, the result in Figure 1 and Figure 2 shows that the increase of water binding capacity of GF dough by hydrocolloids leads on the increased of GF bread specific volume.

Effect of hydrocolloids to flour ratio and types of hydrocolloids on bread texture

Bread texture determines the overall eating experience and, therefore, the quality of the bread. Texture of GF bread is mainly determined by the types of flour, composition of composite flour, and additives (particularly hydrocolloids in this study). In this study, texture of the baked bread, specifically hardness, was tested with *Texture Analyzer*. Hardness is defined as the maximum force during the first cycle of compression, imitating the first bite of the molar digestion. Less incorporation of air into bread dough may lead to increase hardness of bread (Crockett, 2011). Hardness of bread was measured for fresh bread and one-day stored bread. Hardness of various GF bread with hydrocolloid additives is shown in Figure 3. Hardness of control GF bread was much higher than wheat bread. Addition of hydrocolloids reduced hardness of GF bread but still higher than wheat bread.

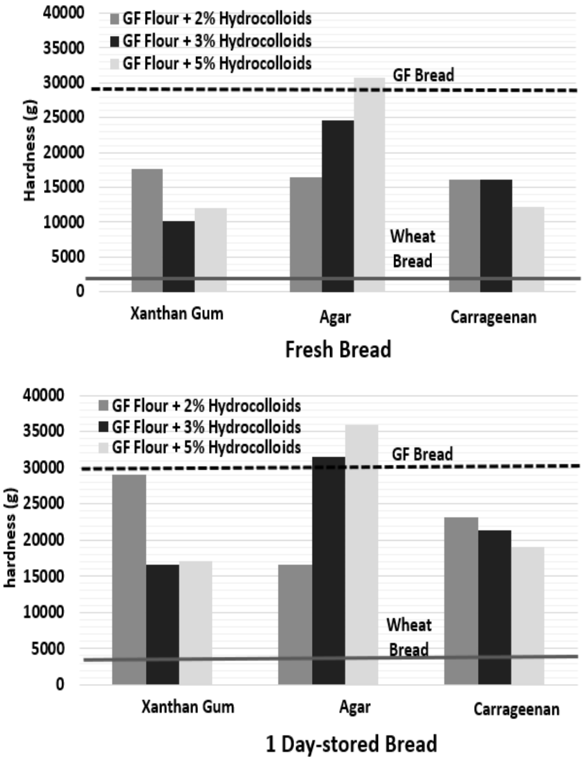

Figure 3. Hardness of GF bread from FERCAF and Rice flour mix (2:1) by the addition of various hydrocolloids

Addition of 3 % xanthan gum resulted on the lowest hardness of the fresh GF bread. In contrast, agar at higher concentration (3 % and 5 %) were not effective to reduce hardness of GF bread. Bread texture test result showed that FERCAF-Rice Flour GF bread, either with or without the addition of hydrocolloids, had higher hardness than wheat bread. Addition of 3 % and 5 % of xanthan gum or 5% carrageenan into FERCAF-Rice Flour resulted on the lowest hardness of GF breads dough and successfully reduced hardness of GF bread from 30,000 to about 15,000-gram force (50% reduction). This result was well correspond to the result from Lazaridou et al. (2007), which showed that increasing level of hydrocolloids from 1% to 2% decreased the loaf volume due to the more rigid and cohesive nature of the dough. Still, hardness of GF bread was lower than wheat bread. After one day stored, GF bread hardness increased due to staling and retrogradation of starch, except for GF-Agar 3 %, which showed more resistance to staling. However, hardness of GF-xanthan gum 3 % and 5 % bread after one-day storage showed similar value to GF-Agar 3% bread. Both control wheat bread and GF bread showed stability against staling and retrogradation after one-day storage.

In general, hardness of GF-hydrocolloid breads was lower than control GF bread, but still higher than wheat bread. Therefore, hydrocolloids successfully improved the texture GF bread, but still could not effectively stabilize GF bread from staling, which lead to the increased of hardness. This phenomenon was different than the result from Guarda (2004), which stated that hydrocolloids can improve wheat bread texture and prevent staling to some extent. This opposite effect might occur due to the different interaction between wheat flour and GF (FERCAF-rice) flour with various hydrocolloids. Berk (2017) observed that hardness of rice – carob bean flour GF cakes were well correlated with specific volume results and cakes with high specific volume had the softest texture. This correlation was similar to FERCAF-rice flour-hydrocolloids mix GF bread.

Effect of hydrocolloids to flour ratio and types of hydrocolloids on bread surface appearance and microstructures

Microstructure of GF bread (control), wheat bread (control), and selected GF-hydrocolloid breads at 60x magnification and 500x are showed in Figure 4. In general, porous structure of bread is well corresponded to volume development and gas retention capacities due to gas expansion during baking. While wheat bread had clear pore structures, control FERCAF-Rice Flour GF bread showed cracks without any clear pores. This suggested that GF bread expansion mechanism was different from wheat bread. As a result, GF bread had lower volume development and had higher hardness than wheat bread. Based on SEM result, GF-xanthan gum bread had high porosity and showed the most bread-like structure. GF-xanthan gum bread and GF-carrageenan bread had smooth surface without visible distinct starch granules present in bread microstructure.

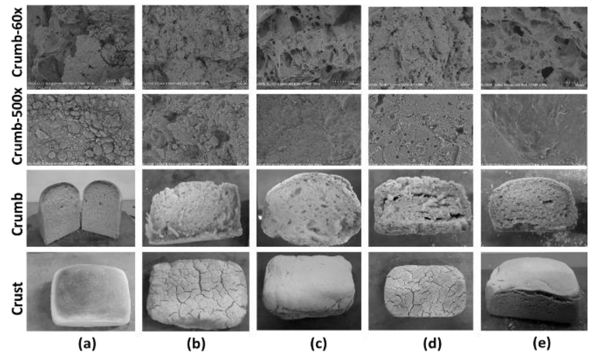

Figure 4: Low-vacuum Scanning Electron Microscopy (SEM) and surface appearance of bread from (a) wheat flour; (b) GF flour (FERCAF- Rice flour = 2:1); (c) GF-Xanthan Gum 3%; (d) GF-Agar 2%; (e) GF-Carrageenan 5%

CONCLUSIONS

Based on the results, hydrocolloids successfully improved GF bread characteristics. Specifically, hydrocolloids addition increased volume specific, increased porosity, and reduced hardness of GF bread. Hydrocolloids successfully improved the texture GF bread, but still could not effectively stabilize GF bread from staling, which lead to the increased of hardness. Among tested hydrocolloids, xanthan gum resulted the best improvement of FERCAF-Rice Flour GF bread. Specifically, GF-Xanthan gum bread has a more bread-like structure. For further application, xanthan gum can be added to GF flour mixture to produce GF bread-premix. In general, addition of hydrocolloids can improve added value and application of GF flour, particularly fermented cassava flour (FERCAF), e.g. for food ingredients or gluten-free food.

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