Optimization of Formulation of Gluten-Free Rice Bread using Response Surface Methodology

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Abstract: ***The effects of rice flour, cornstarch, potato starch and soy bean flour on the quality of gluten-free rice bread were investigated using Response Surface Methodology (RSM). Box-Behnken design consisting of four variables, rice flour, cornstarch, potato starch and soy bean flour in a three-level pattern with 15 runs, was prepared. The design was applied to develop models for the specific volume and sensory score of the gluten-free rice bread. The optimized formulation contained 64.9g rice, 20.3g cornstarch, 10.2g potato starch and 4.9g soybean flour, providing the final product with more uniformed, texture and high sensory score. Texture Profile Analysis(TPA) of the produce with the optimized formulation exhibited low firmness during storage, indicating that the combination of four different cereal floursretarded the retrogradation of gluten-free rice bread.***

Keywords: cornstarch, gluten-free bread, optimization, potato starch, rice flour, response surface methodology

1. Introduction

Bread is commonly made from awheat-flour dough that is cultured with yeast, allowed to rise, and finally baked in an oven. The fermentation by yeast indoughgenerated carbon dioxide which is entrapped in the air pockets commonly found in bread. Wheat flourcontains two water-insoluble, glutenin and gliadinthat form “gluten” as a resulting network. Owing to its high levels of gluten (which give the stable bubble structure of dough), wheat is the most common grain used for the preparation of bread. However, gluten-free breads have been required for people affected by gluten-related disorders such as coeliac disease and non-coeliac gluten sensitivity and the demands have been gradually growing.

Starch is widely used as an ingredient and significantly contributes to texture, appearance, and overall acceptability of cereal based foods (Horstmann *et al.,* 2016). Starch plays an important role in gluten-free bread formulation, due to the ability of starch to form a matrix in which gas bubbles are entrapped, increasing the gas holding capacity of the batters. Abdel-Aal(2009) suggested three mechanisms through which addition of starch influences gluten-free formulations: enhancement of crumb softness, maintenance of the batter consistency during mixing and influencing starch gelatinization during the baking process. Different starches from naturally gluten-free sources such as corn, cassava, potato and rice have been utilized in gluten-free bread formulations. Of these, rice starch as been used as basic ingredient in gluten-free bread, due to its lack of gluten, and easily digested carbohydrate.Riceflour is one of the major ingredients in many gluten-free baking mixes in countries. It is attractive, due to its unique properties, such as hypoallergenicity, colorlessness, and bland taste (Shin *et al.*, 2010). However, it is limited in making bread, since rice does not contain a sufficient amount of gliadin to form gluten. For such a reason, rice proteins cannot contribute the unique properties of wheat dough such as an appropriate water binding capacity, cohesiveness, viscosity, and elasticity (Mezaize *et al.*, 2009). Nevertheless, better technological results were obtained when these raw materials were used together with different starches and cereal flours like cornstarch, rice, soy and buckwheat flour (Gallagher *et al.*, 2004). Therefore, the main strategy for the development of gluten-free bread is to substitute ingredients that are able to mimic the functional properties of gluten.With respect to the ingredients in the bread making process,rice is the most commonly used ingredient, followed bycorn as these are the two most productive cereals around the world (Bourekoua *et al.*, 2016). Besides, starch from tubers such as potato ismost commonly used in the manufacture of gluten-free bread (Masure *et al.*, 2016).Soybean flour has been used to fortify wheat, gluten-free bread quality and to improve the mechanical behaviour of dough (Simurina *et al.*, 2017).

The Box-Behnken experimental design, developed by Box and Behnken in 1987, is a useful method for developing second-order response surface models. The Box-Behnken design is based on the construction of balanced incompleted block designs and requires at least three levels for each factor. In Box-Behnken experimental design, the level of one of the factors is fixed at the center level while combinations of all levels of the other factors are applied (Kocabaş, 2001).

When formulating a mixture of ingredients, the goal is to find the optimal mix that gives a good technological performance. Response surface methodology (RSM) has been successfully applied to investigated the effects of xanthan gum, extruded rice flour and cornstarch (Madhuresh *et al.*, 2013). The mixture of different starches may lead to production of different gluten-free bread through different interaction between ingredient starches. Thus, in this study the formula of rice-based bread with corn, potato starch and soy bean flour was investigated using RSM. Moreover, the formula for rice – based bread has not been optimized using RMS. Thus, the aim of the present study was to use rice flour, cornstarch, potato starch and soy bean in rice-based gluten-free breadmaking and to statistically establish optimal amounts of each ingredient.

2. Materials and Methods

2.1 Materials

Cai Lay Cam rice was grown at Cai Lay district of Tien Giang province. Rice was milled and sifted with screens of 0.25 mm to obtain fine fractions. The rice flour composition was (expressed as dried basis) 9.8% protein, 12.8% moisture, 66.4 mg/100g anthocyanin. Cornstarch (Roquette, France), potato starch (Thai Lan), soybean (Huong Que, Viet Nam) and sugar (sucrose), salt, instant yeast containing natural dough yeast (*Saccharomyces cerevisiae*-Mauri), fresh milk (no sugar – Vinamilk, Viet Nam), whole egg and oil were purchased from local markets of My Tho. Additives were utilizedhydroxypropyl methyl cellulose (ShinEtsu, Japan), and maltodextrin (HBK, Germany).

Gluten-free batter included instant dry yeast 3g, white sugar 10g, salt 1.25g, oil 6g, egg 12g, fresh milk 25g, hydroxypropyl methyl cellulose (HPMC) 1.25g, maltodextrin 10gand tap water 100g. The amount ofrice flour, cornstarch,potato starch,and soy bean were in the range of60 to 70g; 15 to 25g,5 to 15gand 0 to 10g, respectively.

2.2 Methods

Response surface methodology was applied for evaluation of the effects of flour and starch content parameters and their optimization for various responses. Box– Behnken experimental design with three numeric factors on three levels was used. Design consisted of fifteen experimental runs with three replicates at the central point. Various parameters could affect specific volume, sensory bread score. In this work, independent variables used in experimental design were rice flour (60 to 70g), cornstarch (15 to 25g), potato starch (5 to 15g) and soybean (0 to 10g). Experiment design of bread formulations was shown in Table 1.The experiment included specified 2 response variables and 4 experimental factors.

Table 1: Experimental domain with coded values of independent variables used in Box–Behnken design (BBD)

Variable	Coded levels		
	-1	0	1
Rice flour content (g) (X_1)	60	65	70
Cornstarch content (g) (X_2)	15	20	25
Potato starch content (g) (X_3)	5	10	15
Soybean (g) (X_4)	0	5	10

This pane displays the regression equation which has been fitted to the data. The equation of the fitted model was fitted as equation of sensory (Y_1) (Eq. 1) and specific volume(Y_2) (Eq. 2):

$$\begin{aligned} Y_1 = & -89.87 + 2.68X_1 + 0.64X_2 + 0.57X_4 + 0.5X_3 \\ & - 0.02X_1^2 - 0.002X_1X_2 + 0.003X_1X_4 \\ & - 0.001X_1X_3 - 0.009X_2^2 - 0.015X_2X_4 \\ & - 0.007X_2X_3 - 0.03X_4^2 - 0.013X_3X_4 \\ & - 0.01X_3^2 \end{aligned}$$

$$\begin{aligned} Y_2 = & -62.42 + 1.77X_1 + 0.44X_2 + 0.42X_4 + 0.28X_3 \\ & - 0.01X_1^2 - 0.002X_1X_2 + 0.003X_1X_4 \\ & - 0.0X_1X_3 - 0.005X_2^2 - 0.011X_2X_4 \\ & - 0.003X_2X_3 - 0.023X_4^2 - 0.013X_3X_4 \\ & - 0.007X_3^2 \end{aligned}$$

2.3 Laboratory bread baking

For laboratory bread making, half of the total rice flour and boiling water (half of the total water) were mixed until the flour is converted into a stiff paste or dough (about five minutes). Theresultant dough was left to rest until the temperature decreased to 30^{o}C. The yeast previously solved in warm water (35^{o}C) was added to the mixture of the remaining all flours, the other ingredients and water and blended for 10 min in Bear mixer. Then 100 g of the resulting batter was placed in a greased bread pan andfermentedfor 30 minutes at room temperature. Finally, the fermented dough was baked at 175^{o}C for 35 min. After baking, bread loaves were removed from the pans and cooled at room temperature.After 1 hour (h)of cooling, sensory evaluation and determination of specific volume of bread samples were performed, respectively. TPA-test was carried out after 2 h standing at room temperature by CT3 Brookfield. For shelf-life analysis, the bread loaves were packed in polyethylene bags and stored at 4^{o}C for 5 days.The batches were prepared in three replicates.

2.4 Specific volume

Bread loaf volume (cm^3) and weight (g) were determined after 60 min cooling. Loaf volume was measured by small seeds displacement method (Greene and Bovell-Benjamin, 2004). Sesame seeds were poured into container whose volume is known until the bottom was covered. The loaf was placed inside the container which was then filled to the top with more seeds. The extra sesame seeds, which equal the loaf volume, were measured in a graduated cylinder. The specific volume of the loaf was calculated using the following equation (Eq. 3):

Specific volume (cm^3/g) = loaf volume (cm^3)/loaf weight (g) (3)

2.5 Texture profile analysis

Texture Profile Analysis (TPA) was performed with a CT3 Texture Analyzer (Brookfield, USA). Single slice of 25mm or two slices of 12.5mm in thickness are placed under a 38.1mm diameter cylindrical probe (TA4). With the latter, TPA of the crumb was conducted with a constant speed of 2.0 mm/s (pretest speed, test speed, and post-test speed) over a distance of 10.0 mm. The wait time between the first and the second compression cycle was 5 second, and the trigger force was 10g. Triplicate measurements for each sample were made. The peak force of compression was reported as firmness in accordance with the AACC method 74-09

(AACC, 2000).Three of twelve loaves were immediately used for performing the texture and structural analyses on day 0, the remaining four loaves were used for the texture analysis on storage after the 1st day(26 h after baking), the 3rd day (74 h after baking) and the 5rd dayof storage (122 h after baking), respectively.

2.6 Sensory analysis

Breads were sensory evaluated by panel of thirty individuals(aged 18–40) both male and female who were recruited from the students and lecturers of Tien Giang University. The bread crust was removed, and the crumb was cut into cubes before serving to the panelists on coded plates.The panelists who were habitual consumers of bread, were instructed to visually evaluate for the nine-point hedonic scale. Panelists were instructed to visually evaluate for appearance and odor, then take at least three-fourths of bread, and slowly masticate the product before providing overall acceptability of bread, all on a 9-point hedonic scale consist of like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely (Wongklom *et al.*, 2016).

2.7 Chemical Analyses

The water content of the rice flour and breads were determined by the approved AACC method 44-15.02 (AACC, 2000). Protein content of the rice flour was analyzedusing the Kjeldahl method and expressed using the conversion factor N × 6.25 (AOAC, 1980).

2.8 Statistical analysis

The optimum levels of the components in the formulation for gluten-free bread were determined with RSM. The breads were prepared according to the experimental design (Table 1) in order to develop gluten-free bread formulation by using Statgraphics Centurion XVII.The data obtained were statistically treated by analysis of variance (ANOVA) and the means were compared by the Fisher LSD test at a significance level of 0.05. Data were presented as mean of sample sets. Statistical analysis of the results to assess significant differences among samples was performed.

3. Results and Discussion

Rice flour alone is not suitable for bread production. Rice bread has irregular and varying air cells. The source and addition levels of starch influenced the power and stability of fermentation. The principal component analysis showed that hardness revealed great divergences in specific volume and sensory parameters. However, the high specific volume and sensory parameters value have medium hardness. Thus, the optimized recipes for rice flour, cornstarch, potato starch and soybean were developed in order to maximize specific volume and the highest sensory scores of gluten-free breads. In general the breads quality criteria include large volume, soft crumb, and high sensory acceptability for gluten-free bread (Cauvain, 2013).

3.1 Effects of cornstarch, potato starch, rice flour and soybeanon specific volume of gluten-free bread

Bread loaf volume is an important parameter used in the determination and assessment of quality of bread (Matos and Rocell, 2012). The specific volume of the breads produced with various level of rice flour, corn starch potato starch and soybean were shown in Fig. 1. Breads made from various starches sources such as rice flour or cornstarch or potato starch or soybean at high level had the lowest specificvolume. It has been noticed that, by increasing of the amount of starches, they caused an increase dry matter content in dough. Thus, the amount of water used in the gluten-free bread formulation did not increase the bread volume well and the crumb was dense and brittle. In contrast, addition of excess amounts of water led to in irregularly shaped bread with collapsed surfaces. Thus, increasing the proper water content would be expected to enhance starch gelatinization and hydration of the protein, resulting in softer and less gummy bread with improved bread loaf volume.Many authors previously concluded that water positively affects the volume of gluten-free bread if using suitable content (Bourekoua *et al.*, 2016; Różyło*et al.*, 2015; Schoenlechner *et al.*, 2010).

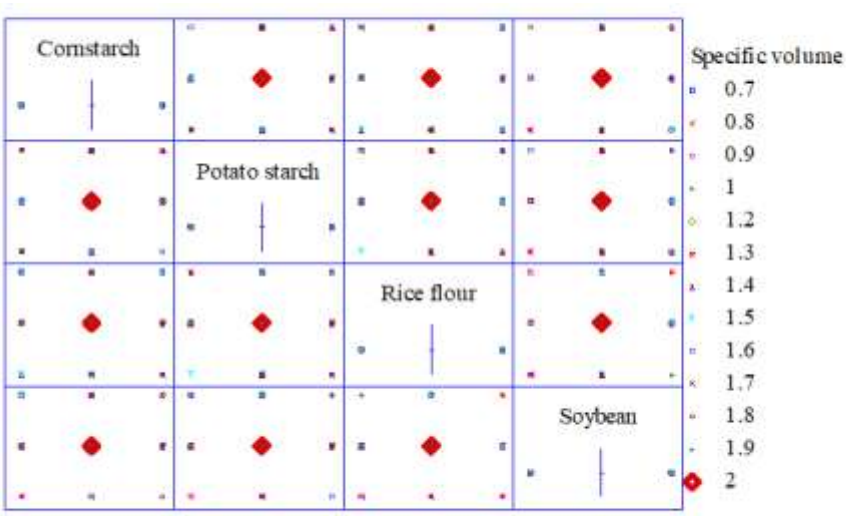

Figure 1: Matrix plot of data variables for effect ofcornstarch, potato starch, rice flour and soybean onspecific volume (cm^3/g) of bread

The amylose content in flour or starch affects the physical properties of gluten-free bread. High amylose in starch tends to have lower swelling power than low amylose starch. The extent of granule swelling of starch reflects the interaction between starch granules and water molecules, which is related to physicochemical properties of starch (Alay and Meireles, 2015). The amylose contents in rice four (16.2%) and potato starch are higherthat in cornstarch. Thus, bread with high amylopectin cornstarch showed high specific volume during fermentation time, but after baking it was easily collapsed. This collapse may be due to a decrease of gas retention capacity and also to the lower consistency of the dough and high plasticity of the structure. The lower consistency causes the bubbles to become unstable, resulting in large holes and the high plasticity and finally in collapse of structure. Addition of proper starches type and contents to bread could be essential for gas retention as well as the expansion of gas bubbles during proofing and baking, and contribute to the structural architecture and mechanical strength of gluten-free bread (Gallagher *et al.,* 2004).

Gluten-free breads formulated without soybean flour showed the lowest quality, having a very dense crumb structure with a corresponding low specific volume.The best results were obtained with 5% soybean flour in the dough formulation. Without soybean flour produced cracks in the crumb, while higher levels yielded a dense crumb structure and a low bread volume.

The combination of medium levels of three factors resulted in the highest specific volume. The obtained results showed that the bread volume was significantly dependent both on the type of raw material and on the amount of flour or starches used in the recipe. The source and addition levels of starch influenced the power and stability of specificvolume. Some starches alleviate some negative actions, in that dispersed particles deform and puncture gas bubbles (Taylor *et al.*, 2006). This is dependent on the interaction with other ingredients in the formulation (Miyazaki *et al.*, 2006).

3.2 Effects of cornstarch, potato starch, rice flour and soybeanon sensory value of gluten-free bread

Sensory analysis with habitual consumers of bread was performed with a one selected bread formulation. The sensory evaluation of the fresh bread was performed from matrix plot of data variables of Figure 2. With respect to the sensory evaluation of each product, quantitative scores information was analyzed by frequencies. The results revealed that all gluten-free formulations were acceptable, since they received scores much higher than 5, ranging from 5.7 to 7.3 (like moderately to like slightly). The relationship between the overall acceptability and independent variables are shown in the equation (1). Breads containing middle levels of the independent variables were rated high due to their good appearance and high specific volume. Matrix plot of data variables of flours shows that up to a certain limit acceptability increased as the independent variable interacted among each other. The combination of medium levels of three factors (cornstarch, potato starch, rice flour and soybean) resulted in the highest scores from 7.1 to 7.3 in terms of overall acceptability.

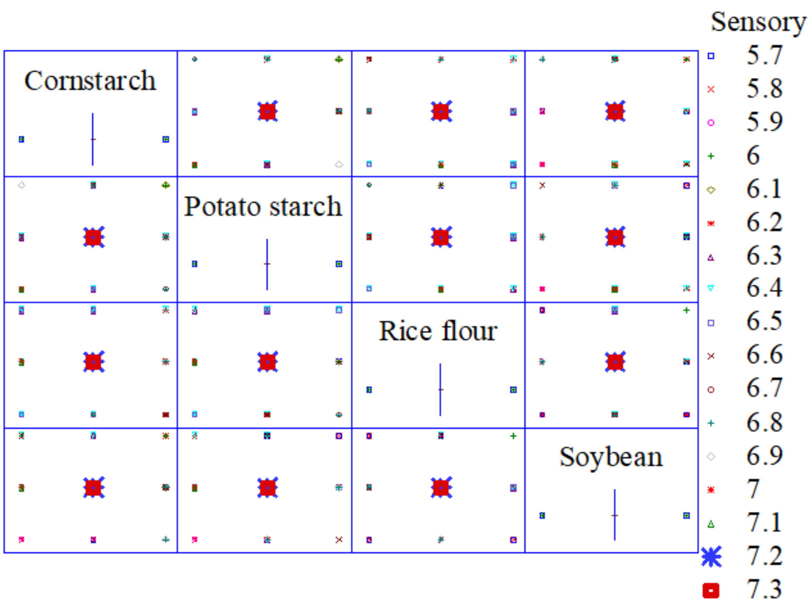

Figure 2: Matrix plot of data variables for effect of cornstarch, potato starch, rice flour and soybeanon sensory value of bread

3.3. Optimization of gluten-free bread formulation

Statistical models have been fit to the response variables. Models with P-values below 0.05, of which there are 2, indicate that the model as fit is statistically significant at the 5.0% significance level. Also of interest is the R-squared statistic, which shows the percentage of variation in the response that has been explained by the fitted model. R-squared values range from 84.38% (specific volume) to 84.57% (sensory value). The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 80.47% (for specific volume) and 80.71 (for sensory value), as shown in Table 2. The optimization of the responses has been determined and are displayed in Table3. At these settings, the response variables generate a desirability index of 97.3%.

Table 2: Analysis of the experimental results

Model	*Sensory*	*Specific volume (cm^3/g)*
Transformation	none	none
Model d.f.	14.00	14.00
P-value	0.00	0.00
Error d.f.	64.00	64.00
Stnd. error	0.21	0.16
R-squared	84.57	84.38
Adj. R-squared	80.71	80.47

Based on the above-described results, it can be asserted that the quality of the gluten-free bread was not dependent on a single main factor and three independent variables were important in defining the characteristics of the bread. Therefore, the next step involved the detectionof the best combination of factors that are able to produce the expected characteristics of the final product. All comments arising from the response surface plots were taken into account in the optimization, considering that the optimal solution arises from a compromise among the different responses (Sabanis *et al.*, 2011).

Table 3: Optimization of the responses

Response	*Optimized*	*Prediction*	*Lower 95.0% limit*	*Upper 95.0% limit*	*Desirability*
Sensory	yes	7.18793	7.04466	7.33119	0.961721
Specific volume (cm^3/g)	yes	1.96922	1.8634	2.07503	0.985225
Optimized desirability					0.973402

Table 4: Factor settings at optimum

Factor	Setting	Optimum response specific volume (cm^3/g)	Optimum response of sensory
Rice flour	64.8515	62.6140	65.9467
Cornstarch	20.2514	17.8806	20.4188
Soybean	4.91564	5.1012	4.8450
Potato starch	10.2421	10.5410	9.6050

The mixture of the potato, cornstarch and rice flour gave good results at intermediate level. The rice flour as major component has a favorite in the mixture due to the favorable characteristics in the specific volume and hardness of bread. The cornstarchis known to be essential for lowering the bread hardness, resulting in more compact bread.

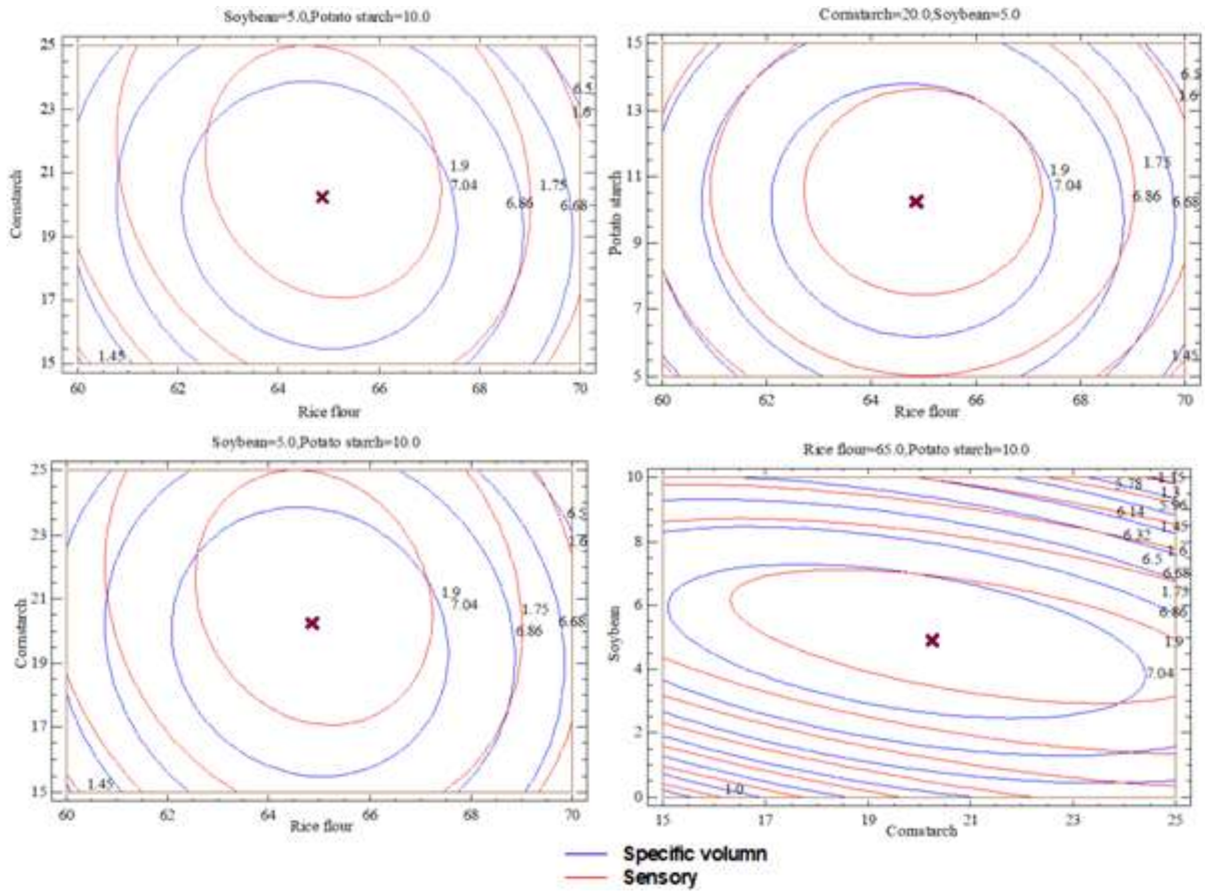

Figure 3: Overlay plot illustrating the numerical optimization

As a result of the optimization step, the best conditions, which were attained for the expected response values were 64.9g rice, 20.3g cornstarch,10.2g potato starch and 4.9g soybean. At these flour concentrations, maximum specific volume was 2 cm^3/g and highest score of sensory evaluation (7.3 - like). The calculated desirability for this formulation was 99,9% for specific volume, 88% for sensory value and 97.3% for the overall optimized desirability (Table 4). This specific volume was higher than that of the gluten-free bread described by Kim *et al.* (2015) and Kadan*et al.* (2001), which yielded 1.86 cm^3/g and 1.9 cm^3/g, respectively. Overall acceptability evaluation depicted that the optimized bread exhibited fine taste, more uniform crumb texture, flavour, color and appearance being rated with 7 scores on a nine point scale. It also was observed that the crumb of the optimized bread had medium size air pores and good uniformity. Panelists commented that this bread “looked more like wheat bread” and that the loaves had “loaf volume and sensory value similar to wheat bread”. The similar results of sensory characteristics of bread were found as reported by Breshears and Crowe (2013).

3.4 Shelf life of gluten-free breads from optimized formulation

The shelf life of bread is mainly influenced by loss of moisture, staling, and microbial deterioration. Of these, staling is the main shelf life-limiting factor. The bread dough prepared under optimized formulation was baked and tested during 5 days storage at 4°Cwas compared with wheat bread. The textural changes in crumb and moisture were presented in Figure 4. In general, the moisture and hardness of bread increased during the storage.

When comparing the moisture of the wheat bread with the optimized bread, gluten-free breads have kept higher moisture levels than wheat breads through 5 days of storage. The moisture of gluten-free bread decreased from 47.0% to 43.8% while that of wheat flour from 44.0% to 41.1%. These results indicated that the water loss of gluten-free rice bread was higher than that of wheat flour during 5 days of storage.The gluten network in wheat bread slows the movement of water and therefore, the absence of gluten in gluten-free bread can result in accelerated moisture migration from crumb to crust (Gallagher *et al.*, 2004). The firmness changes of gluten-free rice bread were exhibited in Fig. 4 (b). The hardness of gluten-free rice bread increased from 1088g to 1741g during 5 days storage, whereas the hardness of wheat bread increased from 968 g to 1707g. Although the hardness the gluten-free bread was slightly higher than that of wheat flour at the beginning of storage,the results demonstrated that the retrogradation of gluten-free rice bread (130.6 g firmness/day)proceeded more slowly than that of wheat flour (147.8 g firmness/day). However, Kadan (2001) found that gluten-free rice bread products exhibit faster rates of staling when compared to related wheat products.The addition of potato starch in the gluten-free rice bread formulation may delay the starch retrogradation. Therefore, bread containing potato starch may appear to be effective factor for reducing hardening and moisture loss of the crumb of gluten-free bread during storage.

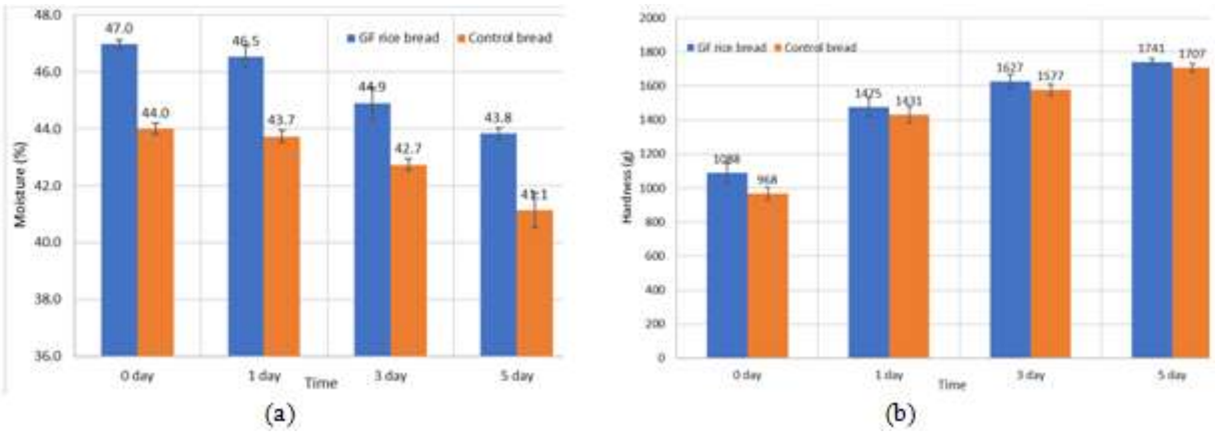

Figure 4: The changes of moisture (a) and hardness (b) in gluten-free rice bread( )and control bread ( ) during storage

4. Conclusion

Development of high quality gluten-free rice bread was successful achieved with four starches of rice, corn, potato starch and soy bean using RSM. The combination of rice flour, cornstarch,potato starch and soy bean employed in this study expressed a great effect on the quality of gluten-free rice bread. The optimized conditions of fourtypes of flour(64.9g rice, 20.3g cornstarch, 10.2g potato starch and 4.9g soybean) were achieved with high quality bread (high overall acceptability scores, high specific volume and low hardness). The RSM with various non gluten starches could be applied in future optimization of gluten-free breads.

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