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Effects of different storage temperatures on rheological properties of bread dough and Organoleptic of bread Incorporated with rubber seeds (*Hevea Brasiliensis*) flour

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Rubber seed is underutilized by-product from rubber seeds tree which contain high protein content. Storage time and temperature of bread dough is known to be negatively affecting the quality of bread. Thus, this study is conducted to determine the organoleptic and rheology properties of the dough incorporated with rubber seed flour at different storage temperature. The formulation were varied from control (C1) 100% bread flour, (C2) 100% rubber seeds flour, bread substituted with 25% 50 and 75% of rubber seed flour (RSF25, RSF50 and RSF 75 respectively). The storage temperature were varied from range of 25°C, 10°C, 4°C and -18°C for 14 days. The straight dough method was used in making the bread. The analyses conducted in this research were colour, sensory evaluation and rheological properties. The color of rubber seed bread for both crust and crumb showed significant different ($p<0.05$) for all bread samples. There were also significant different ($p<0.05$) between bread color stored at 25ºC, 10ºC, 4ºC and -18ºC making from whole bread flour as control and the bread making with the incorporation of rubber seed flour. Rubber seed bread was acceptable by the consumer in term of sensory evaluation. The rheological properties of bread dough store at -18°C showed that bread supplemented with 25% and 50% of rubber seed flour were the most suitable formulation to be used for storage dough. From this research, the usage of the rubber seed flour in bread making can improve the quality of the bread dough.

Keywords: Rubber seed flour, rheological properties, organoleptic properties, dough, bread.

INTRODUCTION

The seeds of rubber tree, *Hevea brasiliensis* has aroused occasional in interest as a source of protein but only oil extracted from the rubber seeds are widely used worldwide. The seed has a high content of semidrying oil which may be used in the paint industry (Anon et al. 1950; Lim, 2012), leaving the press cake as potential source of high protein food for cattle or sheep. According to United States Department of Agriculture (2009), The Recommended Dietary Allowance (RDA) for proteins in humans is 56 g and 46 g for males and females respectively would thus be achieved by the sole consumption of about 300 g rubber seeds per day. Table 1 shows the proximate analysis of the Malaysian rubber seeds.

Based on the Table 1, the moisture content of rubber seeds as determined by the previous study was 3.99%. The rubber seeds also showed high in protein content which was 17.41 g/ 100 g. The fat content was about 68.53% which was in good comparison with 37.30- 50.00% as reported by

Ukpebor et al. 2007.

Table 1: Proximate Analysis of Rubber Seeds

Constituent	Wt (%)
Moisture	3.99±0.01
Protein	17.41±0.01
Fat	68.53±0.04
Ash	3.08±0.01
Carbohydrate	6.99±0.01

Sources: Eka et al. 2010

The differences in fat content compared to previous studies may due to the difference of strain of rubber trees and also the soil and climatic condition of rubber plantation. Ash content is a guidance of the level of inorganics in rubber seed and based on Table 1, the ash content is about 3.08%. The carbohydrate content of rubber seed was 6.99%. From the table, the fat contributed the most percentage of content in the rubber seeds.

Bread is regarded as the most popular food product with a worldwide consumption and with endless variations in recipe, processing and shape. According to Dewenttinck et al. (2008), the bread is a fermented bakery product produced from wheat flour, water, yeast and salt involving a series of operations including mixing, kneading, proofing, shaping and baking. Bread has been popular around the world and is one of the oldest artificial foods, having been of importance since the dawn of agriculture. The proportions of types of used flours and other ingredients vary widely as well as the modes of preparations. Due to those differences, the types, shapes, sizes and texture of the breads differ around the world. Each of the ingredients used in the bread making has their own specific roles. Flour provides the primary structure, starch and protein to the final baked bread. The protein content of the flour is the best indicator of the quality of the bread dough and the finished bread. Water transforms flour into viscoelastic dough that retains gas produces during fermentation and providing the medium for all the chemical reaction to occur. Yeast ferments sugar and produces carbon dioxide and ethanol which result in porous leavened bread. Salt is added to enhance flavour and restrict yeast activity. The commercial bread usually is added with the additives to improve the flavour, texture, colour and also shelf life.

Information on the rheological properties of dough will be useful for predicting the potential application of the wheat flour and also the quality of the end product. Since protein has a major role in the quality of bread, supplementation of wheat dough with rubber seed flour certainly affects rheological properties of the fortified wheat flour dough and its subsequent finished products. These effects can be measured by using rheometer to evaluate the bread making potential and performance characteristics of the fortified flour.

MATERIALS AND METHODS

Raw Materials

The raw materials used are rubber seeds (*Hevea brasiliensis*) that were collected from rubber tree plantation which processed into powder from. Bakery ingredients such as wheat flour, instant dry yeast, sugar and were bought from local supermarket in Jertih, Terengganu.

Preparation of Rubber Seed Flour (MILLING PROCESS)

Seed Material and Flour Production

The rubber seed was collected, screened and washed to ensure that they are free from foreign materials. Then the rubber seeds were dried in the oven at 60ºC until the moisture content of 7% achieved which is safe for storage (Ebewele et al. 2010) and then milled, screened through a mesh sieve and packaged in plastic container and stored in at room temperature prior to analysis.

Powder Yield

The yield of rubber seed flour (RSF) is calculated using the formula given:

% yield = (Weight of obtained RSF / Weight of fresh rubber seeds) x 100

Baking Test

Bread making process

The bread were produced according to the method which described by Maaruf et al. (2011). The RSF obtained were blended with wheat flour (WF) at 25:75, 50:50 and 75:25 levels of substitution for bread production. RSF and WF at 100:0 and 0:100 levels were used as control. The bread recipe consisted of 200 g of each blend, sugar (5%), salt (2%), yeast (4%) and water (60%). The dry ingredients were thoroughly mixed. The mixture were kneaded into smooth pliable elastic-like dough, covered and allowed to ferment for about 45 minutes, baked at 180℃ for 20 minutes. The baked products were cooled and packaged in polythene bags for further analysis. The formulations of the bread were being simplified as shown in Table 2.

Table 2: Formulation of the Bread Incorporated with Rubber Seed Flour.

Ingredients	Control 1 (g)	Control 2 (g)	RSF 75 (g)	RSF 50 (g)	RSF 25 (g)
WF	200	-	50	100	150
RSF	-	200	150	100	50
Water	120	120	120	120	120
Sugar	10	10	10	10	10
Salt	4	4	4	4	4
Yeast	8	8	8	8	8

******WF-Wheat flour, RSF- Rubber seeds flour
*RSF 75 (25% WF: 75% RSF), RSF 50 (50% WF: 50% RSF), RSF 25 (75% WF: 25% RSF)

Preparation of Dough

The prepared dough were stored at 4 different storage temperature which are ambient temperature (25°C), cooling temperature (10°C), chilling temperature (4°C) and freezing temperature (- 18°C) for 14 days.

After 14 days the dough been stored, the dough was brought out and thawed in room temperature for 4 hours. After the dough completely thaws, the dough were divided into 3 pieces of 50 g. Then, the dough was proofed in a proofer at 37°C for 45 minutes and baked in an oven at 180°C for 20 minutes. The baked samples were cooled at room temperature for 1 hour prior to analysis.

Colour Measurement

Crust and crumb colour were measured using the Cromameter Minolta (CR-300) Trimulus Color Analyser, Japan. The colour attributes Hunter L, a and b values were recorded where L* defines lightness, a* denotes red/ green value and b* the yellow/blue color. The L* axis has the following boundaries: L=100 (white or total reflection) and L=0 (black or total absorption). Along the a* axis, the colour measurement movement in the – a direction depicts a shift toward green while + a movement depicts a shift toward red. Along b* axis, - b movement represents a shift towards blue while + b shows a shift towards yellow. Four measurements were taken from each sample.

Sensory Evaluation

Sensory evaluation for fresh bread was performed using 50 panelists comprising of graduate students and staffs members of the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin. The samples were randomly assigned to each panelist. The panelists were asked to evaluate each loaf for appearance, crumb texture, crust and crumb, color, taste, odour and overall acceptability. A 7-point hedonic scale were used where 1 = dislike very much to 7 = like very much.

Rheological Measurement

The rheological measurements of selected sample were conducted using a rheometer (Anton Paar MCR 301, GmbH, Germany) and the analysis was done according to Demirkesen et al. (2010). All measurements were done at 30°C, using parallel plate geometry (50 mm diameter and 1 mm gap). The dough sample was placed between the plates and the edges were carefully trimmed with a spatula. The, frequency sweep experiments were carried out at 0.5% strain rate between 0.1 and 100 Hz. Finally, elastic storage (G') and loss (G") modules values were obtained. All the rheological experiments were performed at least twice and their averages were reported in the study.

Statistical Analysis

Statistical analysis was performed using one way ANOVA to compare the mean values of physical, chemical and sensory evaluation. SPSS statistical software version 14 was used to analyse data and significances were determined at $p < 0.05$.

RESULTS AND DISCUSSION

Rubber seed flour is the product obtained by grinding the whole rubber seed. Based on powder yield calculation the yield of rubber seed flour is 23.65%.

Effect of Rubber Seed Flour on the Colour of Bread

Colour of the food is the first parameter of the quality evaluated by the customers which become the crucial factors in the acceptance of the food. For the crust color of the bread for each of the storage temperature, it showed that there is significant different ($p<0.05$) between the formulation of C2, RSF25, RSF50 and RSF75 with the C1. C1 have the highest mean value of L* which are 60.58 ± 0.40, 66.83±1.90, 69.36±0.35

and 67.33±1.25 at storage temperature of 25°C, 10°C, 4°C and -18°C respectively. For the lowest value of L*, it was observed in C2 with the mean value of 39.28 ±0.13, 37.75±1.57, 42.31±0.49 and 40.85±0.89 respectively. The graph in Figure 1(a) showed that with an increasing percentage of the rubber seeds flour, the L* value will decrease. This indicating that the lower L* value of the bread, the darker the colour of the crust. From the result, C2 with lowest amount of L* value is darkest among the bread while C1 have the lightest crust in comparison among the control. For the formulation with different percentage of rubber seeds flour, RSF75 from each storage temperature were the darkest between RSF25 and RSF50 with the lowest L* value of 42.44±0.31, 45.87±0.75, 47.16±0.09 and 49.50±0.43 respectively. The might because of the high protein content in rubber seeds flour which accelerated more Maillard reaction during the baking process. According to Gomez et al. (2003), the crust characteristics is known to be associated with Maillard reaction, thus flour containing more protein can increase Maillard reaction and causing more browner colour of the bread crust.

By comparing each of the bread with the same formulation at the different temperature, there is significant different ($p<0.05$) between C1 at 25ºC with C1 at 10ºC and 4ºC but no significant different ($p<0.05$) between C1 at 25ºC with C1 at -18ºC. The lightest bread among C1 was at 4ºC with the L* value of 69.36±0.35 and the darkest one was C1 at 25ºC with L* value of 60.58 ± 0.40. The same goes with C2 and RSF25 in term of lightness between the storage temperatures. However, for RSF50, there is significant different ($p<0.05$) with RSF50 at 25ºC and -18ºC but no significant difference with RSF50 at 4ºC. These differences exist might due to the temperature and condition of the storage of bread over its lightness. This finding is in line with the research reported by Giannou and Tzia (2007) and Zulkifli et al., (2019), which proved that frozen storage of dough would increase the brightness of bread due to its white spot formation on the bread crust.

For a*, there is significant different ($p<0.05$) between C2, RSF25, RSF50 and RSF75 with C1 at the different storage temperature. For both RSF25 at 25ºC and -18ºC showing the highest mean value of a* of 7.10±0.47 and 11.71±0.42 respectively meanwhile at 4ºC and 10ºC, the highest mean value of a* were observed in RSF50 with the value of 8.50±0.21 and 7.51±0.20 respectively. The lowest a* value was observed in C1 at 25°C, 10°C, 4°C and -18°C with mean value of 1.12±0.24, -0.99±0.10, -0.76±0.18 and 5.88±0.28 respectively. The a* value indicated the redness of the crust where all the bread with the addition of rubber seeds are redder as compared with the C1. This means, RSF25 at 25ºC and -18ºC and RSF50 at 4ºC and 10ºC are redder compared to another bread. This might due to the colour of the rubber seed itself which is reddish browning (Abu Hasan et al., 2019).

For b*, there is significant different ($p<0.05$) between C2, RSF25, RSF50 and RSF75 with the C1 at each storage temperature. The highest mean was observed in C1 with the value of b* were 16.37±0.37, 19.18±0.55, 20.85±0.22 and 24.41±0.24 at 25°C, 10°C, 4°C and -18°C respectively. Based on comparison of storage temperature, it showed that C1 at -18ºC were less yellow amongst C1. RSF75 have the lowest mean of b* which were 6.60±0.61, 11.95±0.99, 11.20±0.54 and 15.89±0.51 at 25°C, 10°C, 4°C and -18°C respectively.

Based on Figure 1(b), there is significant different ($p<0.05$) of crumb color between C2, RSF25, RSF50 and RSF75 with C1 for each of the storage temperature. C1 have the highest L* values compared to another samples which is ranging from 61.01 to 73.54 where the highest mean was from C1 stored at -18ºC. Thus, C1 at storage temperature of -18ºC have the lightest colour of all the same formulation at different temperature. The lowest mean of L* was C2 with mean of 39.28 ±0.13, 37.75±1.57, 42.31±0.49 and 40.85±0.89 at each storage temperature respectively. Thus, addition of the rubber seeds flour in the bread making will produce more darker bread as flour other than wheat gradually increase the darkness with significant difference among all composite flour (Feili et al. 2013). This can be visually observed since rubber seed flour were brownish compared to C1.

For a*, there is significant different ($p<0.05$) between C2, RSF25, RSF50 and RSF75 with C1 for each of the storage temperature. C2 have the highest a* value at 25 °C which was 5.29±0.33 and 4.89±0.68 meanwhile at 10°C and 4, the highest mean value was from RSF75 with value of 5.75±0.14 and 5.54±0.28, while, RSF25 at -18°C with the value of 5.39±0.30. The lowest a* value were observed in C1 for all storage temperature which ranging from -1.19 to -0.20. This indicated, the crumb colour of the bread with rubber seeds flour are redder compared to the control, C1.

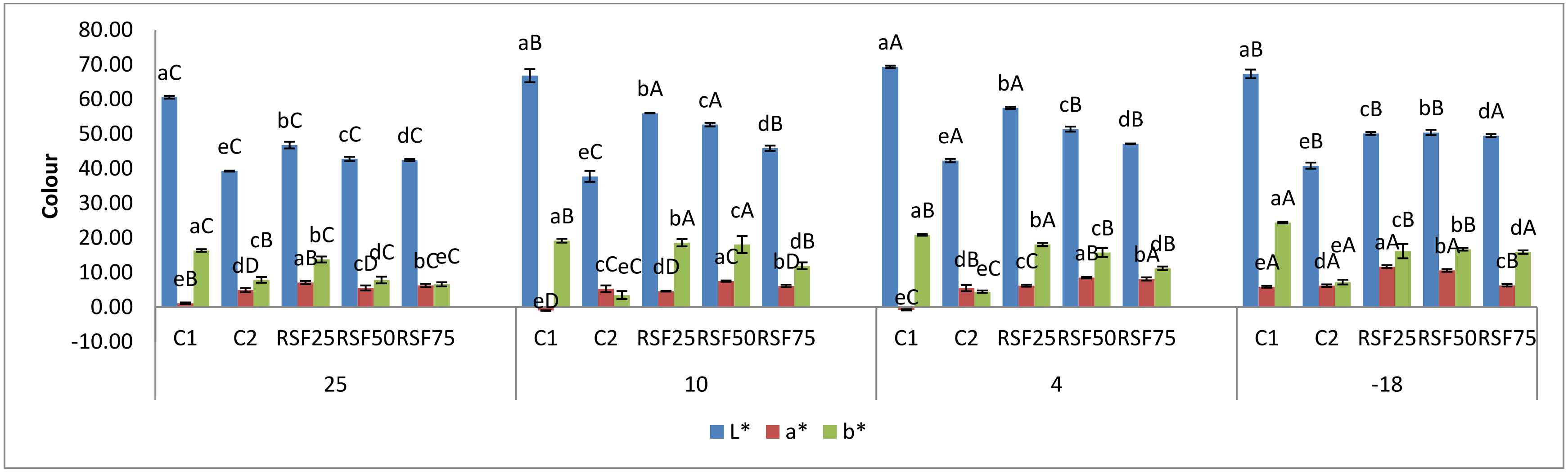

Figure 1 (a): Effect of Rubber Seed Flour at Different Storage Temperature on Colour of Bread Crust

A-C = Significant different (p<0.05) between each of storage temperature

a-e = Significant different (p<0.05) among formulation

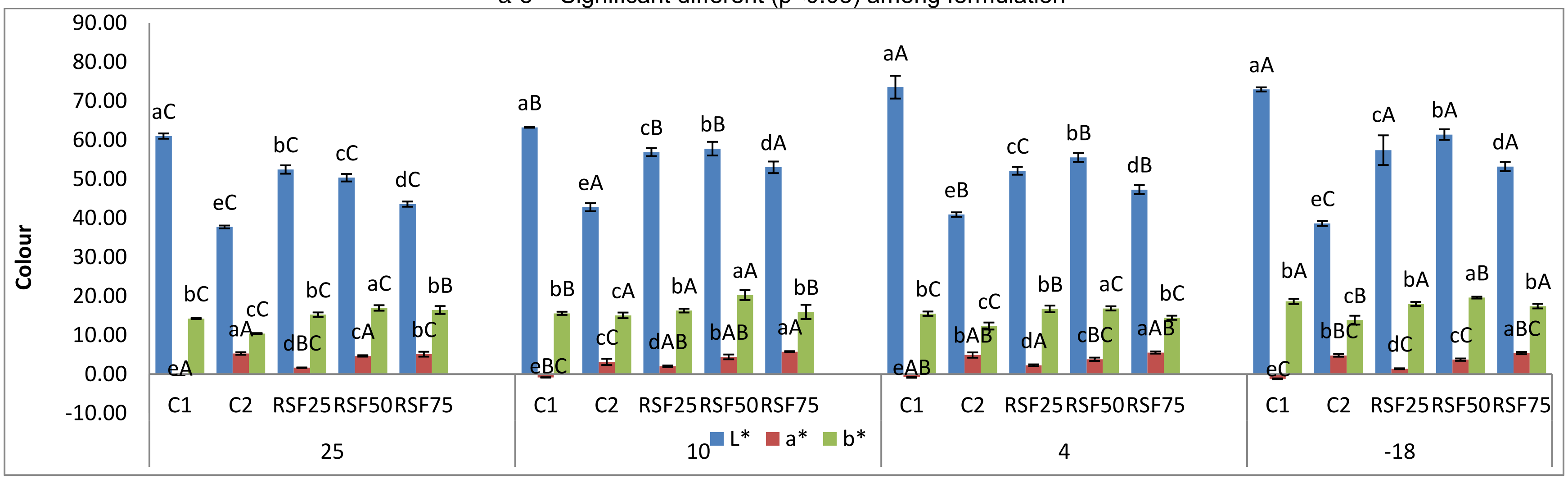

Figure 1 (b): Effect of Rubber Seed Flour at Different Storage Temperature on Colour of Bread Crumbs

A-C = Significant different (p<0.05) between each of storage temperature

a-e = Significant different (p<0.05) among formulation

For b*, there is significant different ($p<0.05$) between C2, RSF25, RSF50 and RSF75 with C1 at each of the storage temperature. RSF50 have the highest mean at 25ºC, 10ºC, 4ºC and -18ºC with the value of 16.96±0.70, 20.26±1.27, 16.85±0.54 and 19.61±0.24 respectively. C2 have the lowest b* at each of the storage temperature with value ranging from 10.37±0.14 to 15.06±0.72. This means that RSF50 is more yellow and C2 is less yellow compared to others.

Sensory Evaluation of bread

Sensory evaluation was conducted in order to evaluate consumer acceptance of the products. According to Figure 2, the overall acceptability of consumers was for C1 with the mean value of 3.52±1.52 compared to other bread which formulated with rubber seed flour. In term of aroma, C1 was preferred by the consumer with the highest mean of 4.06±1.57 while both RSF25 and RSF75 have the lowest mean of 3.52±1.37 and 3.52±1.23 respectively. The crumb colour of RSF75 was preferable compared to C1, C2, RSF25 and RSF50. This might due to brown colour which makes the bread more attractive. According to Matos and Rossel (2013), colour of the bread is an important factor in sensory evaluation depending on their perception of bread.

According to Figure 3, the overall acceptability of the bread was in the range of 3.50±1.34 and 3.82±1.48 where the highest mean was recorded by C1. In term of crust colour, the bread formulated with composite flour was accepted more compared to the control, C1 and C2. However, the crumb colour of C1 and RSF50 were most preferable compared to another by the consumers. The C1 scored the highest mean of 4.08±1.34 meanwhile RSF75 scored the lowest mean of 3.40±1.20 in term of aroma. The texture of bread made up of 75% of rubber seed flour have the higher score of acceptability compared to another bread.

According to Figure 4, the overall acceptability was generally in the same range for each of bread at 4°C however, RSF50 scored the highest mean of 3.72±1.28. In term of crumb colour, crust colour and aroma, C1 scored the highest mean of 4.16±1.28, 4.36±1.35 and 4.18±1.42 respectively compared to other bread. The texture of RSF50 was more likeable by the consumers and this may due to the balance ratio of wheat flour and rubber seed flour in the formulation with the ratio of 50:50.

According to Figure 5, C1 have the highest mean of 4.54±1.42 for the storage temperature of -18°C followed by RSF25 with the score of 4.42±1.50. The crust colour and aroma of RSF25 was more acceptable compared to C1, C2, RSF50 and RSF75. However, for the crumb colour, C1 was more preferable compared to other bread. In term of texture, C2 was least likeable by the consumers which reflected by the mean of 2.98±1.15 and this may due to the fragile and compact structure of the bread when applied the force by the fingers.

According to the sensory analysis which have been conducted, the most acceptable bread was C1 at storage temperature of -18°C and then followed by RSF25 which also at the same storage temperature. This showed that the addition of 25% of rubber seed flour at -18°C gave comparable overall acceptability by the consumers. Thus, the bread with the addition of rubber seed flour are generally accepted by the consumers. The acceptability of bread with addition of rubber seed flour may be improved by using other ingredients which could enhance the flavour, aroma and texture of the bread.

Rheological properties of bread dough

Based on sensory evaluation, the bread dough store at -18ºC was the most acceptable by the consumers. Five samples were subjected to the rheological measurement for further investigation. According to Ferry (1980), dynamic test methods have been developed to determine the rheology of polymers, but they can also be used in determining the viscoelastic properties of food including bread dough (Rutuja, et al. 2012). The rheological response of material is obtained by placing it a pressure, which the associated with strain or strain rate. At the same time, dynamic oscillatory equipment measures the degree of reliability (G', storage modulus) and viscosity (G", loss modulus) (Rutuja et al. 2012). When performed on a linear region, dynamic tests allow for a variety of rheological measurements on a single sample, such as temperature, strain or frequency to be changed. According to the law of power, when G′ overcomes G″ showed dough exhibiting elastic properties, properties that resemble solid (Amemiya and Vibration 1992).

Figure 6 shows the linear viscoelastic modulus of dough samples containing different percentage of rubber seed flour. These figures also include viscoelastic moduli data obtained for wheat dough sample (C1). C2 and RSF75 Storage modulus (G'), loss modulus (G"), storage modulus (after ferment, G'), loss modulus after ferment (G")

Samples showed solid like structure with elastic modulus (G') higher than the viscous modulus (G"). This shows that the texture of C2 and RSF 75 harder compared to C1, RSF25 and RSF50.

According to Leroy et al. (2010); Demirkesen et al. (2010) and Sandeep and Narpinder (2013), if G' is greater than G", the material appears to be dominant as a gel or soft solid and if G' is smaller than G", the study material exhibits fluid-like properties (Rutuja et al. 2012). From figure 6, the rheological readings begin at a frequency of 100 Hz and end at frequency of 0.1 Hz.

Both G' and G" for all samples show the properties of frequency dependence. Where, such moduli data increases with the increasing of the frequency. According to Sandeep and Narpinder (2013), this phenomenon shows that the overall mobility of the chain in the gluten network is relatively high.

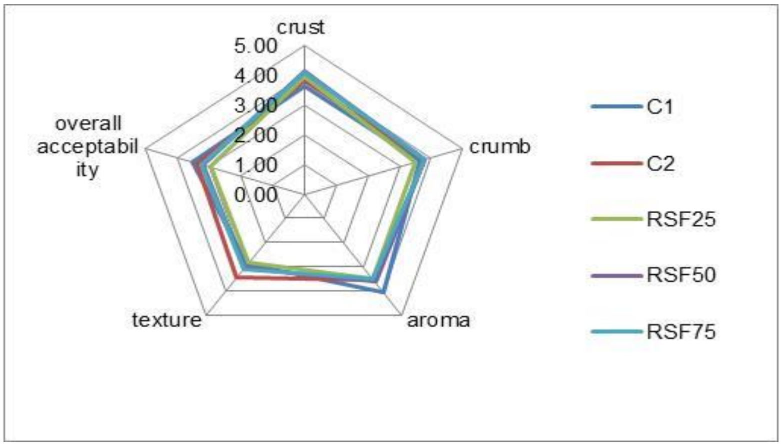

Figure 2: Sensory Evaluation of Bread at Storage Temperature of 25°C

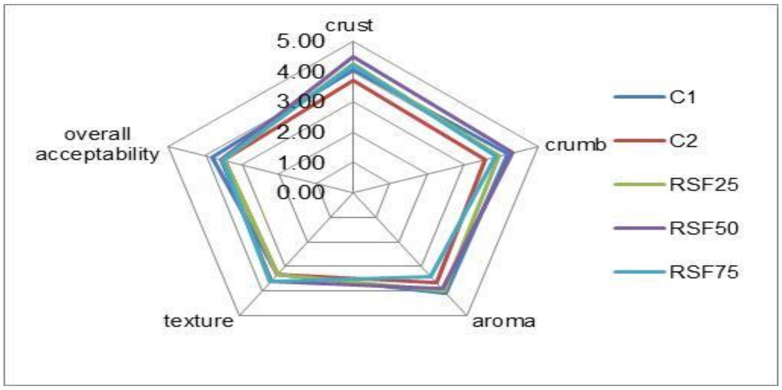

Figure 3: Sensory Evaluation of Bread at Storage Temperature of 10°C

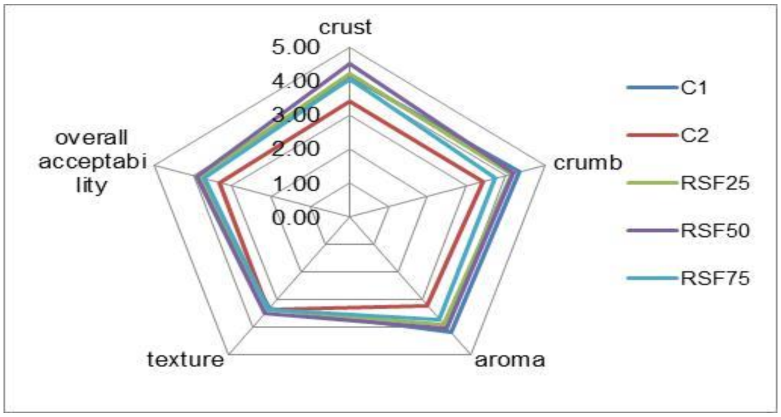

Figure 4: Sensory Evaluation of Bread at Storage Temperature of 4°C

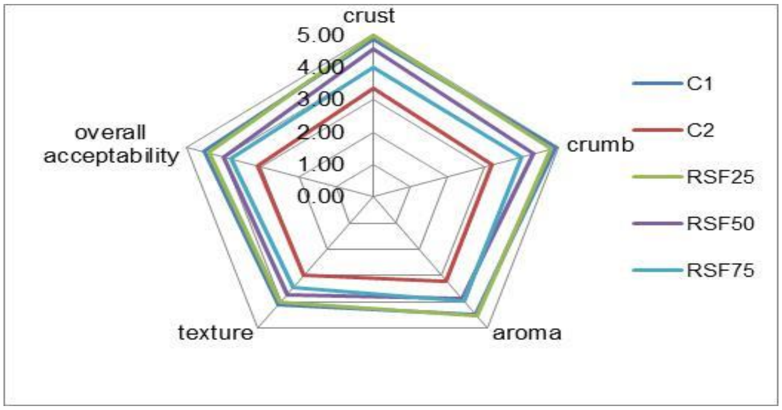

Figure 5: Sensory Evaluation of Bread at Storage Temperature of -18°C

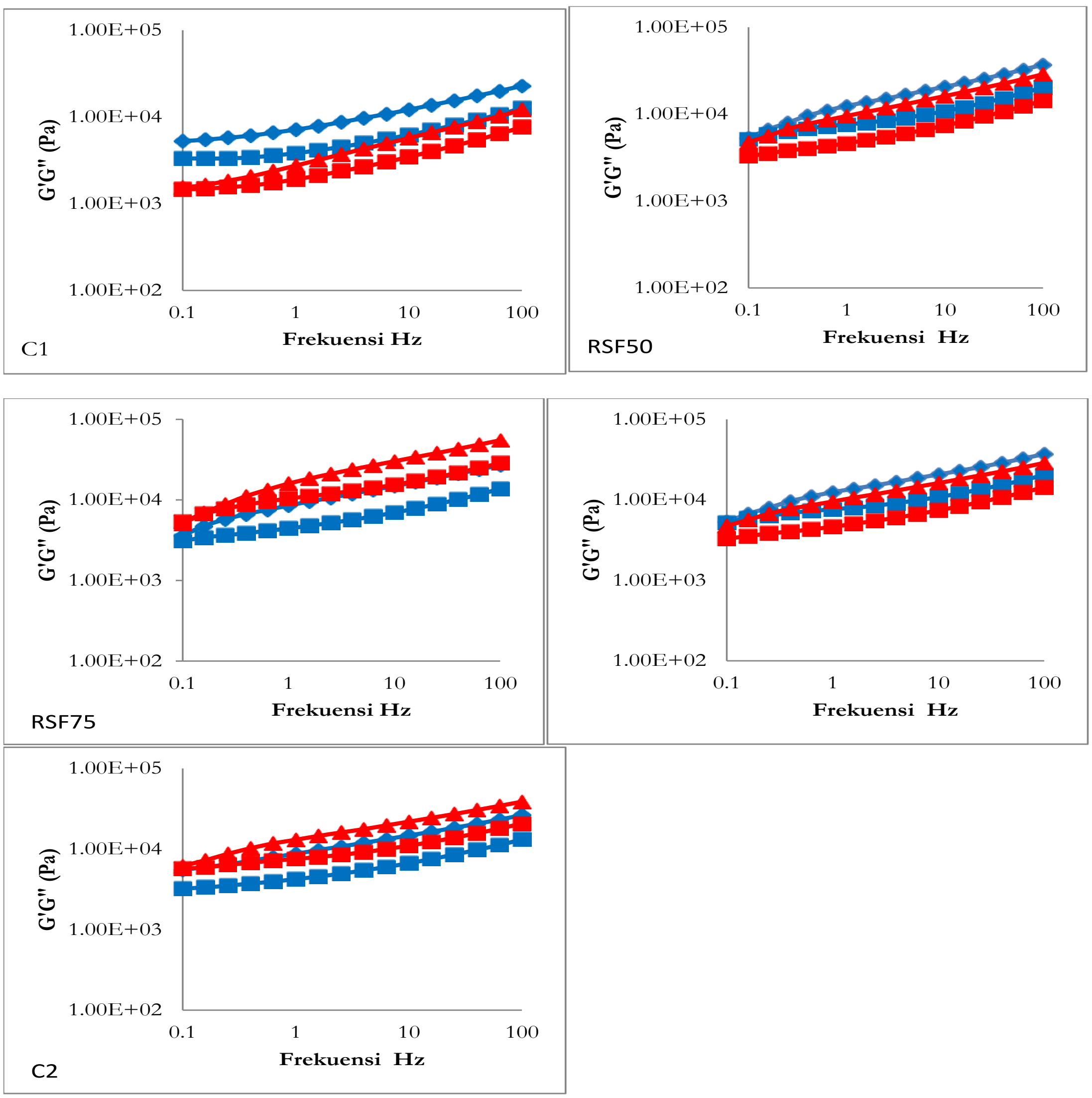

Figure 6: Dynamic frequency sweep test on bread dough with different percentage of rubber seed flour.

CONCLUSION

Rubber seed have a great potential as a new source of protein to replace wheat flour as it have protein content. The incorporation of rubber seed in bread making give the significant different to the colour of the bread. Increasing the percentage of the rubber seeds flour causing the crust colour darker than control bread. At the different storage temperature, the bread making from the whole wheat flour as control and the bread with addition of the rubber seeds flour shows significant different, where rubber seed can improve frozen dough bread in term of its rheological properties. Rubber seeds bread also acceptable by consumer. Thus, the bread incorporated with 25% and 50% of rubber seeds flour at the -18ºC of rubber seeds is the suitable formulation to be used for frozen dough as it gives a better quality of the bread. It is recommended that the further studies on the shelf life testing should be done to determine the shelf life of bread with addition of rubber seeds.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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