



Physico-Chemical Properties of Bread Made of Frozen Dough Incorporated with Rubber Seed (*Hevea Brasiliensis*) Flour Stored at Different Storage Temperature

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Abstract

Rubber seed is an underutilised by-product from rubber seeds and contains high protein. The dough that undergoes freezing negatively affects the quality of the end product. This study was conducted to determine the physical and chemical properties of bread that was made from frozen dough, incorporated with rubber seed flour (RSF) and stored at different storage temperatures. The formulation varied from control 100% bread flour, 100% RSF and bread dough prepared from substituted RSF with percentages of 25%, 50% and 75%. The dough was stored at temperatures of 25 °C, 10 °C, 4 °C and -18 °C for 14 days. Straight dough method was used to make the bread. Analyses were conducted on specific volume, texture, moisture content, water activity, porosity and pH. Results showed that bread formulated with RSF had low moisture content and water activity. However, the specific volume was high in the bread with different formulation and storage temperature. Among the formulation RSF25 showed the most suitable formulation to be used for frozen dough with the specific volume of 5.44 ± 0.007 cm³/g, when stored at lower temperature (-18 °C). The bread made of rubber seeds showed significant difference in texture, pore size and pH. In conclusion, the use of RSF can improve the quality of the bread made from frozen dough.

Keywords: *Hevea brasiliensis*, underutilised by-product, high protein content, frozen dough.

1. Introduction

Rubber trees, also known as *Hevea brasiliensis*, are a tropical plant belonging to the Euphorbiaceae family and are cultivated in many countries, especially in Southeast Asia. These trees originally come from the rain forests of Brazil and South America. Although rubber trees are native of South America, their cultivation is limited throughout different countries due to the high prevalence of leaf blight diseases and other natural predators. Despite the wide use of latex produced by rubber trees, the seeds are the residues from rubber plantations. These rubber seeds contain nutritive values that are suitable for human and animal consumption and biofuel production [1]. In some countries, these seeds are included in the diet of people living near the plantations. Although these seeds have good protein source, they contain cyanogenic glycosides. Nonetheless, this disadvantage can be overcome by proper cooking methods. The seeds are soaked for at least 24 hours with several changes of water and then boiled, uncovered for about half an hour [2]. Recent studies indicated that rubber seeds contain high percentage of protein at 17.41% per 100g [1]. In bread making, wheat flour is the main conventional ingredient due to its gluten fraction that makes the dough elastic by causing the bread to expand and to trap the carbon dioxide generated by yeast during fermentation [3]. Composite flour is used widely to extend the usage of wheat flour in the future without changing the quality of the bread. Previous studies did not report on the usage

of rubber seed flour (RSF), but its high protein content indicated its potential to retain gluten development in bread making. Freezing is commonly used as a deterioration control process for different dough and bakery products because this method is suitable to preserve dough and bread quality. Fresh bread may be produced by using frozen dough technology [4].

However, using frozen dough also can cause problems because the temperature fluctuation can affect the quality of the finished products. A few studies have investigated the effect of temperature fluctuation on frozen dough quality. The temperature fluctuations during storage cause increased rates of quality deterioration particularly due to changes in the structure of ice crystals and recrystallization [5-7]. Phimolsiripol et al. [8] reported that 30 days of frozen storage under extreme fluctuating temperature had caused structural damage and reduced carbon dioxide gas (CO₂) production in the dough.

Thus, the present study was conducted to determine the physical and chemical properties of frozen dough incorporated with RSF at different storage temperatures.

2. Materials and Methods

2.1. Materials and equipments

Raw rubber seeds were collected from rubber tree plantation and then processed into powder form. Wheat flour, instant dry yeast,



sugar and salt were bought from the local supermarket in Terengganu, Malaysia.

2.2. Preparation of the RSF

The rubber seeds were collected, screened and washed to ensure they were free of foreign materials. Next, the rubber seeds were dried in the oven at 60 °C until the moisture content is 7% [9]. The seeds were then milled, screened through a mesh sieve, packaged in a plastic container and stored in bakery laboratory at room temperature prior to analysis.

2.3. Bread-making process

The bread was produced in accordance with the method by Maaruf et al. [10]. The produced RSF was blended with wheat flour (WF) at 25:75 (RSF25), 50:50 (RSF50) and 75:25 (RSF75) ratios of substitution for bread production. RSF and WF at 100:0 (C1) and 0:100 (C2) ratios were used as the 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 was kneaded into smooth pliable elastic-like dough, covered, allowed to ferment in the proofer at 37 °C for approximately 45 minutes and baked at 180 °C for 20 minutes. The baked products were cooled and packaged in polythene bags for further analysis.

2.4. Preparation of the frozen dough

The dough was prepared according to the above method of bread making, and the dough was stored at four different storage temperatures: ambient temperature (25 °C), cooling temperature (10 °C), chilling temperature (4 °C) and freezing temperature (−18 °C) for 14 days.

After 14 days of storage, the dough was thawed at room temperature for 4 hours and then divided into 10 pieces of 30 g each. These pieces were 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.

2.5. Physico-chemical analysis

Measurement of Bread Volume: The loaves were weighed 1 hour after removal from oven (cooling). Volume (ml) was determined by rapeseed displacement method [11].

Pore Size: The porosity of the bread was determined by preparing 4 cm x 4 cm of slices from the middle of the crumb. The image of the bread was taken and analysed using Image J System.

Moisture content: Moisture content was determined by oven drying method [12] to investigate the moisture content in the bread incorporated with rubber seed.

Water Activity: The water activity of samples was analysed using Aqua Lab Water Activity Meter Model 4TE manufactured at USA. The sample was placed in the water activity meter, and the data were obtained in triplicate.

pH: The pH of the fresh and frozen bread was examined by using a pH meter. Approximately 10 g of samples were weighed and homogenised in 50 ml of distilled water. The electrode was immersed in the homogenous solution. The data were obtained in triplicate.

Texture : Texture attributes were measured objectively using TA.XT plus model texture analyser (Stable Micro System Co. Ltd.,

Surrey England) by adopting the standard method AOAC, method 74-09 (AOAC, 2000). The probe was calibrated, and a cube sample (2cm x 2cm x 2cm) was cut from the middle sample. The compression test was selected in texture analysis using a 5 kg load cell, and the sample was compressed to 45% of its original height. The strain required for 45% compression was recorded using the following conditions: pretest speed, 1.0 mm/s; test speed, 1.7 mm/s; post-test speed, 10 m/s; compression distance, 25%; and trigger type, auto 5 g. The data were collected as the average of three readings.

2.6. Statistical Analysis

Statistical analysis was performed using two-way ANOVA to compare the mean values of physical and chemical properties of frozen dough. SPSS statistical software version 20 was used to analyse data, and significances were set at $p < 0.05$ with Tukey's homogenous test.

3. Results and Discussion

3.1. Effect of RSF on the specific volume of bread

Good-quality bread is usually described as soft and voluminous. On the basis of the graph in Fig. 1, the bread made of dough stored at room temperature (25 °C) shows significant difference ($p < 0.05$) with C1 among the formulations of C2, RSF50 and RSF75. However, RSF25 has no significant difference with C1 ($p < 0.05$). A similar trend is observed for the bread made of dough stored at 10 °C and −18 °C. C2, RSF50 and RSF75 showed significant difference ($p < 0.05$) with C1, but RSF25 did not ($p < 0.05$). This result is due to the similar protein content for C1 and RSF25. C1 shows the highest mean value of 4.71 ± 0.22 , whereas RSF50 has the lowest mean value of 3.61 ± 0.72 for the bread made of dough stored at 25 °C.

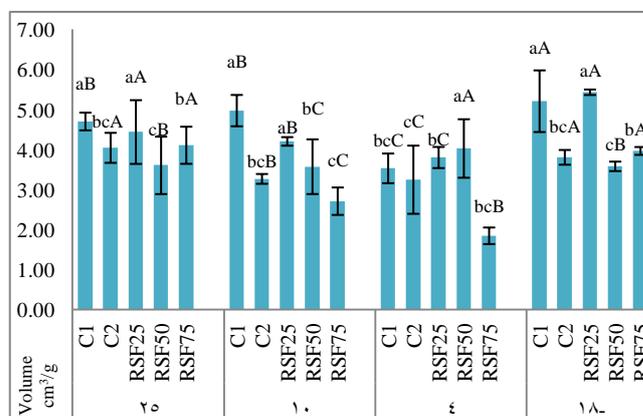


Fig. 1: Specific volume of bread with addition of rubber seed flour at different storage temperature.

A-C = Significant different ($p < 0.05$) between storage temperature
a-c = Significant different ($p < 0.05$) among formulation

For the bread made of dough stored at 10 °C, C1 has the highest mean value of 4.98 ± 0.39 cm³/g, whereas RSF75 has the lowest mean value of 2.27 ± 0.34 cm³/g. For the bread made of dough stored at −18 °C, RSF25 has the highest mean value of 5.44 ± 0.07 cm³/g, whereas RSF50 has the lowest mean value of 3.59 ± 0.12 cm³/g. For the bread made of dough stored at 4 °C, significant difference ($p < 0.05$) is found between RSF50 and C1. However, no significant difference ($p < 0.05$) with C1 is observed among the formulations of C2, RSF25 and RSF75. RSF50 has the highest mean value of 4.21 ± 0.73 cm³/g, whereas C2 has the lowest mean value of 3.25 ± 0.85 cm³/g.

The significant difference in the specific volume is due to the different percentages of the protein in the main ingredient used.

Protein is the main important factor that contributes to the increasing specific volume of the bread. According to Eka et al. [1], the protein content of rubber seed was 17.41 g/100 g; hence, it could produce bread with higher specific volume than that made of bread flour. However, not all bread with high protein percentage has high specific volume because the specific volume varies with the storage temperature. RSF25 has the comparable specific volume at 25 °C, 4 °C and 10 °C with C1; nonetheless, the volume was higher at -18 °C compared to C1. This result indicates the ability of RSF to retain gluten during freezing, thereby increasing the specific volume. According to Ribotta et al. [4], the freezing and storage of dough at -18 °C reduces bread quality, which is reflected by a lower loaf volume. However, in the present study, the incorporation of RSF in bread making can increase the specific volume of frozen dough bread. The result is the same for the storage temperature of 4 °C, where the specific volume of RSF5 is higher than that of C1.

3.2. Effect of RSF on the Pore Size of Bread

Pores are air pockets that are found in the leavened bread and where the carbon dioxide from fermentation creates networks of primarily interconnected void structure [13]. Table 1 shows the increasing cell number of RSF25, RSF50 and RSF75. RSF75 has higher cell number at 350 as compared with C1 with only 44 at 25 °C. This result might be due to the high protein content of RSF that influences the gluten network development. C2 has the highest cell number at 252 among other formulations because it uses only RSF during bread making. The cell size of the bread decreased when the percentage of the rubber seed increased in the formulation. C2 has the lowest cell size at 16 mm², and C1, RSF25, RSF50 and RSF75 have cell sizes of 100, 51, 18 and 10 mm², respectively. Thus, increasing the percentage of the RSF produces compact bread with small cells and pores and increased cell number.

Table 1: Effect of rubber seed flour on pore size of bread.

Temp erature (°C)	Porosity	Formulation				
		C1	C2	RSF25	RSF50	RSF75
25	Cell number	44	252	125	198	350
	Cell size (mm ²)	100	16	51	18	10
10	Cell number	144	348	67	197	145
	Cell size (mm ²)	22	12	126	25	27
4	Cell number	168	167	199	139	336
	Cell size (mm ²)	22	9	39	31	20
-18	Cell number	268	241	77	96	213
	Cell size (mm ²)	13	15	56	40	19

With increasing RSF, the bread made of dough (C2, RSF50 and RSF75) stored at 10 °C has an increased cell number compared with C1. However, the formulation of RSF25 has the lowest cell number at 67 among all other formulations, such as C1 with 144 cell counts and RSF25 incorporated with rubber seed. This result occurs because the temperature of 10 °C disturbs the gluten network development during storage. Slow freezing promotes the extracellular ice crystal formation, whereas fast freezing promotes intracellular ones [14]. Temperature of 10 °C is considered as slow freezing. According to Havet et al. [15], slow freezing greatly damages the gluten network, and the extent of damage depends on the ice crystal size. The cell size of RSF25, RSF50 and RSF75 increased with the increase in the percentage of RSF. This increase might be due to the high fat content of rubber seeds that interrupted the gluten network during storage. However, the cell size of C2 (12 mm²) decreased, and the same result was obtained for C2 with decreasing cell size of 16 mm² at storage temperature of 25 °C.

For the storage temperature of 4 °C, all the formulations of C2, RSF25 and RSF75 showed an increment in cell porosity compared with C1. The result obtained is similar to that at 10 °C; however, the cell number of RSF50 is reduced but that of RSF25 remains the same as observed at 10 °C. This result may be due to the same case as the bread made of dough stored at temperature 10 °C; 4 °C is the common temperature of refrigerator and considered as slow freezing, thereby causing the interruption in the gluten network development in the bread. However, each bread piece has an increase of cell size for RSF25 with 39 mm² and RSF50 with 31 mm², when compared with C1 that has only 22 mm² of cell size. Meanwhile, C2 and RSF75 show the decreasing of cell size of the bread with the value of 9 and 20 mm², respectively.

For the storage temperature of -18 °C, increasing the percentage of RSF also increased the cell number of the bread crumb for RSF25 with 77, RSF50 with 96, and RSF75 with 213 but could not exceed the cell number of C1 at 268 counts. This phenomenon occurs because freezing affects the structure of the gluten network in the bread and decreases the cell number of the bread crumb. At the same time, thawing the dough immediately after freezing becomes the cause for the decreasing cell number. Change in the water or ice distribution in the complex dough could cause reversible and irreversible cellular damage on the yeast's microenvironment [16]. The cell size of RSF25, RSF50 and RSF75 decreased with the increasing percentage of RSF; nonetheless, it was larger than the cell size of C1.

Each bread type with the same formulation was compared at different storage temperatures. C1 has an increase in cell number ranging from 44 to 268; the lowest cell number was recorded to be C1 with 44 counts at 25 °C with the decreasing cell size over each temperature. The same trend was observed in C2 as the cell number of bread crumb increased from 25 °C with 252 counts to 4 °C with 348 counts; however, the cell number decreased during storage temperature of -18 °C with the total count of 241. The result is similar to cell size; the cell size increased at 25 °C, 10 °C and 4 °C but decreased at -18 °C. For the formulation of RSF25, the cell number of the bread ranges from 67 to 199 with the highest number at 25 °C and lowest at 10 °C. For the cell size, no large difference was observed for RSF25 at 25 °C and -18 °C; nonetheless, the cell size of RSF25 at 10 °C was larger than that at other temperatures.

3.3. Effect of RSF on the moisture of bread

Moisture content of the bread is usually associated with the product shelf life upon subsequent storage. Water contributed approximately 40% of standard bread dough and over 35% of baked bread; this finding is in agreement with the research done by Cauvain and Young [17], who stated that the moisture content of bread is approximately 40%. The results show significant difference ($p < 0.05$) in moisture content with C1 and the formulations of C2, RSF25, RSF50 and RSF75. The highest mean value was recorded in C1, and the lowest mean value was presented by C2 for each of bread stored at different storage temperatures. In comparison with the other breads, C1 shows the highest mean value of moisture when stored at 25 °C, 10 °C, 4 °C and -18 °C (30.09±0.50, 32.21±1.44, 33.57±0.50 and 30.92±0.26 per cent, respectively). The lowest mean among the formulations at 25 °C, 10 °C, 4 °C and -18 °C were 15.25±1.14, 15.12±0.49, 18.15±0.51 and 14.89±0.63 per cent, respectively. The moisture content for C1 is 30%–40%, which is in agreement with the standard for the bread product. The graph in Fig. 2 clearly shows the decreasing moisture content with the increasing percentage of RSF for all the bread made from dough stored at different storage temperatures.

When comparing the effect of RSF on the bread made from dough stored at different storage temperatures, significant difference ($p < 0.05$) of C1 at 25 °C, 10 °C and 4 °C is found; however, no significant difference ($p < 0.05$) of C1 was observed at 25 °C and -18 °C. For C1, the highest moisture content was recorded in C1

at 4 °C, and the lowest was at 25 °C and -18 °C. The same result was obtained for RSF75, which shows significant and no significant differences at the similar storage temperature as C1. The highest mean value was $19.64 \pm 0.32\%$ at 25 °C, and lowest mean value was $18.88 \pm 0.45\%$ at -18 °C. Significant difference ($p < 0.05$) of C2 was observed at 25 °C, 4 °C and -18 °C; nonetheless, no significant difference ($p < 0.05$) is seen between C2 at 25 °C and 10 °C. The highest mean value was $18.15 \pm 0.51\%$ at 4 °C, and the lowest mean value was $14.89 \pm 0.63\%$. The same result was obtained for RSF25 at the same storage temperature. The highest mean was recorded at 25 °C and 4 °C; however, the lowest mean is at -18 °C. Significant difference ($p < 0.05$) is observed among RSF50 at 25 °C, 10 °C and -18 °C but none at 25 °C and 4 °C. Both recorded the lowest mean value of $21.59 \pm 0.93\%$ and $21.59 \pm 0.43\%$, respectively.

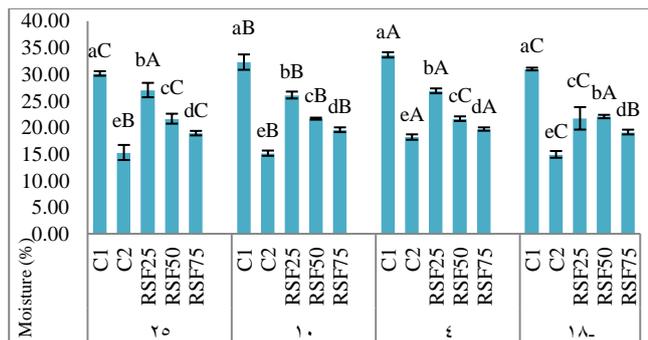


Fig. 2: Effect of Rubber Seed Flour at Different Storage Temperature on Moisture.

A-C = Significant different ($p < 0.05$) between storage temperature
a-e = Significant different ($p < 0.05$) among formulation

Significant differences in bread at different temperatures may occur due to the effect of freezing on the moisture content at different temperatures. The highest moisture content was mostly recorded at storage temperature of 4 °C, and the lowest moisture content was observed mainly in bread made of dough stored at -18 °C. This result is due to the large ice crystal produced by the slow freezing followed by the thawing process, where the water may remain in the bread and increase the moisture content of the bread at 4 °C. The ability of RSF to lower the moisture content at -18 °C may be driven by the low moisture content of RSF itself. Eka et al. [1] reported that rubber seed has low moisture content of 3.99 ± 0.01 compared with 14% of commercial bread flour. The moisture content of the flour affects the moisture content of bread. Thus, the bread could have good quality because its low moisture content promotes longer shelf life than that for conventional bread when stored under similar conditions.

3.4. Effect of RSF on the water activity (Aw) of bread

Water activity can be defined as water in food that is not bound to food molecules and supports the growth of the bacteria. This factor can also determine the shelf life of the bread: bread with low water activity will have a long shelf life. Fig. 3 shows a significant difference ($p < 0.05$) of formulation at different storage temperatures among C2, RSF25, RSF50 and RSF75 with C1. Significant difference ($p < 0.05$) is also observed in the formulation of RSF25 and RSF50. The highest mean value was recorded in C1 ranging from 0.90 to 0.92, and the lowest mean value was recorded in C2 ranging from 0.74 to 0.78 at each storage temperature. The water activity of the bread decreased with the increasing percentage of the RSF in the formulation. This result might be due to the high content of mineral in the rubber seeds, which can bind to more water compared with the bread flour. If the water available in the bread is small, then the microbial growth can be reduced. Certain water activity may present the microbial that causes deterioration in the bread. Thus, increasing the percentage of RSF in

the formulation will decrease the water activity of the bread. Significant difference ($p < 0.05$) is found between the water activity of the same formulation at 25 °C, 10 °C, 4 °C and -18 °C because the different temperature may have a certain effect on the water activity of the bread. The highest water activity was mostly observed in the bread made of dough stored at 4 °C ranging from 0.93 to 0.80, followed by bread made of dough stored at 10 °C, 4 °C and -18 °C.

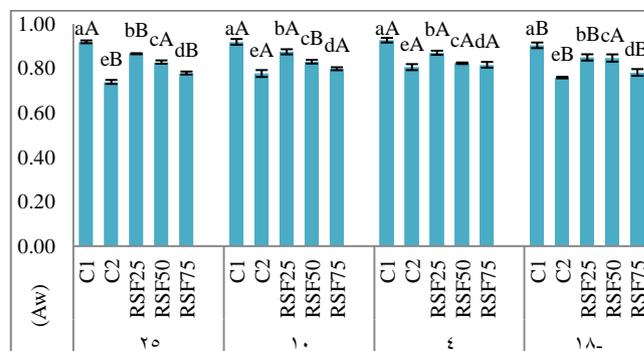


Fig. 3: Effect of Rubber Seed Flour at Different Storage Temperature on Water Activity.

A-B = Significant different ($p < 0.05$) between storage temperature
a-e = Significant different ($p < 0.05$) among formulation

3.5. Effect of rubber seed flour on pH of the bread

pH can be defined as the measurement of the acidity or alkalinity of a solution commonly measured on a scale of 0 to 14. pH 7 is considered neutral, low pH values are acidic, and high values are alkaline or caustic. pH analysis showed a direct measure of acid content $[H^+]$ in the bread. Thus, the acidity of the bread can be determined.

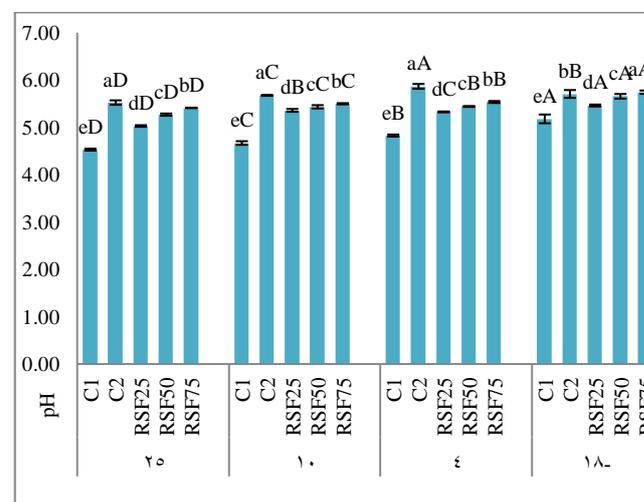


Fig. 4: Effect of Rubber Seed Flour at Different Storage Temperature on pH.

A-D = Significant different ($p < 0.05$) between storage temperature
a-e = Significant different ($p < 0.05$) among formulation

Fig. 4 shows a significant difference ($p < 0.05$) of the samples C2, RSF25, RSF50 and RSF75 with C1; moreover, a significant difference ($p < 0.05$) is found among RSF25, RSF50 and RSF75 with C2 at the temperature of 25 °C, 10 °C, 4 °C and -18 °C. At 25 °C, C2 has the highest mean of 5.52 ± 0.05 , and C1 has the lowest mean value of 4.53 ± 0.02 . The same trend was observed in the storage temperature of 10 °C and 4 °C, where the highest mean was recorded from C2 with the mean value of 5.67 ± 0.01 and 5.86 ± 0.05 , respectively. However, at -18 °C, the highest mean value was observed from RSF75 with the mean of 5.74 ± 0.04 . The lowest mean for the temperature of 10 °C, 4 °C and -18 °C was

recorded at C1, which corresponds to 4.67 ± 0.04 , 4.82 ± 0.02 and 5.18 ± 0.09 , respectively. The fermentation of the yeast is preferable at the temperature of 25 °C and 32 °C [17]. The fermentation rate was low at -18 °C because freezing can destroy the cellular structure of yeast cells, and the fermentation activity of yeast is dramatically lowered after thawing the dough [18]. Thus, the pH values of bread decreased due to the low degree of yeast fermentation.

3.6. Effect of RSF on the texture of bread

Bread texture is an important factor that is evaluated by the consumers and affects the acceptability of the product by consumers. As shown in Table 2, each bread stored at 25 °C, 4 °C and -18 °C shows a significant difference ($p<0.05$) among C2, RSF25, RSF50 and RSF75 with C1; C1 has the highest mean value of hardness ranging from 18.97 ± 7.05 to 26.29 ± 2.30 . However, no significant difference ($p<0.05$) of C2 with C1 at 10 °C was found. Therefore, an increase of the percentage of the RSF in the bread results in a decrease of the hardness.

Table 2: Effect of Rubber Seeds Flour on the Texture of Bread

Temperature (°C)	Formulation	Parameters	
		Hardness (kg/m ³)	Springiness
25	C1	26.29 ± 2.30^{aA}	0.94 ± 0.03^{aA}
	C2	8.30 ± 2.28^{bA}	0.66 ± 0.03^{cA}
	RSF 25	16.72 ± 1.50^{bA}	0.83 ± 0.04^{bcA}
	RSF50	12.30 ± 5.84^{bA}	0.78 ± 0.04^{bcA}
	RSF75	0.60 ± 0.17^{bB}	0.80 ± 0.02^{abA}
10	C1	4.40 ± 0.55^{bB}	0.92 ± 0.02^{aA}
	C2	8.70 ± 2.28^{bA}	0.65 ± 0.08^{cA}
	RSF 25	20.07 ± 6.30^{aA}	0.69 ± 0.17^{bcA}
	RSF50	21.64 ± 6.98^{aA}	0.83 ± 0.10^{bcA}
	RSF75	19.60 ± 0.01^{aA}	0.86 ± 0.08^{abA}
4	C1	18.97 ± 7.05^{aA}	0.90 ± 0.03^{aA}
	C2	2.37 ± 0.41^{bB}	0.62 ± 0.06^{bcA}
	RSF 25	2.72 ± 2.21^{bB}	0.62 ± 0.33^{bcA}
	RSF50	1.86 ± 1.32^{bB}	0.58 ± 0.27^{cA}
	RSF75	2.27 ± 0.02^{bB}	0.80 ± 0.03^{abA}
-18	C1	22.45 ± 3.07^{aA}	0.91 ± 0.06^{aA}
	C2	6.91 ± 4.37^{bB}	0.48 ± 0.24^{cA}
	RSF 25	8.36 ± 1.39^{bB}	0.76 ± 0.16^{bcA}
	RSF50	1.39 ± 0.07^{bB}	0.71 ± 0.08^{bcA}
	RSF75	2.16 ± 0.22^{bB}	0.73 ± 0.03^{abA}

A-B = Significant different ($p<0.05$) between each of storage temperature
a-c = Significant different ($p<0.05$) among formulation

The lowest mean value of hardness value was observed to be C2 stored at 25 °C and 10 °C (8.30 ± 2.28 and 8.70 ± 2.28 , respectively) and RSF50 at 4 °C and -18 °C with the mean value of 1.86 ± 1.32 and 1.39 ± 0.07 , respectively. This result might be due to the high protein of rubber seed that is responsible for forming gluten and retaining the gluten network development during the storage even under fluctuating temperature. Thus, the ability to keep fermenting the gas during the rising of dough is not interrupted, thereby resulting in good bread quality in terms of hardness. Gomez et al. [19] reported that bread hardness is due to the interaction between gluten and fibrous materials.

When comparing the storage temperatures, the bread made of dough stored at 10 °C has higher value of hardness than those at 25 °C, 4 °C and -18 °C do. However, the value was comparable with the bread made of dough stored at 25 °C; meanwhile, the hardness of the bread made of dough stored at 25 °C was similar with bread made of dough stored at 4 °C. The bread made of dough stored at 25 °C and 10 °C was harder than that at 4 °C and -18 °C. For the springiness of the bread, significant difference was found among C2, RSF25 and RSF50 with C1 but none between RSF75 and C1. As shown in Table 2, the highest mean value observed in C1 at each storage temperature ranges from 0.90 ± 0.03 kg/m³ to 0.94 ± 0.03 kg/m³. The lowest mean was observed in C2 with the mean value of 0.48 ± 0.24 kg/m³ to 0.66 ± 0.03

kg/m³. However, no significant difference was observed between each formulation at the storage temperature of 25 °C, 10 °C, 4 °C and -18 °C.

4. Conclusion

RSF has a potential as a bread ingredient to retain gluten development in frozen dough. This is proved through the high specific volume of bread made of dough stored at -18 °C with 25% RSF added into the formulation. Incorporating RSF in bread reduced the moisture content and the water activity of the bread with the increasing percentage of RSF in formulation. Moreover, significant difference was found among the pH and texture with different percentages of RSF and storage temperature. Generally, RSF can be used to improve the quality of bread made of frozen dough.

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