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Development and Physicochemical properties of Breadfruit (*Artocarpus altilis)* Resistant Starch Bread

Zarinah Zakaria[1], Nurul Farahin Mohamod[1], Siti Nuriah Mohd Noor[1], Napisah Hussin[2] and Norshazila Shahidan[1]

[1]Faculty of Bioresources and Food Industry,
Universiti Sultan Zainal Abidin,
Besut Campus, 22200 Besut,
Terengganu, MALAYSIA.

[2]Faculty of Health Science,
Universiti Sultan Zainal Abidin,
Gong Badak Campus,
21300 Kuala Terengganu,
Terengganu, MALAYSIA.

Corresponding author: Zarinah Zakaria,
Faculty of Bioresources and Food Industry,
Universiti Sultan Zainal Abidin,
Besut Campus, 22200 Besut,
Terengganu, MALAYSIA.

Email: zarinah@unisza.edu.my

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ABSTRACT

This study aims to produce breadfruit-resistant starch (BRS) bread with resistant starch concentration levels of 5%, 10% and 15%, and determine the physicochemical properties of the BRS bread produced. This study involved two major phases which were the breadmaking process and physicochemical analysis. A straight-dough method was applied during the preparation of bread, and the data obtained were statistically analyzed using the SPSS software. The results showed that the moisture content of the control bread was higher compared to the moisture content of the breadfruit-resistant starch (BRS) bread, and the BRS bread had a lower volume compared to the control bread. For texture analysis, there was no significant difference ($p>0.05$) in terms of hardness, springiness and chewiness between all samples of bread involved. Both the crumb and crust parts of the bread samples showed a decrease in the lightness color due to the presence of BRS and the occurrence of the Maillard reaction. As conclusion, the obtained results in this study are useful for the bakery and food industries when the functional ingredient is used in products and BRS bread that produced might be assumed as one of healthy fortified bread.

Keywords: Breadfruit resistant starch, Bread, Physicochemical, Maillard Reaction, Functional Ingredients

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan roti menggunakan kanji rintang sukun pada tahap 5%, 10%, dan 15% dan menilai sifat-sifat fizikokimia roti yang dihasilkan dari kanji rintang sukun. Kajian ini melibatkan dua fasa iaitu proses pembuatan roti dan analisis fizikokimia. Kaedah adunan-lurus digunakan dalam penyediaan sampel roti dan data yang diperoleh dianalisis menggunakan perisian statistik SPSS. Keputusan menunjukkan kandungan kelembapan sampel roti kawalan adalah lebih tinggi berbanding sampel roti kanji rintang buah sukun dan isipadu roti kanji rintang sukun lebih rendah berbanding roti sampel kawalan. Analisis tekstur menunjukkan, tiada perbezaan yang signifikan ($p>0.05$) untuk nilai kekerasan, kespringan dan kekenyalan di antara kesemua sampel roti .Kedua-dua isi dan kulit roti menunjukkan penurunan tahap kecerahan disebabkan oleh kehadiran kanji rintang sukun dan reaksi Maillard yang berlaku. Kesimpulannya, keseluruhan keputusan yang diperolehi di dalam kajian ini amat berguna kepada industri makanan dan bakeri apabila ramuan berfungsi digunakan di dalam produk dan roti kanji rintang sukun yang terhasil boleh dianggap sebagai salah satu roti yang ditambah nilai dan berkhasiat.

Kata Kunci: Kanji Rintang Sukun, Roti, Fizikokimia, Tindakbalas Maillard, Ramuan Berfungsi

INTRODUCTION

Bread is one of the oldest and largest consumed staples and is eat up across the universe by all age groups (Bhatt & Gupta, 2015). Bread can be described as a fermented confectionery product which is produced usually from wheat flour, yeast, water, sugar, salt and other ingredients needed in consequences by a series of process that involve mixing, kneading, proofing, shaping and baking (Dewettinck et al., 2008). Since the consumer nowadays are more concerned about their health, they will focus more on consuming products which will raise up their immune systems (Bhatt & Gupta, 2015). The functional food products have been mainly launched in bakery products (Kotilainen et al., 2006; Menrad, 2003) which provide the ideal track by which the functionality can be delivered to the consumer in an acceptable food (Sir et al., 2008) and supplying a product which meets the consumer's needs in terms of appearance, taste and texture (Alldrick, 2007). Due to that, breadfruit can be used as one of the functional ingredient that can be added in bread. Nutrionally, it is favorably compared with the other tropical staples for its mineral and vitamin content such as calcium, iron, magnesium, potassium, niacin and thiamine which the potassium levels are ten times than that found in white rice and can supply the current recommended human daily intake of dietary fibre (Ragone & Cavletto, 2006). Taking into account the nutritional values of breadfruits, there is a growing interest towards developing a breadfruit-based foods and formulations (Turi et al., 2015) and from that, it is not surprising when the majority of research about the breadfruit is focusing on its physicochemical properties in starch and flour form (Nwokocha & Williams, 2010; Wang et al., 2011; Akankabi et al. 2009; Loos et al.1981). Thus, the main objectives of this study were to produce

bread for breadfruit resistant bread (BRS) bread by using 5, 10, 15% of BRS and to determine the physicochemical properties of BRS bread produced.

MATERIAL AND METHODS

Raw Materials

Breadfruit-resistant starch was supplied by the Faculty of Health Sciences (FSK) Universiti Sultan Zainal Abidin (UniSZA). Ingredients like sugar, salt, wheat flour, milk powder, and yeast used to produce the bread samples were bought from a supermarket in Jertih, Terengganu, Malaysia.

Breadmaking Procedure

Breads were produced using the straight-dough method (Bhatt & Gupta, 2015). Five types of bread were used as samples. Commercial white bread from brand A and bread made with 100% high protein wheat flour served as controls, while the other three samples were added with 5%, 10%, and 15% of breadfruit-resistant starch (BRS), respectively. The formulation used for making the bread can be referred in Table 1.

Table 1 Formulation of Breadfruit Resistant starch (BRS)Bread

Ingredients (%)	Control	BRS (5%)	BRS (10%)	BRS (15%)
Wheat Flour	100%	95%	90%	85%
Breadfruit Resistant Starch	-	5%	10%	15%
Water	60%	60%	60%	60%
Milk Powder	1.5%	1.5%	1.5%	1.5%
Yeast	2%	2%	2%	2%
Salt	1.5%	1.5%	1.5%	1.5%
Sugar	8%	8%	8%	8%
Shortening	4%	4%	4%	4%

Proximate Analysis

Moisture Content Analysis

Oven drying method was used to determine the moisture content of the sample. The bread sample was grinded by using blender. Approximately, 3 g of the grinded white bread sample (W2) were spreaded evenly in crucible and were let to dry in an oven for 24 h at 105°C. After drying, the crucible was transferred by partially covering it with lid in the desiccator to let it cool. The crucible containing dried sample were weighed (W3) and all of the reading were recorded. The moisture content was calculated by using the formula.

$$\text{Moisture (\%)} = \frac{W3 - W1}{W2} \times 100$$

Where: W1 = Weight of empty crucible (g)
W2 = Weight of sample (g)
W3 = Weight of crucible + dried sample

Ash Content Analysis

The ash content was determined by using muffle furnace method. The empty crucibles were dried in an oven at 105°C for 4 h and were cooled in desiccator after that period followed by weighing (W1) and labelling. Approximately, 3 g of white bread sample were weighed (W2) and placed in the muffle furnace at 550°C overnight. After 24 h, the muffle furnace was turned off and the crucible was removed and weighed soon after

attaining the room temperature (W3). The reading was recorded. The percentage of ash was determined by using the formula.

$$\text{Ash (\%)} = \frac{W3 - W1}{W2} \times 100$$

Where: W1= Weight of empty crucible (g)
W2 = Weight of sample (g)
W3 = Weight of crucible (g)

Crude Protein Analysis

Protein content of the food sample was determined by using KJEDATHERM system, Gerhardt, Germany. Three stages were involved, which were digestion, distillation and titration. In digestion phase, 1 g of the bread sample was inserted into a digestion tube. Then, 2 tablet of Kjeltabs Cu 3.5 catalyst were added with the 12 mL concentrated sulphuric acid (H_2SO_4). The bread sample, catalyst and the (H_2SO_4) were digested until the color of the sample change from black to clear blue or green indicating the reaction is ended. The digestion process took time for about 60 to 90 min. The digestion tubes were cooled for about 10 to 20 min. In distillation process, the samples were neutralized by using sodium hydroxide (NaOH). The receiver solution which were; 25 mL of 4% boric acid and 10 drops on indicator solution were filled into the conical flask. The receiver solution and digestion were placed in the distillation unit and the safety door was closed. The desired program was chose and 70 mL distilled water were dispensed into the tube automatically followed by the 50 mL of 40% (NaOH). This distillation process took approximately 4 min. The receiver solution in the distillate flask turned green in colour after distillation process indicating that there was a presence of alkali. Titration was the last process conducted. The distillates were titrated with the standardized hydrochloric acid (HCl) and borate ion were converted into nitrogen, (N) in sample until the colour of the mixture turns to pink or red. The volume of HCl used for sample and digested blank was recorded.

$$\text{\% N} = \frac{0.1 \times (A - B) \times 14.007 \times 100}{\text{Weight of sample (g)} \times 1000} \times 100$$

Where: 0.1 = Molarity of HCl used
A = Volume of HCl used to titrate sample (mL)
B = Volume of HCl used to titrate blank (mL)
14.007 = Molecular weight of Nitrogen

$$\text{\% of Crude Protein} = \text{\% N} \times \text{Protein Factor (5.7)}$$

Crude Fiber Analysis

Gerhardt Fibertherm Automated Fiber Analysis System (Germany) was used to determine the crude fiber content in white bread samples. Empty fiber bags and crucible were dried in an oven at 105°C for about 4 h. After drying, the fiber bags were cooled in desiccators before weighing (W2) process. Approximately, 1 g of bread sample (W1) was placed in each of the fiber bags. Glass spacer was inserted onto the Fibertherm and the fiber analysis was run. After the process was done, the samples were dried again at the same temperature for about 24 h. The dried samples were cooled in desiccator before weighing (W3). The samples in the crucible were burned in the muffle furnace at 550°C for 6 h. The samples then were left to cool in the desiccator before the next weighing process (W4). All the results that obtained were recorded for further calculation use.

$$\text{\% Crude Fiber} = \frac{(W3 - W2 - W4)}{W1} \times 100$$

Where: W1 = Weight of sample (g)
W2 = Weight of fibre bag (g)
W3 = Weight of crucible + sample + fibre bag (g)
W4 = Weight of crucible + ash (g)

Crude Fat Content Analysis

Fat Soxhlet method was used to determine the fat content in the breadfruit resistant bread samples. The extraction cups were dried at 105°C for 1 h, and were cooled in the desiccator, labeled and weighed (W2). Approximately, 3 g of samples (W1) were placed inside the thimbles. The thimble cup then was transferred into the extraction cups contained 150 mL petroleum. The extraction cups then were attached to the Fat Soxhlet extractor machine for 2 h. After that, the extraction cups were dried overnight and cooled in desiccators before weighing process (W3). The percentage of fat was calculated by using the formulas as shown below:

$$\% \text{ Fat} = \frac{W3 - W2}{W1} \times 100$$

Where: W1 = Weight of sample (g)
W2 = Weight of extraction beaker (g)
W3 = Weight of extraction beaker + fat (g)

Carbohydrate calculation

Percentage of carbohydrate in samples was determined by subtracting the 100% with the percentage of crude protein, crude fat, ash and moisture content that were obtained after the analysis conducted.
Carbohydrate (%) = 100 % - (protein % + fat % + ash % + moisture %)

Physicochemical Properties Test

Texture Profile Analysis (TPA)

The Texture Profile Analysis of the bread was done by using a TA.XT plus model texture analyzer (Stable Micro System Co. Ltd., Surrey England) 1 h after baking. Firstly, texture analyzer was calibrated with 5 kg weight and the height was depends on the bread size before the test was run. The cylindrical probe was used for this analysis which was in 36 mm diameter in size. Then, the bread sample was placed at the center of the Texture Analyzer machine and the probe head was adjusted until it touched the surface of sample. The maximum force to compress the bread at a certain length of time was recorded. The textural properties (hardness, springiness, chewiness and cohesiveness) of bread were extracted from the curved obtained. The test was conducted in duplicates and the total average value was calculated and reported.

Colour Analysis

The colour of the crust and crumb of the bread was determined by using the Konica Minolta CR-400 chromameter (USA). Crust colour was measured at the surface of the bread and its crumb at the center part of the bread after cutting it into half. The colour intensity were expressed as L*, a* and b* values which the L* value indicated the lightness represented from white (100) to black (0). While a* value represented the red (+a) or green (-a) and b* represented yellow (+b) or blue (-b).

Determination of Volume, Specific volume and Density

The volume of the breadfruit resistant starch bread was determined by using VolScan Profiler machine VSP 600; Stable Micro System Ltd., Surrey, UK). TheVolScan Profiler was a bench top laser based scanner that measures the volume of bread and bakery products. The product was mounted at each end by a suitable mounting device tailored to the specific product. Parameters entered into the software by the operator for each batch under test included the sample ID name, date, flour weight, bread type and batch code. This assessment was done rapidly and the result was obtained in a period ranging from a few seconds. The product was then automatically weighed

and an eye-safe laser device scanned vertically to measure the contours of the product at selectable intervals whilst the product rotated. Once the test was completed, the volume, length, maximum width, maximum height, height at the maximum width and width at the maximum height was quickly determined. The density was then derived from dividing bread weight (g) by its volume (cm^3).

Determination of Pore Size Distribution

The pore size distribution of the bread samples were determined by using the Image J software version 1.44c. Based on Abràmofff et al (2005), ImageJ open-source was a Java-based imaging that read and wrote the image files of bread sample, and operations on individual pixels, image regions, whole images and volumes of the bread samples. Volumes, called stacks in ImageJ, were an ordered sequences of images that can be operated upon as a whole. Images were saved in a TIFF format, and the central area of the crumb was selected for further analysis. To obtain a black and white threshold, the image was converted into 8-bit and binary segmentation was performed. The software was used to calculate the percentage of pore area in the whole area of the examined slice, which was the porosity and the medium pore size using the calibration ruler.

Statistical Analysis

The data that will be obtained were analysed by using the SPSS. The calculated mean was compared by using the One Way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Proximate Analysis

Brand A and BRS 10% bread samples were found to have significantly ($p<0.05$) lower moisture content compared to the control with mean values of 35.88 and 37.29, respectively. This might be due to the reduction of moisture content in the bread crumbs which is attributed to the migration of moisture from the wetter bread crumbs to the drier bread crust (Baik & Chinachoti, 2001), together with technical problems that occurred during the baking process which had led to overbaking. Giovanelli et al. (1997) stated that after baking, water migrates from crumb to crust, and some water evaporates. RS 10% and RS 15% showed significantly ($p<0.05$) higher ash content than the control. Bhore (2010) stated that breadfruit is a rich source of carbohydrates, vitamins and minerals. It can be concluded that the higher the concentration of the breadfruit-resistant starch (BRS), the higher the mineral content of the bread sample. Brand A bread showed significant ($p<0.05$) difference in terms of content compared to the control bread. This may be due to the fortification of food process using specific vitamins and minerals. White bread is one of the products that are fortified with iron (Murtagh & Aisling, 2008). BRS 15% and BRS 5% bread were found to have significantly ($p<0.05$) lower protein content than the control sample. Between all samples, BRS 15% bread had the lowest mean protein value which was 7.94% compared to the control sample at 8.63%. This might be due to the low protein content in BRS. Tan et al. (2017) stated the breadfruit starch contains very low amounts of protein and lipids, which are similar to the results from other studies (Wang et al., 2011).

All of the bread samples involved were found to have significantly ($p<0.05$) higher fat content than the control bread, indicating that the control bread had the lowest mean value for fat content. The lipid contents in breadfruit vary between reports at 0.08 g - 4.90 g per 100 g of fresh or cooked food, but most studies on fats in general had reported values of less than 2 g per 100 g, thus this study's findings can be deemed as similar to the results obtained in previous experiments (Turi et al., 2015). The high content of fat in brand A bread compared to the other bread samples may be due to the other additional ingredients used. Brand A, BRS 15% and BRS 10% bread had significantly ($p<0.05$) lower fibre content compared to the control bread. This may be caused by the damage of a significant fraction of starch granules during the milling or grinding process where the breadfruit flour was produced before the resistant starch was extracted. The mechanical damage to the starch granules' structure greatly affected its properties and caused it to lose its birefringence, have a higher rate of water absorption, and is more susceptible to (fungal) enzymic hydrolysis (Goesaert et al., 2005). There was no significant ($p>0.05$) difference found between the carbohydrate level content of all samples.

According to Nonaka (1997), the carbohydrate content in food products is related to the products' moisture content and level of water absorption. The reaction between water and carbohydrate molecules plays an important role in bread-making and results in insignificant carbohydrate content (Zuwariah et al., 2009). So, it is

not impossible for the carbohydrate content to be insignificant in samples. The energy values obtained in Table 2 show that there were significant ($p<0.05$) differences in the energy content of all bread samples, and the control bread had the lowest mean for energy value. The mean of energy value also increased as the concentration of the resistant starch added to the bread was also increased. According to Juarez-Garcia et al. (2006), resistant starch (RS) has a reduced caloric content and is characterized by its physiological effects that make it comparable to dietary fibre.

Table 2 Proximate Analysis of Breads

Compositions	Control	Brand A	RS 5%	RS 10%	RS 15%
Moisture (%)	$41.15^{a}\pm0.03$	$35.88^{c}\pm0.19$	$40.45^{a}\pm0.03$	$37.29^{b}\pm0.97$	$40.78^{a}\pm0.24$
Ash (%)	$1.05^{c}\pm0.07$	$3.04^{a}\pm0.00$	$1.24^{c}\pm0.14$	$1.49^{b}\pm0.11$	$1.57^{b}\pm0.03$
Protein (%)	$8.63^{c}\pm0.01$	$8.50^{a}\pm0.00$	$8.31^{c}\pm0.26$	$8.49^{b}\pm0.02$	$7.94^{b}\pm0.02$
Fat (%)	$0.57^{c}\pm0.00$	$2.60^{a}\pm0.00$	$1.31^{b}\pm0.03$	$1.66^{b}\pm0.21$	$1.16^{bc}\pm0.54$
Fiber (%)	$6.10^{a}\pm0.79$	$1.40^{b}\pm0.00$	$5.00^{a}\pm0.84$	$2.16^{b}\pm0.06$	$2.03^{b}\pm0.01$
Carbohydrate (%)	$48.60^{b}\pm0.02$	$49.20^{ab}\pm0.00$	$48.68^{b}\pm0.11$	$49.87^{a}\pm0.42$	$48.54^{b}\pm0.76$
Energy (kcal/g)	$234.08^{e}\pm0.17$	$258.00^{a}\pm0.00$	$239.75^{c}\pm0.87$	$248.35^{b}\pm0.17$	$236.35^{d}\pm1.71$

Means in the same row with diferent superscript are significantly ($p<0.05$) different

Texture Profile Analysis

The hardness of brand A bread was found to be significantly ($p<0.05$) higher compared to the other analysed bread samples (Table 3). This is also closely related to the breads' moisture content where storage time affects moisture loss. The brand A bread sample had already gone through a lengthy distribution journey to the retail outlet. Thus, it is proven that the higher the concentration of resistant starch, the higher the hardness of the bread. This result was in agreement with the experiment conducted by Sanz-Penella et al. (2010) where a significant increase ($p<0.05$) in hardness was noticed in bread samples that were added with resistant starch from modified pea starch. The bread samples were found to have insignificant ($p>0.05$) differences in terms of their springiness. This result is possible and is in agreement with the results from Sanz-Penella et al. (2010) which showed that even with the addition of resistant starch, the springiness of bread was not significantly influenced ($p>0.05$). The 5%, 10% and 15% BRS breads were found to have similar and lower cohesiveness mean values than the control (0.89) at 0.89, 0.84 and 0.81, respectively. This is in agreement with the results from Sanz-Penella et al. (2010) which expressed that the cohesiveness of breads with 10%, 20% and 30% flour replacements using resistant starch from modified pea starch was lower compared to the control sample, thus giving evidence that the matrix structure of protein had loosened. According to Bourne (2002), chewiness is the product of firmness, cohesiveness, and springiness. The textural properties obtained from the experiment showed no significant ($p>0.05$) difference in terms of the chewiness of all samples. Since cohesiveness is also influenced by firmness, cohesiveness and springiness, it is not impossible that there is no significant difference ($p>0.05$) in the chewiness value as the values for hardness, cohesiveness and springiness were also different for each bread sample.

Table 3 Texture profile Analysis

Bread	Hardness	Springiness	Cohesiveness	Chewiness
Control	783.77^{b}±260.91	0.97^{a}±0.01	0.89^{a}±0.02	682.48^{a}±239.66
Brand A	2869.87^{a}±172.26	0.86^{a}±0.15	0.51^{b}±0.02	1261.37^{a}±352.57
BRS 5%	579.34^{b}±600.38	0.63^{a}±0.41	0.89^{a}±0.07	414.14^{a}±516.51
BRS 10%	940.86^{b}±666.87	0.92^{a}±0.01	0.84^{a}±0.07	703.86^{a}±447.82
BRS 15%	1238.53^{b}±988.63	0.95^{a}±0.02	0.81^{a}±0.05	921.95^{a}±682.76

Mean in the same column with different superscript are significantly ($p<0.05$) different

Colour Analysis

Table 4 showed that the L* (lightness) values of the BRS breads were found to be significantly ($p<0.05$) lower than the control and brand A breads. This shows that when there is an addition of resistant starch during bread formulation, the lightness of bread crust is decreased after the baking process.

This result is in agreement with an experiment conducted by Sankhon et al. (2013) where the colour of the crust showed a significant decrease ($p < 0.05$) in L value when using Parkia flour supplemented bread. The colour had also changed from light-brown (control) to darker brown in the 40% Parkia flour bread. The L* values of BRS 10% and BRS 15% crumbs were found to be significantly ($p<0.05$) lower than all other bread samples. This result is in agreement with the result obtained by Sankhon et al. (2013) who showed a decrease in L* value as the percentage of Parkia flour was increased. The lower L* values obtained may be due to the Maillard browning and caramelisation processes which happened due to the distribution of water and the reaction between reducing sugars and amino acids (Kent & Evers, 1994). Both a* values for the crumb and crust of the BRS breads were found to be significantly ($p<0.05$) higher than the control and brand A breads. This may be due to the after-baking process effects on the resistant starch such as moisture, heat and autoclave treatment which caused the browning of the samples (Zuwariah et al., 2009). Additionally, the operating conditions applied during baking for example, temperature, air speed, relative humidity, and modes of heat transfer (Sankhon et al., 2013) may also affect the a* values. The b* value for crust showed a decrease as the lightness value also decreased. This shows that there is a relationship between the L* value and the b* value of the bread crust. The BRS 15% bread crumb had the significantly ($p<0.05$) highest b* value compared to the other bread samples including the control bread, and 15% was the highest concentration level of BRS added. This result might be due to the yellow colour of the breadfruit-resistant starch itself.

Table 4 Colour value of Breads

Parameter	Control	Brand A	RS 5%	RS 10%	RS 15%
Crust					
L*	49.16^{a}±2.96	52.87^{a}±7.72	45.52^{ab}±0.87	33.46±^{b}7.79	42.85±^{ab}0.49
a*	15.93^{ab}±0.02	11.65^{c}±2.85	16.85^{a}±0.33	12.85^{bc}±1.27	16.18^{ab}±0.78
b*	35.14^{a}±1.92	33.41^{a}±1.36	33.70^{a}±1.03	27.86^{c}±3.37	30.39^{ab}±1.86
Crumb					
L*	76.48^{a}±2.84	78.51^{a}±0.75	73.91^{a}±0.95	53.76^{c}±3.33	67.37^{b}±1.00
a*	-1.52^{c}±0.08	-1.47^{c}±0.03	-1.00^{b}±0.08	0.19^{a}±0.21	0.34^{a}±0.13
b*	15.11^{c}±0.81	13.68^{d}±0.08	16.90^{b}±0.49	17.44^{b}±0.02	20.89^{a}±0.16

Mean in the same row with different superscript are significantly ($p<0.05$) different

Volume Analysis

Comparing the volume of bread among all bread samples involved, the data showed significant ($p<0.05$) differences between the samples of breads (Table 5). Meanwhile, there were significant ($p<0.05$) differences in the specific volume and density of the BRS breads when compared with the control and brand A samples. The control bread had the highest mean volume among all samples. This may be due to the level of protein in high protein flour. The loaf volume is affected by the quantity and quality of protein in the flour (Ragaee & Abdel-Aal, 2006) as well as proofing time (Zghal et al., 2002). The volumes of BRS breads were significantly ($p<0.05$) lower that the brand A and control samples, and this might be due to the addition of resistant starch during bread formulation. Based on an experiment conducted by Rosell and Santos (2010), the inclusion of resistant starch (RS) and fibre blend during bread formulation induced a reduction in the specific volume of bread obtained by conventional breadmaking. It is shown in Table 5 that there was a significant ($p<0.05$) difference between the density of bread for brand A and the control bread sample, and the values were lower than those of the BRS bread samples indicating that the density will increase when resistant starch is present in the bread. Density is the inverse of specific volume and it has an enormous effect on the mechanical behaviour of bread crumbs. This means that as density increases, the specific volume will decrease (Scanlon & Zghal, 2001).

Table 5 Volume, Specific volume and Density of Bread

Bread	Volume (mL)	Specific Volume (mL/g)	Density (g/mL)
Control	$1871.42^{a}\pm0.45$	$3.68^{a}\pm0.00$	$0.27^{d}\pm0.00$
Brand A	$1624.82^{e}\pm1.00$	$3.68^{a}\pm0.00$	$0.27^{d}\pm0.00$
BRS 5%	$1742.37^{c}\pm0.34$	$3.39^{c}\pm0.00$	$0.30^{b}\pm0.00$
BRS 10%	$1643.43^{d}\pm3.45$	$3.14^{d}\pm0.01$	$0.32^{a}\pm0.00$
BRS 15%	$1783.12^{b}\pm1.31$	$3.61^{b}\pm0.00$	$0.28^{c}\pm0.00$

Mean in the same column with different superscript are significantly ($p<0.05$) different

Pore Size Distribution

Based on Table 6 and Figure 1, it was found that the BRS 15% bread sample had the highest number of pores. This might be due to the effect of the punching and rounding process to remove gases from the dough after fermentation which promotes the generation of more pores or gas cells and the formation of thinner gluten fibrils and sheets through the destruction and reformation of the gluten matrix (Eliasson & Larsson, 1993; Bushuk et al., 1997). It might also be related to the presence of the fibre blend (resistant starch) which had disrupted the crumb structure due to the physical disruption of the gluten network which favours crumb collapse during storage at low temperatures, consequently yielding lower specific volume (Mandala et al., 2009).

Table 6 Bread porosity

	Control	Brand A	BRS 5%	BRS 10%	BRS 15%
Pore count	578	444	362	481	631
Pore area (mm^2)	0.958	1.957	1.354	1.231	0.894

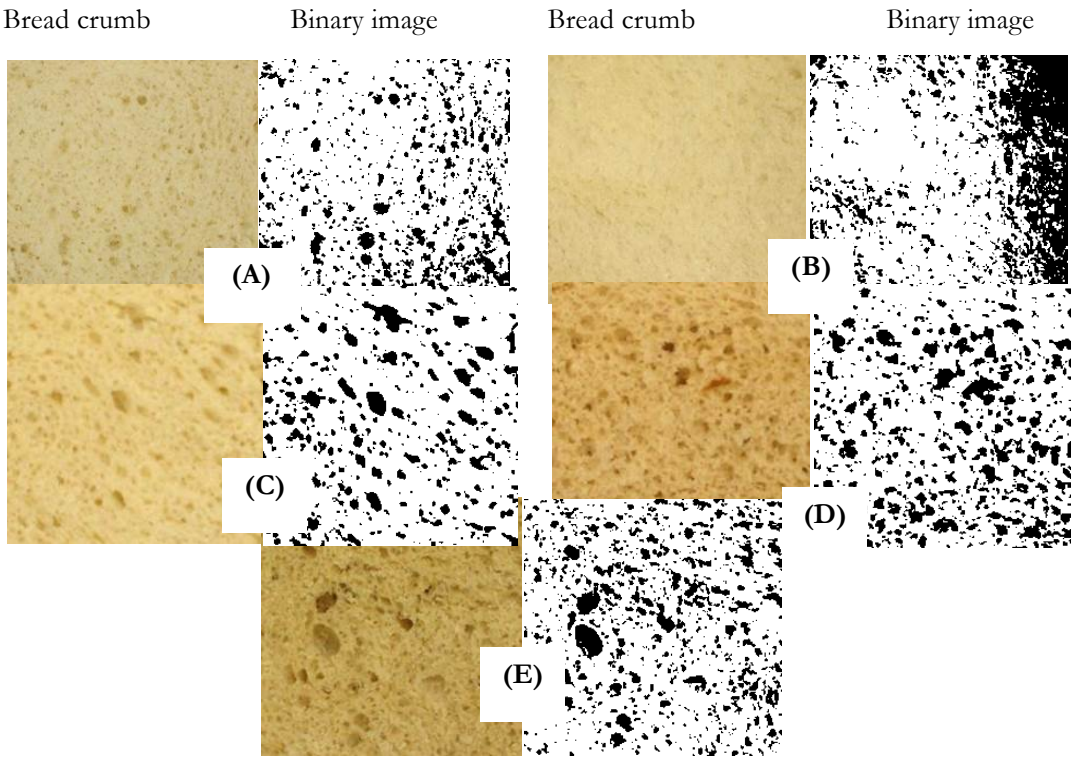


Figure 1 Image of crumb of bread samples and binary image from ImageJ
A) Control; B) Brand A; C) BRS 5%; D) BRS 10%; E) BRS 15%

CONCLUSIONS

In conclusion, the BRS added during the bread formulation did gave several impacts on the physical and chemical characteristics of bread. The moisture content of the control bread was the highest compared to the moisture content of all other bread samples. Both the crumb and crust parts of the bread samples showed a decrease in the lightness of bread due to the presence of BRS and the occurrence of the Maillard reaction. Processing conditions such as baking would affect and destroy natural starch such as BRS and was approved when the addition of resistant starch concentration did not cause an increase in the fibre content. The obtained results are useful for the bakery and food industries and also for food product development when the functional ingredients were used in products.

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