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Nutritional Value and Physicochemical Properties of White Bread Incorporated With *Hevea Brasiliensis* (Rubber Seed) Flour

Nutritional Value and Physicochemical Properties of White Bread Incorporated with *Hevea brasiliensis* (Rubber Seed) Flour

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ABSTRACT

This study was conducted to determine the nutritional value and physicochemical properties of white bread incorporated with rubber seed (*Hevea brasiliensis*) flour (RSF). Rubber seeds were dehulled, oven dried, ground and sieved to produce RSF. The formulation of the bread included two controls, namely, C1 (100% wheat flour) and C2 (100% RSF), and bread incorporated with 25% RSF (RSF25), 50% RSF (RSF50) and 75% RSF (RSF75). The proximate compositions of wheat and RSF in the composite flour and bread samples were determined using standard procedures. The physical characteristics (specific volume, water activity, colour analysis of the crust and crumb, texture analysis and pore size) of the controls and supplemented breads were assessed. Increasing the amount of RSF from 25% to 100% significantly increased ($p < 0.05$) the ash, protein, fibre and fat contents of the bread samples and significantly decreased ($p < 0.05$) their carbohydrate and moisture contents. The specific volume increased and the water activity decreased as the added amount of RSF increased. The preferred percentage range of the specific volume was 25%–50%. Colour analysis revealed that the colour of the crust and crumb of the bread darkened when the percentage of RSF supplemented in the formulation increased. Thus, (RSF25) showed the best acceptability based on the nutritional value and quality of the bread.

Keywords: rubber seed flour, composite flour, bread, proximate composition

ABSTRAK

Kajian ini dijalankan untuk menentukan nilai nutrisi dan ciri fiziko-kimia roti putih dengan penambahan tepung biji getah. Biji getah dibuang kulit, dikeringkan menggunakan oven, dikisar dan diayak untuk menghasilkan tepung getah (RSF). Formulasi roti bervariasi daripada dua kawalan, C1 (100% tepung gandum) dan C2 (100% tepung biji getah), tepung biji getah 25% (RSF25), tepung biji getah 50% (RSF50) tepung biji getah (RSF75). Komposisi tepung gandum dan tepung getah, tepung komposit dan sampel roti ditentukan menggunakan prosedur yang ditetapkan. Ciri-ciri fizikal (isipadu spesifik, aktiviti air, analisis warna untuk kulit dan isi roti, analisis tekstur dan saiz liang) ditentukan dalam sampel roti. Peningkatan pertambahan tepung biji getah dari 25% hingga 100% RSF menunjukkan peningkatan yang ketara ($p < 0.05$) dari segi kandungan abu, protein, serat dan lemak sementara terdapat pengurangan yang ketara ($p < 0.05$) dalam kandungan karbohidrat dan kandungan kelembapan. Ciri-ciri fizikal menunjukkan isipadu spesifik yang tinggi dan aktiviti air yang rendah apabila ditambah tepung getah tetapi untuk isipadu spesifik, peratusan yang baik adalah antara 25–50% RSF. Untuk analisis warna, warna kulit dan isi roti terbentuk apabila berlaku peningkatan peratusan RSF dalam formulasi. Oleh itu, disimpulkan bahawa roti yang ditambah tepung getah 25% (RSF25) memberikan roti dengan penerimaan yang terbaik berdasarkan nilai nutrisi dan kualiti roti.

Kata kunci: Tepung biji getah, tepung komposit, roti, nilai nutrisi, isipadu spesifik

INTRODUCTION

Despite the abundance of rubber (*Hevea brasiliensis*) seeds, they are generally left unused and disposed of. Nonetheless, rubber seeds have a number of applications, such as planting materials and oil for biodiesel and seed cake that can be used as animal feed (Okafor and Anyanwa, 2006; Achinewhu and Akpapunam, 1985; Chin et al., 1977). Rubber seeds are abundant in Malaysia. However, they are wasted in rubber plantations even though rubber seeds have potential for human consumption as a food ingredient. With economic importance, rubber seeds are also exploited in Malaysia primarily for latex. Although they are considered as a good protein source, they are disadvantageous because of the presence of cyanogenic glucoside, a toxic factor (Duke and Ducellier, 1993). They also contain linamarin, a cyanogenic glucoside. The hydrolysis or cyanogenesis of linamarin by the endogenous enzyme linamarase (α -glucosidase) forms glucose and acetonecyanohydrin that in turn decomposes into hydrogen cyanide and acetone (Idibie et al., 2007; Sornyotha et al., 2006). Nevertheless, linamarin can protect plants from herbivores, animals and insect feeders (Siritunga and Sayre, 2004), but can be overcome by soaking for 24 h, boiling or storage for at least 2 months (Narahari and Kothandaraman, 1983). The cyanide content is greatly reduced to a safe level by drying (Okafor et al., 2004). In some places, rubber seeds are

consumed without adverse side effects. For example, in Jerantut, Pahang, Malaysia, people consume rubber seeds in their daily dish known as 'Asam Rong'. Boiled and drained rubber seeds are eaten by Indians in the Amazon Valley in South America (Njwe *et al.*, 1988).

According to the Malaysian Rubber Board (2009), rubber trees start to produce seeds at the age of 4 years, but approximately 800 seeds (1.3 kg) produced twice a year are considered as waste. In 2007, rubber plantations covered 1,229,940 hectares of land (Malaysian Rubber Board, 2009). Eka *et al.* (2010) reported that an annual production of 1.2 million metric tons is regarded as waste and that this industry is the second-largest agricultural sector after oil palm in Malaysia.

According to the Grain and Feed Annual report (2016), Malaysia imported approximately 1.62 million tons of wheat flour to supply the consumer demand in 2016 and 2017. More than 1.45 million tons of wheat flour has been consumed by Malaysians, and the minimum consumption is approximately 2.31 kg per household every month (Halim, 2015). In 2014, the Malaysian Government subsidised RM 185 million for wheat flour (KPDNKK, 2014). Although the subsidy was removed in 2016, the government still shoulders subsidies of RM 1.35 for 1 kg of wheat flour for household consumptions. This arrangement has been a huge setback to food and beverage (F&B) businesses, restaurants and other food businesses because high costs definitely affect their revenues (News Straits Times, 2016). This study aimed to produce rubber seed flour (RSF) as an alternative to wheat flour to reduce the cost incurred by the government and F&B businesses. Considering that a rubber seed kernel contains 21.5% crude protein which is a possible source of gluten formation, particularly in baking and noodle production (Ravindran and Ravindran, 1988), we conducted this study to determine the physicochemical properties and nutritional value of RSF as a food ingredient.

MATERIALS AND METHODS

RSF preparation (milling process)

Seed material and flour production

The shells of the rubber seeds were removed and washed to ensure that they were free of any foreign materials. The rubber seeds were oven dried at 60 °C until the moisture content reached 7%, which is safe for storage (Nwabanne and Omoniyi, 2010), milled, sifted through a 250 μ m mesh sieve, packed in a plastic container and stored at room temperature prior to analysis.

Composite flour preparation: Wheat flour was supplemented with 25% RSF (RSF25), 50% RSF (RSF50) and 75% RSF (RSF75). In addition, 100% wheat flour and 100% RSF were used as the controls. Each treatment was mixed thoroughly to achieve uniformity of the blends.

Physicochemical properties of RSF

Physical Properties of RSF
Colour Measurement. The colour of RSF was examined to evaluate its whiteness, which determines the presence of bran particles. The colour of RSF was identified with a Konica Minolta CR-400 chromameter. Colour intensity was expressed as L*, a* and b*, where L* is the lightness from white (100) to black (0), a* is red (+a) or green (-a), and b* is yellow (+b) or blue (-b).

Proximate composition analysis

The proximate compositions (moisture, ash, protein, fat and fibre) of wheat flour, RSF, composite flour and bread samples were determined using standard procedures (AOAC, 2005). The carbohydrate content was determined in terms of its difference. Each formulation of flour and bread was analysed in triplicates.

Baking test

Bread-making process

The breads were produced in accordance with the method described by Maaruf *et al.* (2011). The obtained RSF was blended with wheat flour at 25:75, 50:50 and 75:25 levels of substitution for bread production. RSF and wheat flour at 100:0 and 0:100 levels were used as the controls. The bread recipe consisted of 100 g of each blend, sugar (5%), salt (2%), yeast (2%) and water (60%). The dry ingredients were thoroughly mixed. The

mixture was kneaded into a smooth pliable elastic-like dough, covered, allowed to ferment (45 min) and baked at 180 °C for 20 min. The baked products were cooled and packaged in polythene bags for further analysis.

Physicochemical properties of the breads

The physical characteristics, such as specific volume, water activity, colour, texture analysis and pore size, of the bread samples were evaluated.

Specific volume. The specific volume of the bread was obtained by dividing the loaf volume by its corresponding loaf weight (cm³/g) through rapeseed displacement method. Triplicate measurements were conducted before each sample was analysed.

Water activity. The water activities of the samples were analysed using an Aqua lab water activity meter model 4TE (USA). The samples were placed in a water activity meter, and data were obtained in triplicate.

Colour measurement. The colours of the crust and crumb were examined using a Konica Minolta spectrophotometer CR-400 (Japan). The colour of the crust was detected on the surface of the bread, whereas the crumb colour was examined at the centre of the bread after it was cut into halves. Colour intensity was expressed as L*, a* and b*, where L* is the lightness, a* is the red/green value, and b* is the yellow/blue value. The boundaries of the L* axis were L = 100 (white or total reflection) and L = 0 (black or total absorption). Along the a* axis, colour measurement movement in the -a direction depicts a shift towards green, and +a movement represents a shift towards red. Along the b* axis, -b movement denotes a shift towards blue, and +b shows a shift towards yellow. Three measurements were taken from each sample.

Pore size. The pore size of the bread was determined by preparing 4 cm × 4 cm of slices from the middle of the crumb. The images of the crumb were captured using a Canon EOS 60D and analysed on Image J System.

Texture analysis. The texture properties (hardness, gumminess, chewiness, cohesiveness, springiness and resilience) of the bread crumb were measured using a single-arm texture analyser (Model TA.XT Plus, Stable Microsystem, Surrey, UK). All of the samples were prepared and baked on the day of the test. A cube crumb sample with dimensions of 2.0 cm × 2.0 cm × 2.0 cm was sliced from the middle of the bread by using a bread knife and placed centrally beneath the cylinder probe to achieve a consistent flat surface at all times. Texture analysis was performed through a compression test by using a 5 kg load cell, and the sample was compressed to 45% of its original height. The strain required for a 45% compression was recorded under the following conditions: pretest speed, 1.0 mm/s; test speed, 1.7 mm/s; posttest speed, 10 mm/s; compression distance, 25%; and trigger type, auto 5 g. Data were analysed using Texture Expert Version 1.05 (Stable Micro System Ltd, Surrey, UK). All of the tests were conducted in triplicates, and the average values were reported.

Statistical analysis

Data were analysed using SPSS 20 General Linear Procedure (SPSS Inc., USA). The calculated means were compared through one-way ANOVA and Duncan's multiple range tests at a significance level of p<0.05.

RESULTS AND DISCUSSION

Proximate composition of flour

Table 1 presents the proximate compositions of the composite flours and the controls (C1 and C2). The composite flour and controls had moisture, ash, protein, fat, fibre and total carbohydrate contents in the range as tabulated. The means of the ash, protein, fat and fibre contents increased as the percentage of RSF increased, whereas the means of the moisture and carbohydrate contents did not increase. Rahman et al. (1999) reported that the differences in the samples were due to seed maturation, environments and variety sources.

Our results presented significant differences (p<0.05) amongst the samples in terms of moisture content. C1 (wheat flour) displayed the significantly highest moisture content (12.11%). The difference in the values was due to the nature of the seeds. Our results were consistent with the findings of Makinde and Akinoso (2014),

who demonstrated that the moisture content of wheat flour (4.83%) is slightly higher than that of RSF described by Eka et al. (2010) and Ukhun and Uwatse, (1988) (3.99% and 3.0%, respectively). The drying rate is another factor affecting the moisture content of composite flour. During flour processing, rubber seed was dried overnight at 60 °C to induce water evaporation and dry the seeds. Abduh et al. (2016) agreed that other factors that influence moisture content are the storage time and relative humidity of rubber seeds.

Table 1 Proximate composition of composite flour

Composition (%)	Moisture	Ash	Protein	Fat	Fiber	Carbohydrate
C1	12.11±0.95 ^a	0.56±0.05 ^d	13.18±1.57 ^d	1.11±0.55 ^e	0.87±0.37 ^e	73.04±1.19 ^a
C2	3.60±0.59 ^e	3.29±0.10 ^a	22.51±1.1 ^a	50.91±6.6 ^a	14.40±2.9 ^a	19.69±5.6 ^e
RSF25	11.17±0.8 ^b	1.30±0.09 ^c	14.17±1.4 ^d	14.01±1.6 ^d	3.85±1.57 ^d	59.34±2.9 ^b
RSF50	8.59±0.49 ^c	2.25±0.63 ^b	16.82±1.2 ^c	26.12±0.7 ^c	7.14±1.83 ^c	46.22±2.48 ^c
RSF75	6.11±0.71 ^d	2.60±0.74 ^b	19.17±2.4 ^b	36.94±1.8 ^b	11.04±2.6 ^b	35.18±3.4 ^d

a-e* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

The ash content of C1 significantly differed ($p < 0.05$) from those of RSF25, RSF50, RSF75 and C2. RSF50 and RSF75 did not significantly vary from the other samples. C2 had the highest ash content (3.29%), whereas C1 had the lowest (0.56%). Eka et al. (2010) indicated that rubber seeds have an ash content of 3.08 g/100 g. Oyekunle and Omode (2008) found that a rubber seed meal contains approximately 3.5%–5.0% of ash, and ash content is associated with mineral content. This result indicated that the mineral content of RSF was higher than that of wheat flour. Our findings agreed well with those of Eka et al. (2010), who showed that RSF contains 0.85 mg/g Ca, 0.01 mg/g Fe and 9.29 mg/g Mg.

Protein content is a key parameter of composite flour. Flours containing high protein contents can produce high-quality breads in terms of specific volume, texture and pore size. In Table 2, the protein content of RSF50, RSF75 and C2 significantly differed, whereas the protein content of RSF25 was not significantly different from that of C1. C2 presented the highest mean protein content (22.51%), whereas C1 (13.18%) and RSF25 (14.17%) exhibited the lowest values. A high-protein flour typically contains 12%–15% of protein content, and bread flour is formulated to enhance gluten elasticity. Eka et al. (2010) demonstrated that rubber seed contains a high amount of glutamic acid (16.13%) and a low amount of cysteine (0.78%) and that these essential amino acids are crucial in human life because our body is unable to produce these amino acids. The dietary requirement for protein is the minimum intake which satisfies metabolic demands and maintains the appropriate body composition and growth rates, considering any inefficiency of digestion and metabolic consumption (Eka et al., 2010). According to FAO/WHO (1991), a sufficient amount of dietary protein must be supplied to meet the metabolic demand. Therefore, RSF25 might be a good protein source for producing high-quality bread in terms of texture and volume because of the added protein content to the flour compared with that of C1. Such formulation could be applied to food products other than bread.

The results showed that the fat contents of RSF25, RSF50 and RSSF75 significantly differed from those of C1 and C2. C2 had the highest fat content C2 (22.51%), whereas C1 had the lowest (1.11%). In painting and varnishing, rubber seed oil is used as an alternative to linseed oil (Eka et al., 2010). The content of fat, particularly from unsaturated fatty acid, in rubber seeds plays a vital role in human health. Mohd-Setapar (2013) reported that the percentage of alpha-linolenic acid (ALA) of rubber seed oil (35.48%) is higher than those of soybean oil (8.18%) and olive oil (0.59%). Rubber seed oil also has a considerable amount of oleic acid (22.95%). Rubber seed oil contains a high content of ALA which is an omega-3 fatty acid; as such, it is recommended for daily consumption (Aigbodion and Bakare, 2005). Therefore, rubber seed oil shows great potential for the development of different products and applications in numerous industries, such as food and pharmaceutical industries.

Fibre cannot be digested, and an adequate intake will positively affect the human digestive system. The samples presented significant differences in fibre content ($p < 0.05$; Table 1). C2 had the highest fibre content (14.4%), whereas C1 had the lowest (0.87%). This result was consistent with the reported crude fibre content in rubber seed by Udo et al. (2016), Njwe et al. (1988) and Sovanno (2002) (5.88%, 4.0% and 4.7%, respectively). By contrast, an increased supplementation of RSF decreased the carbohydrate content in RSF. The samples significantly varied ($p < 0.05$), and C1 presented the highest carbohydrate content (73.4%).

Colour analysis of RSF

Colour is an important factor in determining flour quality. Miskelly (1984) indicated that flour colour is affected by various factors, such as milled kernel type and flour yield, and by the flour composition, including the ash, protein, pigment and damaged starch contents. The dominant colour can reflect the natural pigments of carotenoids or xanthophylls (Humphries et al., 2004) and the protein molecular composition (Ohm et al., 2008). L^* of RSF25, RSF50 and RSF75 significantly differed from those of C1 and C2. An increased percentage of rubber seeds decreased L^* because of the nature of rubber seeds which become brown after they are blended. This result was confirmed by the finding that C1 had the highest L^* (95.73), whereas C2 had the lowest (62.69; Table 2). The same trends were observed in a^* (redness) and b^* (yellowness). a^* of RSF25, RSF50 and RSF75 significantly differed from that of C1 and C2. C1 displayed the lowest colour (\square 0.54), whereas C2 showed the highest colour (4.97) because wheat flour undergoes several processing procedures, such as milling and bleaching, causing it to appear white. In general, the colour of flour affects the colour of the finished product, and bright white flour is desirable for many products.

Table 2 Colour of rubber seed flour with different percentage of RSF

Composite flour	L^*	a^*	b^*
C1	95.73±0.50 ^a	-0.54±0.10 ^e	1.60±1.13 ^e
C2	62.69±0.56 ^e	4.97± 0.58 ^a	29.63±0.32 ^a
RSF25	87.84±0.79 ^b	0.49±0.17 ^d	13.63±0.95 ^d
RSF50	78.64±0.78 ^c	2.00±0.14 ^c	19.83±0.20 ^c
RSF75	72.62±0.69 ^d	3.51±0.21 ^b	23.39±3.06 ^b

a-e* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

Proximate composition of the breads

Table 3 presents the proximate compositions of the composite flours and the controls. Moisture content is as an important indicator of shelf life upon subsequent storage. The results revealed that RSF25, RSF50 and RSF75 significantly differed ($p < 0.05$) from C1 and C2 in terms of moisture content. Conversely, RSF50 was not significantly different from RSF75 and RSF25. C1 presented the highest mean value (32.65%), and C2 had the lowest (14.49%). The moisture contents of the flour and the bread were correlated (Table 1). A decreasing trend in moisture content resulted in an increasing percentage of RSF because of the ratio of the cooperation between wheat flour and RSF in the formulation. A low moisture content of the bread could also be due to the low moisture content of the composite flour. Thus, its shelf life can be prolonged compared with that of conventional bread stored under the same conditions by controlling the microbial and enzymatic activities that cause the deterioration of bread.

Ash content is an indicator of inorganic compound contents in a sample (Oyekunle and Omode, 2008). In our study, the ash contents of RSF25, RSF50 and RSF75 significantly differed from those of C1 and C2. By contrast, RSF50 did not significantly vary from RSF75 and RSF25. In Table 4, RSF contained high amounts of nutrients which might be applied to food production. The ash content of the bread progressively increased as the percentage of RSF increased because raw rubber seed has a high ash content. A small amount of minerals is vital for the proper function of the human body (Eka et al., 2010). As such, bread incorporated with high ash contents could help improve health conditions.

Table 3 Proximate analysis of bread incorporated with rubber seed flour

Bread Samples	Moisture	Ash	Protein	Fat	Fiber	Carbohydrate
C1	32.65±1.06 ^a	1.63±0.23 ^d	9.97±0.23 ^d	0.09±0.07 ^e	0.19±0.07 ^d	55.67±0.98 ^a
C2	14.49±0.22 ^d	3.94±0.22 ^a	20.69±1.27 ^a	36.02±1.36 ^a	19.12±3.73 ^a	24.85±1.86 ^e
RSF25	23.17±1.79 ^b	2.90±0.40 ^c	13.28±0.67 ^c	14.32±3.91 ^d	5.48±0.65 ^c	46.34±5.04 ^b
RSF50	21.65±3.21 ^{bc}	3.06±0.31 ^{bc}	14.67±0.52 ^c	18.28±1.30 ^c	6.68±0.46 ^c	42.34±2.58 ^c
RSF75	19.03±3.24 ^c	3.38±0.28 ^b	17.65±2.15 ^b	25.78±2.07 ^b	12.68±3.27 ^b	34.16±3.83 ^d

a-d* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

Protein is an essential macronutrient for muscle mass building. Our results revealed that the protein contents of RSF25, RSF50 and RSF75 significantly differed from those of C1 and C2. The increase in protein content resulted in an increased percentage of RSF, although no significant difference was detected between RSF50 and RSF25. Amongst the samples, C1 had the lowest protein content (9.97%), and C2 had the highest protein content (20.69%) because the protein composition in wheat flour was lower than that in RSF. Udo *et al.* (2016) reported that raw rubber seed contains 23.31% of crude protein. The protein contents in the bread were slightly lower in all of the samples than in the composite flour because of the mixing of other ingredients and the various processes, such as proofing, baking and cooling, that bread experienced. These processes also caused enzyme breakdown and protein denaturation. The increased percentage of the protein content was due to the increased percentage of RSF. Zeleny (1971) suggested that a minimum of 11% of protein is needed in the production of yeast-leavened bread. Protein is also necessary to produce gluten and prepare elastic dough which extends and traps air (Zeleny, 1971).

In bread making, fat not only provides flavour but also produces soft texture in bread. Our results revealed that the fat contents of RSF25, RSF50 and RSF75 significantly differed ($p < 0.05$) from those of C1 and C2 (Table 3). C2 presented the highest mean fat content (36.02%), whereas C1 had the lowest mean fat content (0.09%). The increased levels of RSF incorporated in the bread increased the fat content because of the reduced amount of wheat flour in the formulation. Aigbodion and Bakare (2005) reported that rubber seed oil contains oleic acid (22.95%), linoleic acid (37.28%) and linolenic acid (19.22%) which is also known as omega-3. Rubber seed oil is also composed of omega-3 fatty acids which are beneficial to human health (Aigbodion and Bakare 2005). Omega-3 can be consumed from fish oil and vegetable oil, and many people are unaware that rubber seed oil is also a source of omega-3. Babatunde and Pond (1987) stated that rubber seed oil can be used for food and industrial production because of its high essential nutrients. Hence, bread supplemented with RSF can be considered a source of high essential nutrients.

Fibre intake has increased since the food industry promoted the advantages of including fibre in food products (Redgwell and Fischer 2005). Fibre is a roughage derived from plant cell walls. Fibre is composed of soluble and insoluble dietary fibre. Redgwell and Fischer (2005) reported that the primary sources of fibre include polysaccharides, oligosaccharides, lignin, cellulose and its derivatives, fruits, vegetables, oil seed fractions (defatted meals and hulls) and fractions of cereal grains. The fibre contents of RSF25, RSF50 and RSF75 significantly differed from those of C1 and C2. C2 had the highest mean fibre content (19.12%), whereas C1 had the lowest fibre content (0.19%). The crude fibre and ash contents of the bread samples increased as the level of supplementation with RSF increased. Bread supplemented with RSF is good for human diet and offers the health benefits associated with fibre consumption because bread supplemented with RSF has a high fibre content. Dietary fibre intake enhances beneficial physiological effects, such as maintaining health and protection from diseases, such as constipation, blood cholesterol attenuation and blood glucose attenuation (Boyer and Liu, 2004; Redgwell and Fischer, 2005).

In Table 3, the carbohydrate contents of RSF25, RSF50 and RSF75 significantly differed ($p < 0.05$) from those of C1 and C2. C1 had the highest carbohydrate content (55.67%), whereas C2 had the lowest carbohydrate content (24.85%). Supplementing wheat flour with a high percentage of RSF reduced the carbohydrate content of the bread samples. Wheat flour contributed the highest carbohydrate content (73.04%) to the bread samples, and this value was greater than that reported by Makinde and Akinoso (2014) (53.90%). The high carbohydrate content in bread is due to the high carbohydrate content in flour. Carbohydrate is important in diet. Kent (1975) indicated that bread is rich in carbohydrates (45%–58%). In our study, the increased supplementation of wheat flour with RSF significantly affected the chemical quality of the composite bread, suggesting that wheat flour was the main contributor of the carbohydrate content in bread.

Physicochemical properties of the bread samples

The physical characteristics of the bread samples incorporated with RSF were assessed to determine the quality of the bread. Triplicate samples were analysed in terms of specific volume, water activity, colour analysis, pore size and texture. The physical characteristics of the bread samples containing different percentages of RSF supplementation compared with those of the controls are provided in Tables 4 and 5 and Figures 1 and 2.

Effect of RSF on the specific volume of the bread

Dough is created through flour hydration and structural changes caused by mixing (Belton, 2005). The stability of gas cells which are essential for the volume of bread during proofing is influenced by the composition and mechanical properties of the layer at the air–viscoelastic matrix interface, particularly during dough processing (Belton, 2005).

Table 4 Specific volume, pore size and water activity of bread supplemented with different percentage rubber seed flour

Bread Samples	Specific volume (cm ³ /g)	Pore size		Water activity
		Cell number	Average size (mm ²)	
C1	2.93±0.04 ^b	451	5.20	0.84±0.04 ^a
C2	1.73±0.30 ^c	50	66.37	0.75±0.04 ^b
RSF25	3.43±0.43 ^a	357	24.27	0.80±0.03 ^a
RSF50	2.80±0.52 ^b	244	50.70	0.85±0.04 ^a
RSF75	2.00±0.34 ^c	137	33.32	0.82±0.05 ^a

a-c* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

During proofing, the stability of gas cells is essential for the specific volume of bread (Shehzad et al., 2010). In the current study, the specific volume of the bread samples ranged from 1.73 cm³/g to 3.43 cm³/g. The specific volumes of RSF25, RSF75 and C2 significantly differed from that of C1, whereas no significant difference was observed between RSF50 and C1. The highest specific volume of 3.43 cm³/g was found in RSF25. This parameter decreased as the percentage of the added RSF increased except RSF25. Excessive RSF in the formulation can shorten the network of gluten because of the high fat content. As such, this phenomenon prevents the development of gluten and eventually affects the specific volume of bread. The protein content of RSF25 with gluten in wheat flour compared with that of C2 without wheat flour supplemented in the formulation caused an increase in specific volume. Moreover, the protein contents of RSF50 and C1 were the

same. The increased specific volume of RSF25 was also due to the retention of more carbon dioxide in the dough, which provided the high volume of the bread. The gluten fraction is responsible for the dough elasticity by causing it to extend and trap carbon dioxide generated by yeast during fermentation. Gluten coagulates under the influence of heat during baking and thus serves as the framework of loaves, which become relatively rigid and do not collapse (Mongi *et al.*, 2011). By contrast, an excessive amount of RSF in composite flour formulation resulted in a decrease in specific volume. The high protein content of rubber seeds can be applied to other food products. Therefore, RSF supplementation in bread making should be limited to 25%–50% to produce acceptable bread with specific volume characteristics that are comparable with those of 100% wheat bread.

Effect of rubber seed flour on the pore size of the bread

Pores are air cells found in bread leavened by yeast in which carbon dioxide from fermentation creates a void structure. Cell structures vary because of differences in dough mixing and yeast action during fermentation (Shehzad *et al.*, 2010). During fermentation, dough expands as a result of yeast's action which is responsible for the increased porosity and altered stability of alveolar structures (Belton, 2005). In our study, the number of cells increased as the percentage of RSF decreased. C1 recorded the highest number of cells ($n = 451$), whereas C2 yielded the lowest number of cells ($n = 50$) (Table 4). The number of cells in the bread was affected by the mixing process, and the fermentation process generated cells with different sizes. In addition to the low cell count in C2, the high fat content in RSF, which shortened the gluten development and ultimately reduced the air trapped inside the pores, was accounted for this phenomenon.

Effect of rubber seed flour on the water activity of the bread

Water activity can be defined as activity of water that is not bound to food molecules and supports the growth of bacteria and mould (Frazier and Westhoff, 1978). Water activity determines the shelf life of food products. The lower the water activity was, the longer the shelf life of the bread would be. In Table 4, C1 and C2 significantly differed in terms of water activity, whereas RSF25, RSF50 and RSF75 were not significantly different from C1. C2 exhibited the lowest water activity (0.75) possibly because of the high mineral content in RSF that could bind more water than wheat flour could do. In most foods, water activity is more than 0.95, thereby providing sufficient moisture to support the growth of bacteria, yeasts and moulds. The amount of available moisture can be reduced to a point at which the growth of microorganisms is inhibited (Lazaridou *et al.*, 2007). Lazaridou *et al.* (2007) stated that water activity is an important factor in determining the firmness (hardness) of bread crumbs during storage. A low water activity is associated with an increase in the firmness of breads (Ho *et al.*, 2013). Miyazaki *et al.* (2004) also revealed that differences in the water activity of bread crumbs during storage are minimal, implying that retrogradation is retarded, and bread staling occurs slowly.

Effect of RSF on the colour of bread

Colour is an important criterion for the initial acceptability of baked products by consumers (Makine and Akinoso, 2014). Zanoni *et al.* (1995) reported that colour develops at the later stages of baking and can be used to assess the completion of baking. Surface colour depends on the physicochemical characteristics of raw dough (water content, pH, reducing sugars and amino acid content) and operating conditions applied during baking (Zanoni *et al.*, 1995). The colours of the crust and crumb, which were measured by L^* , a^* and b^* , are summarised in Figures 1 and 2. L^* values represented the lightness of the bread by levels 0–100. The redness of bread was denoted by a^* . b^* indicated the yellowness of the bread. The L^* value of bread crust showed that RSF50, RSF75 and C2 significantly differed from C1, whereas RSF25 was not significantly different from C1. RSF50 and RSF75 also showed no significant difference. C1 had the highest L^* reading (57.76), whereas C2 had the lowest (31.23), indicating that C2 was darker than C1. Logically, the dark colour of the rubber seed would be reflected by the dark colour at the end of baking. No correlation was found between the bread colour and the flour colour, as stated in Table 3. The colour darkened as the RSF level increased because of the Maillard browning reaction caused by the reaction amongst wheat proteins, the added sugar and caramelisation, all of which were influenced by the distribution of water and the reaction between the added sugars and amino acids (Kent and Evers, 1994). The results revealed the same trend of L^* of the colour of the bread crumbs.

In terms of a^* of crust colour, RSF25, RSF50, RSF75 and C2 were not significantly different from C1. a^* of the crust was higher than that of the crumb. During baking, the crusts of the products firstly absorbed the heat which in turn passed through the interior of the bread through conduction and radiation. Temperature during baking also contributed to the crust colour with a higher a^* than that of the crumb.

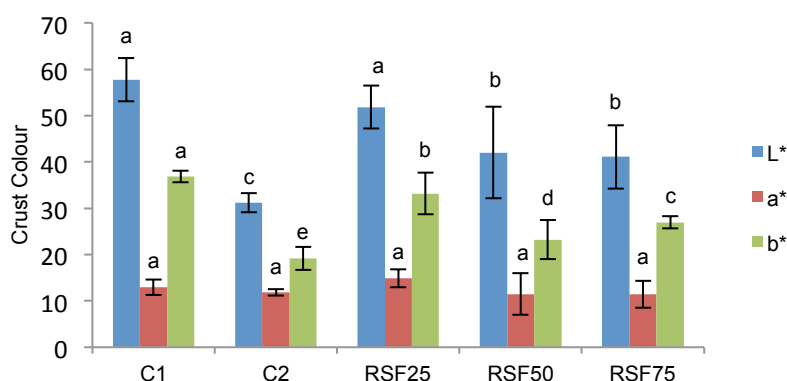


Figure 1 Crust colour of bread supplemented with composite flour

a-e* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

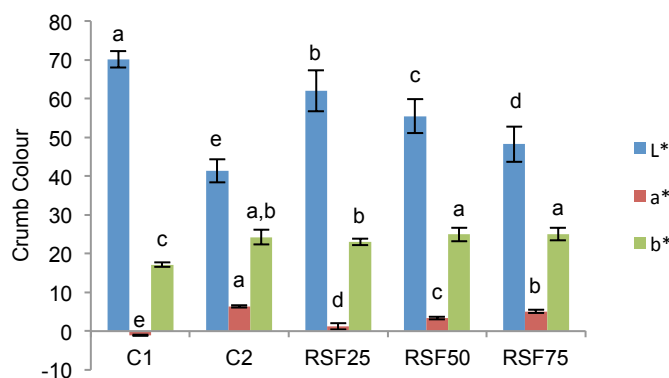


Figure 2 Crumb colour of bread supplemented with composite flour

a-e* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

Moreover, the crust became hotter as water on the surface of the bread dough evaporated due to exposure to high temperatures. For the crumb, RSF25, RSF50, RSF75 and C2 were not significantly different from C1 in terms of a*. C1 had the lowest mean value (-1.07), and C2 had the highest a*(6.38), implying that the colour of RSF bread crumbs was reddish. The colour of rubber seed was originally brown, resulting in the brown colour of the crumb after baking. C1 showed such value because of the presence of a white pigment and the absence of red pigment in the crumb. L* was higher in the crumb than in the crust, whereas a* (redness) and b* (yellowness) in the crust were higher than those in the crumb, demonstrating the browning effect consistent with the values reported by Makinde and Akinoso (2014).

Effect of RSF on bread texture

Bread texture is one of the important characteristics evaluated by customers and one of the factors affecting product acceptability by consumers. Hardness is the force required to compress a material by a given amount and defined as the peak force during the first compression cycle (Malcom et al., 1981). In our study, C2 and RSF75 significantly differed from C1, whereas RSF25 and RSF50 were not significantly different from C1 (Table 5). RSF50 was not significantly different from all of the samples. C2 and RSF75 were the hardest amongst all of the samples because the high fat content in RSF might interfere with the formation of gluten by coating the

protein in flour that is responsible for forming gluten. Thus, the ability to retain fermented gas during the rising of dough decreases because fat shortens the gluten network and hardens bread. Lee *et al.* (2008) indicated that the hardness of bread may also be influenced by moisture distribution during storage. In terms of gumminess, RSF25, RSF50 and RSF75 significantly differed from C1 and C2, whereas C1 and C2 showed no significant difference from each other. Springiness refers to the height that the food recovers during the time that elapses between the end of one compression cycle and the start of the second compression cycle. Resilience is the ability of a product to regain its original height (Serphil and Servet, 2006). In terms of springiness and resilience, RSF25, RSF50 and RSF75 did not significantly differ from C1 and C2. Chewiness is the energy required to chew a solid sample to a steady state of swallowing and obtained on the basis of product hardness, cohesiveness and springiness (Bourne, 2002). Cohesiveness is defined as the ability of food products to withstand deformation or compression between the teeth before the food structure breaks (AACC, 2000). In our study, RSF75 and C2 significantly differed ($p < 0.05$) from C2, whereas RSF25 and RSF50 had no significant difference ($p > 0.05$) from C1. The increased percentage of RSF in the formulation caused an increase in cohesiveness.

Table 5 Texture properties of bread samples

Bread Samples	Hardness	Gumminess	Chewiness	Springiness	Cohesiveness	Resilience
C1	472.40±5.07 ^b	346.73±24.89 ^c	287.13±30.75 ^c	0.83±0.03 ^a	0.75±0.03 ^a	0.47±0.02 ^a
C2	1575.59±785.66 ^a	347.51±48.20 ^c	229.31±38.94 ^c	0.66±0.02 ^a	0.41±0.05 ^c	0.32±0.17 ^a
RSF25	234.96±1.96 ^b	175.41±5.42 ^d	167.07±16.31 ^c	0.95±0.06 ^a	0.75±0.03 ^a	0.34±0.00 ^a
RSF50	1104.42±44.84 ^{ab}	792.50±3.59 ^b	676.61±18.86 ^b	0.64±0.29 ^a	0.68±0.05 ^{ab}	0.36±0.02 ^a
RSF75	1930.52±29.06 ^a	1181.64±68.37 ^a	951.09±124.82 ^a	0.80±0.06 ^a	0.61±0.04 ^b	0.29±0.05 ^a

a-d* Mean values with the same letter are not significantly different ($p < 0.05$) between the bread supplemented with different percentage of rubber seed flour

CONCLUSION

Composite bread samples with various RSF supplementation levels were nutritionally superior (higher protein, fat and crude fibre) to white bread. A high percentage of the incorporated RSF in the formulation resulted in low moisture and carbohydrate contents in the bread. The supplementation of wheat flour with RSF also significantly affected the specific volume, water activity, pore size, colour and texture of the freshly baked bread. Supplementation of 25% RSF into wheat flour provided the bread with the most preferable formulation based on the nutritional value and quality of the bread in terms of volume, texture, water activity and pore size. Therefore, RSF has a high potential for food applications and may be used in noodles, spaghetti and pasta. RSF also has a high fat content. As such, further investigation should be conducted to determine the fat composition in the bread. A shelf life study should also be performed to predict the period when the bread supplemented with RSF is safe to consume and deemed acceptable by consumers.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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