

Effect on Physicochemical and Sensory Attributes of Bread Substituted with Different Levels of Matured Soursop (*Anona muricata*) Flour

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Abstract—Soursop (*Anona muricata*) is one of the underutilized tropical fruits containing nutrients, particularly dietary fibre and antioxidant properties that are beneficial to human health. This objective of this study is to investigate the feasibility of matured soursop pulp flour (SPF) to be substituted with high-protein wheat flour in bread. Bread formulation was substituted with different levels of SPF (0%, 5%, 10% and 15%). The effect on physicochemical properties and sensory attributes were evaluated. Higher substitution level of SPF resulted in significantly higher ($p < 0.05$) fibre, protein and ash content, while fat and carbohydrate content reduced significantly ($p < 0.05$). FESEM showed that the bread crumb surface of control and 5% SPF appeared to distribute evenly and coalesced by thin gluten film. However, higher SPF substitution level in bread formulation exhibited a deleterious effect by formation of discontinuous gluten network. For texture profile analysis, 5% SPF bread resulted in the lowest value of hardness. The score of sensory evaluation showed that 5% SPF bread received good acceptability and is comparable with control bread.

Keywords—Bread, Physicochemical properties, Scanning electron microscopy (SEM), Sensory attributes, Soursop pulp flour.

I. INTRODUCTION

SOURSOP pulp (*Anona muricata L.*) is a well-known tropical fruit that is distributed in Southeast Asia, Africa and Americas. It is one of the exotic fruit that offer very-pleasant, aromatic and slightly-acidic juicy flesh. Soursop pulp is rich with carbohydrate, proteins, minerals, dietary fibre and bioactive compounds [1].

Dietary fibre demonstrated beneficial effects in protection against heart disease and cancer, normalization of blood lipids, regulation of glucose absorption and insulin secretion and prevention of constipation and diverticular disease [2]. The polyphenolic compound in soursop has been reported to prevent diseases associated with oxidative stress, such as cancer, arteriosclerosis and neurodegenerative diseases [3].

The pulp is commonly consumed as fresh fruits, or widely used for the manufacturing of powders, fruit bars, fruit juice blends, nectars, syrups, preserves and ice-cream [4]. However, soursop is considered as underutilized fruit as it is being rejected in the market due to its external injury during post-

harvesting or uneven shape and as it softens very rapidly during ripening and becomes mushy [5].

Wheat flour is being used widely in food products such as bread, cookies, noodles and cakes. However, utilization of white wheat flour in food products containing low nutritional value due to milling processing. Thus, the use of whole wheat flour in food products is increasing due to its health benefits including reducing the risk of coronary heart diseases and certain type of cancers as it provides higher dietary fibre, minerals, vitamins and antioxidant properties [6].

Bread products are well accepted worldwide due to its nutritional content and sensory attributes among cereal and cereal products. Generally, bread is rich with carbohydrate, protein, minerals e.g. calcium and iron, and vitamins e.g. B-complex vitamins [7]. The main ingredients of bread products are high protein wheat flour, yeast or other leavening agent and salt. Optional ingredients are being added to improve the processing method or to produce specialty and novelty breads which often containing an increased nutritional value. Bread products are the best matrix to deliver various functional components for the consumer at an acceptable level. Various researches have been conducted to enhance the nutritional content of bread such as dietary fibre [8], [9], plant sterol esters [9], omega-3 fatty acids [10] and antioxidants [11].

The main objectives of this study is to develop novelty bread product with different substitution level of matured soursop pulp flour (0%, 5%, 10% and 15%) so as to enhance its nutritional content. The physicochemical and sensory attributes of bread substituted with different levels of soursop pulp flour were evaluated to determine its nutritional value and acceptability.

II. MATERIALS AND METHODS

Matured soursop was collected from a local farm in Kuala Pilah, Negeri Sembilan, Malaysia. The ingredients for bread were purchased from bakery store in Senawang, Negeri Sembilan, Malaysia.

A. Preparation of Matured Soursop Pulp Flour

Matured soursop (*Anona muricata*) fruits were manually peeled and washed with running potable water. The core and the seeds were removed, then the pulp was cut into small pieces and air-dried at 60°C until the moisture content was 4-5%. Dried soursop pulp was ground by using Waring blender to pass through US70 (212 µm dia.) and stored in an airtight opaque container at $\leq 5^\circ\text{C}$ prior to use.

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B. Preparation of Bread Samples

Bread samples were prepared by slight modification of the Sponge and Dough Method procedure in AACC (2000). Matured soursop pulp flour (SPF) was substituted with wheat flour at four different levels (0%, 5%, 10%, 15% w/w). All ingredients were mixed and baked at 165°C for 25 min. Bread samples were allowed to cool at ambient temperature for an hour prior to analyses.

C. Proximate Composition

Bread samples were analysed for moisture (Method 44-15A), crude protein ($N \times 6.25$) (Method 46-13), crude fibre (Method 32-10), crude fat (Method 30-25) and ash (Method 08-01) content according to AACC (2000) [12].

Carbohydrate content was determined by difference:
[100 – (moisture+crude protein+crude fibre+crude fat+ash)]

Calorie value of bread samples was calculated according to the following equation:

$$\text{Calorie value (kcal/100g)} = (\% \text{ crude protein} \times 4) + (\% \text{ carbohydrate} \times 4) + (\% \text{ fat} \times 9).$$

All analyses were performed in triplicates.

D. Physical Analysis

1. Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) was conducted by using TA.XT2 Plus Texture Analyzer Uni axial (Surrey, UK). Mode operation of 'Measure Force in Compression' with 5kg load cell was performed on 2 slices of 1.25cm thickness to with a 25mm cylindrical probe. The resulting force-deformation curve was analysed for hardness, springiness, gumminess and chewiness parameter.

The analysis was carried out in triplicates.

2. Colour Analysis

The crust and crumb colour of bread samples were measured in L^* , a^* and b^* by using Chromameter CR-100 (Konica Minolta, Japan). The L^* , a^* and b^* were measured by placing the instrument near to the surface of bread samples.

Colour analysis was performed in triplicates.

3. Field Emission Scanning Electron Microscopy (FESEM)

The micro structural of starch granules in breadcrumb substituted with different levels of SPF were studied by using Field Emission Scanning Electron Microscopy (FESEM). The microstructure of the samples were viewed on a Leo Supra 50VP FESEM (Carl-Zeiss SMT, Oberkochen, Germany) at 5.00kV (accelerating voltage). FESEM images of each samples were taken at different magnifications and the appropriate magnifications was selected.

E. Sensory Analysis

Sensory evaluation of freshly prepared bread samples were evaluated by using 9-point hedonic scale. The score of different sensory attributes i.e. colour, taste, aroma, softness, appearance and overall acceptability was recorded by 30 trained panellists. Four differently coded samples were served to the panellists in temperature controlled (21°C) isolated sensory booths under artificial daylight.

F. Statistical Analysis

Statistical analysis of the result was analysed by using analysis of variance (ANOVA) and significant differences among means of triplicate analysis at ($p < 0.05$) was determined by Duncan's multiple range test.

III. RESULTS AND DISCUSSIONS

A. Proximate Analysis

Analyses of main constituents in bread samples substituted with different levels of matured soursop flour (SPF) are presented in Table I.

The result shows that with higher SPF substitution level, the moisture, protein, ash and crude fibre content of bread increased significantly ($p < 0.05$), while fat and carbohydrate content decreased significantly ($p < 0.05$). According to [13], addition of non-wheat flour increased the level of fibrous material which subsequently altered the continuous gluten matrix in wheat flour and modified the extent of water absorption during dough formation [14]. Water content played a significant role in the final quality of the bread as it affects the gelatinization of starch in bread [15].

Higher SPF substitution level in bread formulation resulted in significant increased ($p < 0.05$) of protein content (Table I). [1] reported that matured soursop pulp contains higher protein content of 7.34%, compared to ripen soursop (2.91%), which may contributed to the increased amount of protein content with higher SPF substitution level.

The edible part of soursop is considered as a good source of fibre, especially soluble pectin and cellulose [16]. Soursop contained minerals such as potassium, sodium, iron, magnesium, calcium, phosphorus and zinc [1].

Higher SPF substitution level in bread is resulted in increased of ash content which attributed by the minerals present in SPF (Table I). Study conducted by [17] reported similar result of increased amount of moisture, ash, protein and dietary fibre in bread formulated with 100% of unripe banana flour. The increased of SPF substitution level in bread subsequently decreased the carbohydrate and calorie value significantly ($p < 0.05$).

TABLE I
PROXIMATE COMPOSITION OF BREAD SUBSTITUTED WITH DIFFERENT LEVELS OF MATURED SOURSOP PULP FLOUR (SPF)

Composition (g/100g)	Control	5% SPF	10% SPF	15% SPF
Moisture	26.65±0.34 ^d	28.87±0.39 ^c	32.02±0.19 ^b	32.49±0.25 ^a
Fat	5.63±0.02 ^a	5.01±0.03 ^b	4.55±0.03 ^c	4.14±0.03 ^d
Protein	4.39±0.12 ^d	6.25±0.19 ^c	7.25±0.05 ^b	11.68±0.47 ^a
Ash	2.71±0.03 ^d	2.80±0.03 ^c	2.89±0.02 ^b	2.99±0.01 ^a
Crude fibre	10.55±0.39 ^c	11.76±0.07 ^b	12.47±0.40 ^{ab}	13.25±0.62 ^a
Carbohydrate	50.07±0.24 ^a	45.30±0.38 ^b	40.82±0.41 ^c	35.45±0.77 ^d
Calorie (kcal/100g)	268.53±1.22 ^a	251.33±1.22 ^b	233.26±1.39 ^c	225.75±1.65 ^d

SPF: Soursop Pulp Flour

Values are expressed as mean±s.d. *n*=3.

Means with different letters in the same row are significantly different (*p*<0.05).

TABLE II
TEXTURE PROFILE ANALYSIS (TPA) OF BREAD SUBSTITUTED WITH DIFFERENT LEVELS OF MATURED SOURSOP PULP FLOUR (SPF)

Textural characteristics	Control	5% SPF	10% SPF	15% SPF
Hardness (g)	446.07±15.79 ^c	305±18±85.97 ^d	629.31±61.22 ^b	888.76±19.81 ^a
Springiness (mm)	0.95±0.01 ^a	0.93±0.01 ^a	0.93±0.01 ^a	0.76±0.02 ^b
Gumminess (g)	331.42±41.45 ^b	253.10±67.55 ^b	370.15±36.71 ^b	656.25±12.69 ^a
Chewiness (g mm)	314.91±85.84 ^b	236.05±61.32 ^b	281.51±34.47 ^b	607.38±10.61 ^a

SPF: Soursop Pulp Flour

Values are expressed as mean±s.d. *n*=3.

Means with different letters in the same row are significantly different (*p*<0.05).

TABLE III
COLOUR ANALYSIS OF BREAD SUBSTITUTED WITH DIFFERENT LEVELS OF MATURED SOURSOP PULP FLOUR (SPF)

Parameter	Control	5% SPF	10% SPF	15% SPF
<i>Crust colour</i>				
Lightness (L*)	64.55±1.00 ^a	62.51±14.80 ^{ab}	60.02±0.76 ^{ab}	57.96±1.55 ^b
Redness (a*)	11.48±0.27 ^a	6.88±1.01 ^c	8.48±1.31 ^{bc}	10.10±0.44 ^{ab}
Yellowness (b*)	37.96±0.44 ^a	28.81±2.11 ^b	29.81±0.19 ^b	30.81±0.19 ^b
<i>Crumb colour</i>				
Lightness (L*)	68.59±0.52 ^a	62.73±1.04 ^b	56.62±1.40 ^c	55.06±0.57 ^c
Redness (a*)	-0.03±0.07 ^b	2.72±0.22 ^b	3.23±0.12 ^b	4.69±0.22 ^{ab}
Yellowness (b*)	18.16±0.19 ^a	17.94±0.60 ^a	17.01±0.67 ^b	18.73±0.33

SPF: Soursop Pulp Flour

Values are expressed as mean±s.d. *n*=3.

Means with different letters in the same row are significantly different (*p*<0.05).

TABLE IV
SENSORY EVALUATION OF BREAD SUBSTITUTED WITH DIFFERENT LEVELS OF MATURED SOURSOP PULP FLOUR (SPF)

Formulations	Appearance	Colour	Taste	Texture	Overall acceptability
Control	6.73±1.14 ^a	6.63±1.40 ^a	6.77±1.10 ^a	6.37±1.27 ^a	6.80±1.10 ^a
5% SPF	6.23±1.31 ^a	6.10±1.16 ^{ab}	6.47±1.31 ^a	6.20±1.24 ^{ab}	6.33±1.09 ^a
10% SPF	5.53±1.25 ^b	5.77±1.14 ^{bc}	5.40±1.81 ^b	5.57±1.50 ^{bc}	5.57±1.25 ^b
15% SPF	5.27±1.60 ^b	5.23±1.52	5.07±1.99 ^b	5.27±1.78 ^c	5.03±1.54 ^b

SPF: Soursop Pulp Flour

Values are expressed as mean±s.d. *n*=30.

Means with different letters in the same column are significantly different (*p*<0.05).

B. Texture Profile Analysis (TPA)

Texture profile analysis (TPA) of bread substituted with different levels of SPF is summarized in Table II. Substitution of SPF at 5% in bread formulation resulted in the lowest value of hardness parameter while the highest value of hardness was recorded in bread of 15% SPF. Meanwhile, the value of springiness of bread decreased significantly at 15% SPF substitution level. The result indicated that the bread of 15% SPF required more time to recover to its original shape. Gumminess and chewiness are the secondary parameter. The result showed that gumminess and chewiness increased significantly (*p*<0.05) with higher SPF substitution level in

bread formulation. However, there is no significant difference (*p*>0.05) of gumminess and chewiness in control and 5% SPF bread samples. Substitution of SPF in bread formulation increased the fibre content and dilutes the wheat gluten content, which subsequently weakened the strength of bread matrix [18].

C. Colour Analysis

Colour is an important characteristics in bread products in addition to texture and aroma due to its contribution to consumer preference. Table III shows the result of crust and crumb colour of bread substituted with different levels of SPF.

Bread substituted with higher SPF substitution level

resulted with darker crust and crumb colour (dark brown). Darker crust and crumb colour of bread with higher SPF substitution level are directly influenced by the colour of substituted flour. The a^* (yellowness) value of control bread was negative (-0.03), which indicated that red hues were not present in the crumb. The value of a^* in bread crumb significantly increased ($p < 0.05$) with higher SPF substitution level. Study conducted by [19] reported the a^* value of bread crumb changed from $-a^*$ to a^* values as the wheat flour was partially substituted with whole waxy wheat flour. The yellowness of crust resulted with significant decreased ($p < 0.05$) with higher SPF substitution level in bread formulation.

D. Field Emission Spectroscopy Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) technique was applied to study the microstructure of breadcrumb substituted with different levels of SPF. This technique allowed a qualitative description of the structural cell and their distribution. From the FESEM analysis, the surface of bread control and 5% SPF appeared to distribute evenly and coalesced by thin gluten film (Figs. 1 (a) and (b), Figs. 2 (a) and (b)). However, at higher SPF substitution level, the gluten membrane, structure that resulted from adhesion of small starch granules, exhibited a deleterious effect by formation of discontinuous gluten network (Figs. 3 (a) and (b), Figs. 4 (a) and (b)).

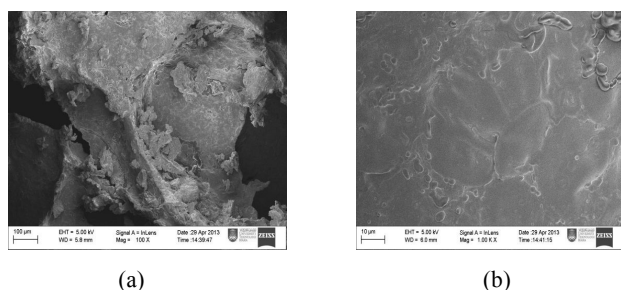


Fig. 1 (a) Control bread under 100x magnification; (b) Control bread under 1000x magnification

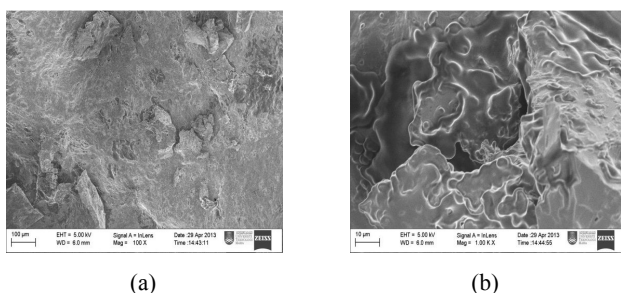


Fig. 2 (a) Bread (5% SPF) under 100x magnification; (b) Bread (5% SPF) under 1000x magnification

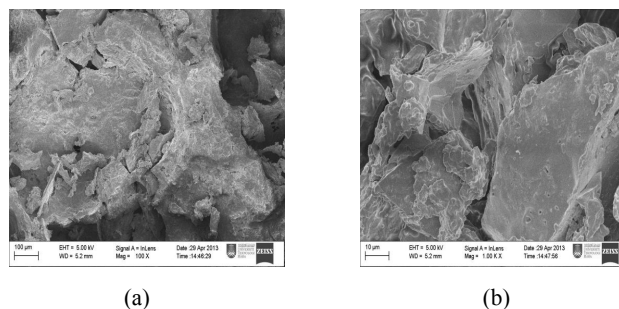


Fig. 3 (a) Bread (10% SPF) under 100x magnification; (b) Bread (10% SPF) under 1000x magnification

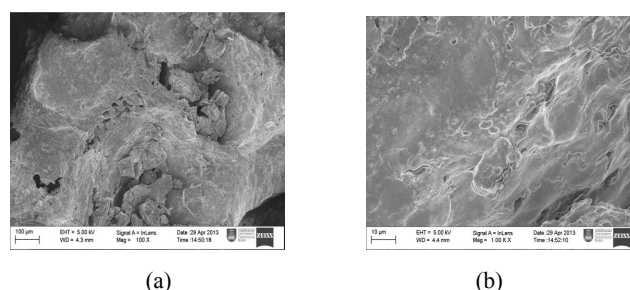


Fig. 4 (a) Bread (15% SPF) under 100 x magnifications; (b) Bread (15% SPF) under 1000x magnifications

The microstructural of 10% and 15% SPF breads imparted distinct distinguishable open pore structures, described as a bicontinuous matrix, which is built up by starch and protein. Similar microstructure observation in the gluten-free rice and low-protein starch breads has been reported by [20].

The uneven surface of bread at higher SPF substitution level was attributed by the presence of higher dietary fibre content. According to [19], dietary fibre diluted the protein content in bread formulation and formed a discontinuous and irregular matrix around the starch granules, which interfered with optimal gluten matrix formation during dough mixing. It also affects the formation of an elastic network of cross-linked gluten molecules during baking, and resulting in easy disruption of gluten network and low loaf volume of bread.

E. Sensory Evaluation

Preference test (9-point hedonic scale) was carried out by 30 panellists to determine the acceptability of the bread substituted with different substitution levels of SPF. The score of sensory evaluation of bread samples is shown in Table IV.

From the result, the score for each attributes decreased significantly ($p < 0.05$) at higher SPF substitution level in bread formulation. Control and 5% SPF breads resulted with no significant different ($p > 0.05$) for all attributes. However, bread of 10% and 15% SPF were having significantly lower ($p < 0.05$) score for all attributes than control and 5% breads. The increased of SPF substitution level in bread formulation produced bread with darker colour and were less preferred by the panellists. For overall acceptability, it was found that bread with 5% SPF is being accepted by the panellist and comparable with control bread.

IV. CONCLUSIONS

In conclusion, matured soursop pulp flour (SPF) is a good source of dietary fibre, minerals, protein and low fat content to enrich the nutritional value of food products. With higher substitution level of SPF in bread formulation, resulted in an improved nutritional quality and properties such as dietary fibre, ash, protein and reduce the fat content, which subsequently reduced the calorie content. Texture Profile Analysis (TPA) and sensory evaluation resulted that substitution of SPF at 5% produced bread that comparable with control and good acceptability score. Field Emission Scanning Electron Microscopy (FESEM) showed that the surface of control and 5% SPF bread appeared with evenly distributed and coalesced by thin gluten film. Therefore, matured soursop pulp flour could be further utilized to enhance the nutritional value in various food products particularly dietary fibre, minerals and protein sources.

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