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INFLUENCE OF UNRIPE BANANA FLOUR INCORPORATION ON THE PHYSICAL, ANTIOXIDANT PROPERTIES AND CONSUMER ACCEPTABILITY OF BISCUITS

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ABSTRACT

The purpose of this work was to evaluate the influence of incorporating unripe banana flour (UBF) in biscuit in order to determine its physical, antioxidant and sensory properties. Two unripe non-commercial banana cultivars (*Luvhele* and *Mabonde*) were used. Wheat flour was replaced by 10, 15, 20 and 30% of UBF. Functional properties of UBF, physical properties, polyphenolic compounds, antioxidant activity and consumer acceptability of baked biscuits were determined. Functional properties of UBF revealed decrease in water and oil absorption capacity, foaming capacity and increase in bulk density at $p < 0.05$. Weight, thickness, fracturability and hardness of composite biscuits significantly increased at $p < 0.05$. The diameter and spread ratio decreased with the inclusion of UBF. The colour of the biscuits (L*, b* and hue values) also decreased. However, the addition of UBF increased the a* value, colour differences and total phenolic content of biscuits. The total flavonoid content of *Luvhele* biscuits increased while that of *Mabonde* biscuits decreased. The antioxidant properties of biscuits were enhanced with UBF addition. Consumer acceptability results revealed that biscuits with 10% UBF level were more acceptable by panellists with regards to taste, crispness and overall acceptability. Results show that UBF can be utilised as functional ingredient in bakery products such as biscuits.

Keywords: Polyphenolic compounds, Flour, Biscuits, Sensory evaluation, Functional properties

INTRODUCTION

Banana is amongst the most consumed tropical fruit in the global market (**Ambrose-Dawn & Lekshman, 2016**). Generally, banana can be classified into two cultivars, either as commercial or non-commercial cultivars. The non-commercial cultivars are also called indigenous varieties since they are cultivated in home gardens by rural communities for consumption (**Anyasi *et al.*, 2013**). *Mabonde* (*Musa AAA*) and *Luvhele (Musa ABB*) are indigenous banana varieties mostly planted in Limpopo province, South Africa (Anyasi *et al.*, 2014). The onset of maturity because of the climacteric nature of the banana fruit makes it to have short shelf life and vulnerable to spoilage by microorganisms. The colour of banana fruit changes quickly by impacts of sunlight and physical bruises, therefore, post-harvest handling is essential **(Anyasi *et al.*, 2014; Yani *et al.*, 2013**). It has been reported that banana fruit at its mature unripe stage is a greater source of carbohydrates containing dietary fibre, lignin, cellulose, hemicellulose and resistant starch (**Sarawong *et al.*, 2013; Ritthiruangdej *et al.*, 2011**). Bioactive compounds such as beta-carotene, ascorbic acid, tocopherol, dopamine, gallocatechin, phenolic acids as well as flavonoids have been identified in banana (**Someya *et al.*, 2002**). Moreover, **Tan-EE et al.** (**2012**) indicated that banana is an antioxidative food and it is also a rich source of minerals. However, information regarding antioxidant properties that exist in non-commercial bananas and their products in South Africa is still lacking.

Norhidayah *et al.* (2014) describe composite flours as the combination of two or more different flours. Recently, there has been a massive interest in fruits that can be incorporated in products formulation to improve the quality characteristics of products such as crispiness and crunchiness but simultaneously, producing healthier food products that can benefit consumers. This initiative has reinforced the utilisation of flour from locally available crops such as sweet potato, cassava, Bambara groundnut, banana, beans, yam, coco yam and other indigenous crops. Wheat is a dominant ingredient utilised to produce various types of bakery products (**Oyeyinka *et al.*, 2014**). However, nutritional deficiency of celiac disease has been linked to the consumption of bakery products made merely of wheat (**Adeola & Ohizua 2018**). Considering the health benefits found in banana, its combination with wheat flour as composite flour to produce baked products such as biscuits may boost nutritional health standard of consumers and lessen risks factors of celiac disease.

Biscuits are described as ready-to-eat food and the process of producing biscuits consists of basic ingredients: flour, fat, sugar, salt and eggs (**Adeola & Ohizula, 2018**). Generally, biscuits are recognised as hard and crunchy. The most important processes during baking of biscuits are caramelisation and Maillard reactions causing most desirable golden colour changes which make the product more appealing to consumers (**Segundo *et al.*, 2017**). Texture is related to the physical properties and is recognised as one of the most essential sensory attributes for bakery products since it influences consumer acceptance (**Pereira *et al.*, 2013**). Changes in ingredients and processing methods causes texture variation of baked products. Biscuits might be the right bakery product to incorporate the health promoting compounds (**Bhat et al., 2020**). Utilisation of unripe banana flour (UBF) in biscuits requires research attention due to recent growing demand for baked products with high nutritional value.

Various research studies have been conducted on the utilisation of unripe commercial banana flour to produce baked products such as biscuits, muffins, cakes, rusks, waffles and bread but information is scarce with reference to indigenous banana varieties as the source of ingredients (**Adeola & Ohizula, 2018; Segundo *et al.*, 2017; Oyeyinka *et al.*, 2014; Norhidayah *et al.*, 2014**). Therefore, utilisation of non-commercial bananas such as *Luvhele* and *Mabonde* as functional ingredients in baked products is one of the novel strategies to enhance the nutritional value, physical, antioxidant and sensory properties of the biscuits. Currently, the food industry has a mandate to manufacture economical, nourishing and beneficial foods (**Bolanho *et al.*, 2014**). To accommodate these demands, the bakery industry has the opportunity of utilising indigenous horticultural crops. The aim of this study was to determine the impact of incorporating unripe UBF on the physical, antioxidant properties and consumer acceptance of biscuits.

MATERIALS AND METHODS

Sample collection and preparation

Two unripe indigenous banana cultivars (*Luvhele* and *Mabonde*) were purchased at the local homes around Thohoyandou, South Africa. Wheat flour, white sugar, margarine, eggs, baking soda and baking powder were all purchased from Shoprite Supermarket in Thohoyandou, South Africa. Unripe bananas of each cultivar were washed and peeled, fruits pulps sliced into small pieces of about 4 mm. Fruit pulps were then spread over oven pans covered with aluminium foil, thereafter air dried in the oven for 12 h at a temperature of 70 °C. Fruit pulps were dried and ground (Retsch ZM 200miller Haan, Germany) for 2 min at 16000 x g to obtain flour and were then sifted through 100 mesh sieve, separately stored in low density zipper plastic bags under controlled temperature of -20 °C until they were used.

Biscuits making

Method of recipe for making biscuits was adopted from **Adeola & Ohizula** (**2018**): flour (500 g), margarine (250 g), sugar (100 g), eggs (2 large), baking powder (7.5 g) and baking soda (3.75 g). Sugar and margarine were mixed to form a fluffy mixture. Eggs were cracked, beaten then added to a fluffy mixture and blended thereafter added to a mixture of composite flours, baking powder and baking soda in the bowl while continuously mixing until soft dough was obtained. The dough was kneaded on the flat surface to smoothens it and a rolling pin was used to roll out the dough into sheet and desired shapes were obtained by using plastic cutters. Cut shapes were transferred to greased baking trays and the baking process was for 25 min in a pre-heated oven at 150 °C until a golden brown colour was achieved. The same procedure was used to produce formulated biscuits with UBF inclusion of 10, 15, 20 and 30% as well as the control sample (biscuits without UBF). Afterwards, biscuits were cooled for 40 min at room temperature and packed in low-density zipper bags until they were used. Biscuits without banana flour (100% wheat) were used as control samples. To validate results, the baking process and analyses of biscuits were replicated three times.

Functional properties of banana flour

Water and oil absorption capacity of UBF were measured based on the method by **Oyeyinka *et al*.** (**2014**). Approximately one gram of each flour sample was added independently to 10 mL distilled water or sunflower oil (Sp. gr. 0.92) in a centrifuge tube. Tap water or sunflower oil was added to rigorously wet the mixture and incubated for 30 min at room temperature and then centrifuged (Hettich Zentrifugen Universal, 320R, Germany) for 30 min at 2000 x g. Water absorption capacity or oil absorption capacity was determined as a percentage of water or oil absorbed by one gram of the flour sample.

Foaming capacity was measured following the method of **Ohizua *et al.*** (**2017**). Approximately one gram of weighed UBF sample was mixed with 25 mL of distilled water in a measuring cylinder. The solution was mixed well and vigorously shaken to foam and the volume of the foam was recorded after 30 s. Foaming capacity was measured as the percentage increase in volume.

The bulk density of UBF was determined using the method of **Mpotokwane *et al*.** (**2008**) whereby each flour sample was added up to 100 mL mark in a measuring cylinder and then weighed. Using the finger, the measuring cylinder was then continuously tapped on top of the table until it reached a constant volume. Afterwards, the bulk density was measured by dividing the weight of flour by the volume of the flour.

Physical properties of biscuits

An analytical weighing balance (0.01 g accuracy) was used to determine the weight of samples in triplicates and measured in grams. The diameter of banana flour was measured by placing three biscuits edge to edge. The total diameter (mm) was measured using Vernier calliper. Biscuits were twisted at an angle of 90° to measure the new diameter. Three biscuits were placed on top of one another to determine their thickness. The total height of biscuits was determined in mm using Vernier calliper. For spread ratio of biscuits, the average value of diameter was divided by average value of thickness. The texture of biscuits was measured by TA-XT plus texture analyser (model 12260, Stable Micro systems, UK) and 2 mm diameter, and 3 cm long probe was screwed on the chunk. According to **Adeola & Ahizula** (**2018**), the test distance required for the procedure is supposed to be pre-test at a speed of 1.0 mm/s, test speed of 0.5 mm/s and post-test of 10 mm/s). The analyser was set to determine the first bite force of biscuits. The force time was analysed for hardness and fructurability to reach the peak. Texture was measured in triplicate.

Instrumental colour analysis of biscuits

The colour attributes of biscuits were measured by Spectrophotometer Lovibond (model no: LC 100, RM 200, China) with the parameters L* (Lightness/darkness), a*(redness/green), b*(yellowness/blue), Chroma (hue saturation) and hue angle recorded. The colour difference was determined using the formula: $\Delta E = \sqrt{\Delta a*^2 + \Delta b*^2 + \Delta L*^2}$

Phenolic compounds and antioxidants activity of biscuits

Extract preparation

The method described by **Atmani et al. (2009)** was used to carry out the extraction process. About one gram of each UBF sample was mixed with 100 mL 80% methanol in a 250 mL conical flask. Orbital shaker was used to carry out the extraction process for 12 h at room temperature. Afterwards, Whatsman no.1 filter paper was used to filter each UBF extracts and evaporated. The extracts were stored in a closed plastic container at 4 °C until used.

Total phenol content

The total phenolic content of biscuits was measured using Follin-Ciocalteau colometric method (**Singleton *et al*., 1999)**. Approximately 0.2 mL of obtained extracts was oxidised with 1 mL Folin-Ciocalteu reagent. The mixture was neutralised with 10 mL of 7% sodium carbonate after 5 min. Distilled water was added to the volume of 25 mL to dilute the mixture. Afterwards, the mixture was rested for 90 min at room temperature and a spectrophotometer at 725 nm was used to read the absorbance of the mixture. Total phenolic content was measured as mg of gallic acid equivalent (GAE) per gram of the sample.

Total flavonoid content

The total flavonoid content of biscuits extract was measured using the spectrophotometric method with a slight modification (**Zhishen *et al*., 1999**). Briefly, about 3 mL of distilled water was used to dilute 0.5 mL aliquot of the sample in aqueous methanol. The mixture was added into 0.3 mL of 5% sodium nitrite then shaken well and 0.6 mL of 10% aluminium chloride was added after 5 min at room temperature. Further, after 6 min, 2 mL of 1 M sodium hydroxide was added and the absorbance of the extracts was read through a spectrophotometer at 510 nm. A Blank was prepared using acidified methanol and total flavonoid content results were calculated and measured in milligram of catechin equivalents per gram of flour extract (mgCE/g)

Free radical scavenging activity (DPPH)

The method of **Brand-Williams *et al*.** (**1995**) was used to measure the DPPH (2,2-diphenyl-1-picryl-hydrazyl) activity of biscuit extracts. Briefly, 0.5 mL phenolic extracts were mixed with 4.5 mL of 60 μM DPPH dissolved in methanol. The mixture was thoroughly shaken using vortex equipment and allowed to rest in a dark room for 30 min. The absorbance of the extracts was determined using spectrophotometer at 517 nm against a solvent blank and results were measured as percentage inhibition of the DPPH radical.

Ferric ion reducing antioxidant power (FRAP)

The FRAP of the biscuits were measured following the method of **Oyaizu** (**1986**) with some modifications. Briefly, 2 g of the extract was dissolved in a suitable 1 mL methanol solvent in a test tube. Afterwards, 1 mL 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide solution were added. The mixture was incubated for 20 min in a water bath at 50 ℃. The reaction was terminated by adding 1 mL of 10% trichloroacetic acid to the resulting mixture and quenched for min using running water. The resulting mixture was centrifuged at 5000 x g for 10 min. Afterwards, 2 mL aliquot was withdrawn from the top layer of each mixture, 2 mL distilled water as well as 0.4 mL of 0.1% ferric chloride solution. Spectrophotometer at 700 nm was used to measure the absorbance of the extracts and results were measured in mg gallic acid equivalents (GAE) per gram of flour sample.

Sensory evaluation of composite biscuits

Permission to conduct sensory characteristics of biscuits was requested from the university Research and Innovation Committee and the project number SARDF/18/FST/09/0811 was allocated. Seventy (70) regular consumers of biscuits, including students and employees were recruited and screened within the university campus. Participants were males and females who were not allergic to wheat gluten and banana. Four composite biscuits and control samples were presented to each panellist coded with three different digit numbers obtained from a Table of Random Numbers. Panellists were expected to rate the consumer acceptability of the composite and control biscuits based on colour, aroma, taste, texture and overall acceptability using 9-point hedonic scale (1, dislike extremely; 9, like extremely). Panellists were given tap water to cleanse the mouth before tasting the next biscuit sample.

Statistical analysis

All analyses were conducted in triplicates and mean ± standard deviation (SD) was used to present the results. The collected data was analysed statistically using SPSS software version 23. One-way analysis of variance (ANOVA) was employed to further analyse the statistical significance of the generated data. Differences were considered significant at $p < 0.05$

RESULTS AND DISCUSSION

Functional properties of composite flour samples

Table 1 summarises the functional properties of UBF samples. There was an increase in the ability of the composite flour to take up water with increase in UBF incorporation for both cultivars. The value for water absorption capacity (WAC) of composite flour varied from 1.0 to 1.40 (*Mabonde*) and 1.17 to 160 (*Luvhele*) g/g, respectively. The increase in WAC of composite flour might attributed to high carbohydrate and low protein contents of UBF as carbohydrates have been observed to substantially affect the WAC of food (**Oyizua *et al*., 2017**). Variation in the WAC of UBF was due to different structures of protein in addition to the availability of various types of hydrophilic carbohydrates in wheat and composite flour (**Kaur *et al*., 2017**). The results indicate favourable WAC and this make the flour to be a desirable raw material for use in the manufacture of ready to eat and baked products. **Mahlako *et al*.** (**2019**) reported similar results of high WAC for wheat, prickly pear and banana flour. Oil absorption capacity is important since it plays a role as a flavour retainer, increase mouth feel of food, appetising and storage life especially in baked or meat products where absorption of fat is desirable (**Asif-Ul-Alam *et al*., 2014**). It measures the performance of food product to take up oil (**Oyizua *et al*., 2017**). There was significant difference between wheat and composite flour at $p < 0.05$. However, the capacity of flour to take up oil decreased as the level of UBF incorporation increased, ranging from 2.00 (control), 1.07 to 1.60 (*Mabonde*) and 1.17 to 1.60 (*Luvhele*) g/g. According to **Anyasi *et al*.** (**2015**), wheat flour has a strong affinity of oil absorption than water hence wheat flour results were higher when compared with composite flour. **Julianti *et al*.** (**2015**) found similar results on composite flour from sweet potato, maize, soybean and xanthan gum. Foaming capacity is important in determining the capacity of the flour to foam which depends on the availability of flexible protein molecules that reduce the surface tension of fluid (**Asif-Ul-Alam *et al*., 2014**; **Oyizua *et al*., 2017**).

Table 1 Functional properties composite flour

Cultivars	WAC (g/g)	OAC (g/g)	Foaming capacity (%)	Bulk density (g/ml)
Control	$1.00^{a}±0.01$	$2.00^{c}±1.00$	$9.93^{g}±3.34$	$0.29^{a}±0.01$
WM_1	$1.00^{a}±0.01$	$1.60^{b}±0.40$	$8.38^{f}±1.47$	$0.40^{b}±0.12$
WM_2	$1.13^{ab}±0.12$	$1.53^{b}±0.12$	$6.36^{c}±2.30$	$0.57^{c}±0.08$
WM_3	$1.30^{b}±0.10$	$1.53^{b}±0.42$	$6.18^{b}±2.16$	$0.64^{cd}±0.11$
WM_4	$1.40^{bc}±0.20$	$1.07^{a}±0.23$	$5.24^{a}±2.61$	$0.72^{d}±0.06$
WL_1	$1.17^{ab}±0.15$	$1.73^{b}±0.64$	$8.13^{e}±5.16$	$0.71^{d}±0.09$
WL_2	$1.33^{b}±0.12$	$1.73^{b}±0.31$	$6.99^{d}±0.39$	$0.79^{d}±0.13$
WL_3	$1.33^{b}±0.12$	$1.67^{b}±0.31$	$6.94^{d}±2.40$	$0.84^{de}±0.12$
WL_4	$1.60^{c}±0.20$	$1.53^{b}±0.23$	$6.89^{d}±3.01$	$0.90^{e}±0.04$

Values are expressed as mean ± SD (n = 3) of triplicate determinations. Values with different superscripts in a column are significantly different from each other ($p < 0.05$). Control = 100% wheat flour. WM_1 = 90% wheat + 10% *Mabonde*, WM_2 = 85% wheat + 15% *Mabonde*, WM_3 = 80% wheat + 20% *Mabonde*, WM_4 = 70% wheat + 30% *Mabonde*, WL_1 = 90% wheat + 10% *Luvhele*, WL_2 = 85% wheat + 15% *Luvhele*, WL_3 = 80% wheat + 20% *Luvhele*, WL_4 = 70% wheat + 30% *Luvhele* flours, WAC= water absorption capacity. OAC= oil absorption capacity.

The values of foaming capacity of composite flour significantly decreased ($p < 0.05$) as the amount of UBF inclusion increased, ranging from 8.38 to 5.24 (*Mabonde*) and from 8.13 to 6.89 (*Luvhele*). Inadequate electrostatic repulsions and unrestricted connection between protein molecules might have contributed to the low foaming capacity of composite flour (**Appiah *et al*., 2011**). **Asif-Ul-Alam *et al*.** (**2014**) found out that highly ordered globular molecule gives low foaming capacity while flexible proteins do the opposite. Therefore, wheat flour had more flexible proteins than UBF. The potential of flour to foam is influenced by the availability of changeable protein molecules which contribute in reducing the surface tension of water. Foaming capacity is an important parameter to determine the potential of utilising flour as whipping agent in whipped food products (**Cheng and Bath, 2016**). **Oyizua *et al*.** (**2017**) obtained similar results on the functional characteristics of unripe cooking banana, pigeon pea and sweet potato blends and **Cheng and Bath** (**2016**) on composite wheat and legume (*Pithecellobium jiringa Jack*) flour. The Bulk density (BD) of composite flour was significantly different ($p < 0.05$) at various levels of inclusion for both banana cultivars. The BD increased as the incorporation of UBF increases. *Mabonde* composite flour at 30% level had the BD value of 0.90 g/ml while *Luvhele* composite flour at 10% level had the lowest BD of 0.40 g/ml. The highest BD of composite flour in 30% *Mabonde* inclusion shows that this flour has the potential of being used as thickening agent in food industry due to its capacity to decrease the thickness of blend which is a principal factor in recuperating and infant feeding (**Chandra *et al*., 2015**). The increase in BD might be attributed to particle size of the banana flour, which is a good physical property in food systems (**Sulieman *et al*., 2019**). Moreover, increase in BD is beneficial because it will offer exceptional advantage during packaging since more flour will be packaged within a constant volume (**Mahloko *et al*., 2019**). Results are in line with those by **Ndife *et al*.** (**2014**) on wheat-soy composite flour blend.

Physical characteristics of biscuits

Table 2 depicts physical characteristics of UBF biscuits. There was an increase in weight of composites biscuits with UBF inclusion levels ranging from 6.39 to 8.93 (*Mabonde*) and 6.37 to 8.22 g (*Luvhele*). The high BD of composite flour could be a contributing factor to the higher weight of composite biscuits. The increase in the weight of composite biscuits might also be linked to the dietary fibre of banana flour added having the capacity to hold together water molecules that inhibit loss of moisture during the baking process. Similar results were obtained by **Adeola and Ohizua** (**2018**) on biscuits produced from blends of flour of unripe cooking banana, pigeon pea, and sweet potato. The diameter of formulated biscuits significantly decreased ($p < 0.05$) with the increase in UBF incorporation ranging from 45.54 to 42.94 mm (*Mabonde*) and from 45.80 to 43.64 mm (*Luvhele*). **Sah *et al*.** (**2011**) indicated that the high amount of protein content in the flour contribute to the decrease in diameter of composite biscuits. These results agree with the report of **Tiwari *et al*.** (**2011**) on wheat and pigeon pea biscuits. The decrease in diameter is also in agreement with **Srivastava *et al*.** (**2012**) who indicated that low diameter was due to increase in fibre content of composite biscuits. The thickness of biscuits significantly increased with the increase in UBF incorporation ranging from 11.0 to 13.53 mm (*Mabonde*) and 10.49 to 13.0 mm (*Luvhele*). The increase in the thickness of composite biscuits might have been influenced by the hydrophilic nature of the UBF added. The obtained results agree with the report of **Ferial and Abu-Salem** (**2011**) who discovered that the incorporation of Bambara groundnut flour to wheat flour significantly increases the thickness of the biscuits. The maximum value of spread ratio (4.59) in control and minimum spread ratio (3.0) in 30% *Luvhele* level were observed in the composite biscuits. The influence of incorporating composite flour to wheat flour might have contributed to the low spread ratio of biscuits since there is formation of aggregations with high number of hydrophilic sites competing for the restricted unbound water in the dough of the biscuits (**Shalin and Sudesh, 2005**). Low spread ratio of composite biscuits could be attributed to higher amount of protein content indicating substantial water binding capacity which in the end hinders the spread of the biscuits (**Chang and Bhat, 2016**). Similar results were reported by **Yousaf *et al*.** (**2013**) wherein the substitution of wheat flour with gram flour decreased the spread ratio of the composite biscuits. Textural qualities hardness and fracturability are desirable attributes for biscuits (**Adeola & Ohizua, 2018**). The fracturability of composite biscuits significantly ($p < 0.05$) increased with the UBF incorporation levels ranging from the lowest 597.83 g at 30% of *Mabonde* to the highest 1156.12 g at 10% of *Luvhele*. The addition of UBF in composite biscuits resulted in high values of fracturability which is an indication of formation of looser matrix in the dough containing lower quantity of protein (**Oksuz & Karakaş, 2016**). Results suggest that baking conditions, type and quantity of ingredients used influenced the fracturability and hardness of *Mabonde* better than *Luvhele* composite biscuits (**Adeola & Ohizua, 2018**). Biscuits with higher *Mabonde* flour will sustain their shape during transportation and be easy to break in the mouth. These results are in line with a report by **Adeola & Ohizua** (**2018**) on biscuits prepared from flour from unripe cooking banana, pigeon pea, and sweet potato.

The hardness of the composite biscuits ranged from 471.55 to 647.73 g (*Mabonde*) and from 348.22 to 541.94 g (*Luvhele*). The hardness of composite biscuits significantly increased with the UBF inclusion levels at $p < 0.05$ while the control sample had the lowest value of 176.14 g. The high values of hardness in composite biscuits could be as results of the dilution of wheat protein with banana flour protein because the interaction of the two protein make biscuits compressed, thereby, improving the hardness (**Kulthe *et al*., 2017**). Moreover, **Hoseney and Rogers** (**1994**) indicated that the interconnection of protein and starch due to hydrogen bond also contributes to the hardness of biscuits. The increase in hardness in this study is in line with the report of **Kulte *et al*.** (**2017**) on wheat and pearl millet biscuits and **Gandhi *et al*.** (**2001**) on wheat and defatted soybean flour.

Table 2 Physical characteristics of composite biscuits

Treatment	Weight (g)	Diameter (mm)	Thickness (mm)	Spread ratio	Texture Fracturability (g)	Hardness (g)
Control	$6.33^{a}\pm0.36$	$48.60^{f}\pm0.39$	$10.00^{a}\pm0.28$	$4.59^{g}\pm0.15$	$410.56^{a}\pm0.66$	$176.14^{a}\pm0.94$
WM_1	$6.39^{a}\pm0.55$	$42.94^{a}\pm0.18$	$11.00^{c}\pm0.13$	$4.54^{g}\pm0.11$	$1146.29^{e}\pm1.33$	$647.73^{e}\pm1.40$
WM_2	$6.54^{b}\pm0.27$	$43.60^{b}\pm0.59$	$11.17^{c}\pm0.08$	$3.97^{e}\pm0.04$	$1037.95^{de}\pm1.20$	$625.79^{e}\pm1.16$
WM_3	$8.48^{d}\pm0.43$	$44.69^{c}\pm0.19$	$13.53^{g}\pm0.06$	$3.50^{c}\pm0.04$	$895.18^{cde}\pm0.82$	$484.64^{c}\pm1.14$
WM_4	$8.58^{d}\pm0.64$	$45.54^{d}\pm0.42$	$13.01^{f}\pm0.11$	$3.00^{a}\pm0.00$	$597.83^{ab}\pm0.76$	$471.55^{c}\pm1.12$
WL_1	$6.37^{a}\pm0.25$	$43.64^{b}\pm0.38$	$10.49^{b}\pm0.02$	$4.27^{f}\pm0.01$	$1156.12^{e}\pm2.00$	$541.94^{d}\pm1.17$
WL_2	$6.55^{b}\pm0.22$	$44.78^{c}\pm0.92$	$11.23^{d}\pm0.15$	$4.04^{e}\pm0.09$	$771.26^{bcd}\pm1.88$	$480.73^{c}\pm1.10$
WL_3	$7.72^{c}\pm0.12$	$45.60^{e}\pm0.51$	$12.66^{e}\pm0.05$	$3.61^{d}\pm0.01$	$732.51^{bc}\pm1.76$	$459.68^{c}\pm1.07$
WL_4	$7.84^{c}\pm0.13$	$45.80^{e}\pm0.22$	$13.00^{f}\pm0.10$	$3.36^{b}\pm0.01$	$614.26^{abc}\pm1.68$	$348.22^{b}\pm1.04$

Values are presented as mean ± SD of three measurements. Means with different letters in a column are significantly different at the level of $p < 0.05$. Control = wheat biscuit. WM_1 = 90% wheat + 10% *mabonde*, WM_2 = 85% wheat + 15% *Mabonde*, WM_3 = 80% wheat + 20% *Mabonde*, WM_4 = 70% wheat + 30% *Mabonde*, WL_1 = 90% wheat + 10% *Luvhele*, WL_2 = 85% wheat + 15% *Luvhele*, WL_3 = 80% wheat + 20% *Luvhele*, WL_4 = 70% wheat + 30% *Luvhele* flours,

Colour profile of biscuits samples

The surface colour of biscuit samples with regards to L*, a*, b*, Hue, Chroma and ΔE values is presented in Table 3. The L* values of composite biscuits significantly decreased with inclusion levels of banana flour in both cultivars ranging from 56.83 to 47.73 in *Mabonde* biscuits sample and 51.77 to 47.53 in *Luvhele* biscuit samples. The highest lightness (L*) value (56.83) was observed in control biscuits. The decrease in L* values shows that formulated biscuits had darker colour at higher levels of UBF incorporation. The composition of ingredients, velocity of air in the baking oven and brown pigments that resulted from the Maillard reaction might have contributed to the darker colour of composite biscuits. The Maillard reaction relies on the quantity of reducing sugars and amino acids or proteins that occur on the surface of baked products including the temperature and time of baking (**Pereira *et a*l., 2013**). However, the redness (a*) values of composite biscuits showed opposite trend. The a* values increased with the incorporation levels of UBF ranging from 5.83 to 8.43 in *Mabonde* biscuit samples. However, the b* value of UBF incorporated biscuits were significantly lower than the control sample. Results agree with findings by **Pereira *et al*.** (**2013**) on Maria type biscuits. **Chevallier *et al.* (2000)** indicated that protein content can be negatively compared with lightness of biscuits, suggesting that non-enzymatic (Maillard) browning plays the important role in forming colour. Moreover, the non-enzymatic browning and caramelisation of sugar promote the generation of brown pigments during the baking process. Maillard browning is formed by the interaction between proteins in wheat and sugar added whereas caramelisation is influenced by the dispersion of water and the reaction of sugar added as well as amino acids (**Noorfarahzilah *et al*., 2014**). Chroma measures how intense the surface colour is and Hue angle is used to define specific colours with the same lightness (**Ducamp-Collin *et al*., 2008**). Chroma values increased with the level of UBF addition ranging from 69.53 to 73.67 in *Mabonde* biscuits and from 70.27 to 73.03 in *Luvhele* biscuits. This might be due to the increased pigment concentration as the inclusion of UBF increases; high values of Chroma induce high colour intensity of samples as judged by human eye (**Pathare *et al*., 2012**). Hue values showed a decrease trend with the incorporation of UBF in the biscuit samples. According to **Pathare *et al*.** (**2012**) a high Hue angle shows a lesser yellow character; therefore, it can be presumed that as the levels of UBF was added biscuits sample became darker. Total colour difference (ΔE) shows the magnitude of colour difference between composite and control biscuits. Colour difference showed a significant difference ($p < 0.05$) amongst biscuits samples with the control having the lowest value of 51.33 and *Luvhele* biscuits sample having the highest value of 57.10 at 30% level. The increase in ΔE may be due to inclusion of UBF whereby the dark colour of composite biscuits increased with the percentage increase of UBF. **Krishnan *et al*.** (**2011**) found similar results for finger millet seed coat composite biscuits.

Table 3 Colour attributes of composite biscuit

Treatment	L^*	a^*	b^*	Hue (^{0})	Chroma	ΔE
Control	$56.83^{d}\pm3.07$	$4.27^{a}\pm0.76$	$26.43^{d}\pm1.59$	$26.80^{d}\pm1.73$	$80.87^{d}\pm1.03$	$51.33^{a}\pm1.96$
WM_1	$51.30^{bc}\pm0.78$	$5.83^{b}\pm0.55$	$19.97^{a}\pm1.37$	$24.23^{cd}\pm0.87$	$69.53^{a}\pm2.02$	$55.43^{bc}\pm1.96$
WM_2	$50.57^{abc}\pm2.40$	$6.23^{b}\pm0.99$	$20.17^{a}\pm3.19$	$24.00^{bcd}\pm0.61$	$70.20^{a}\pm0.44$	$55.53^{bc}\pm2.38$
WM_3	$48.97^{abc}\pm3.23$	$8.20^{cd}\pm0.44$	$22.47^{ab}\pm0.60$	$21.13^{ab}\pm3.33$	$72.80^{bc}\pm0.46$	$55.57^{bc}\pm1.40$
WM_4	$47.73^{ab}\pm0.59$	$8.43^{d}\pm0.91$	$22.80^{abc}\pm0.72$	$20.80^{a}\pm1.47$	$73.67^{c}\pm0.78$	$53.73^{ab}\pm1.82$
WL_1	$51.77^{c}\pm1.17$	$7.01^{bc}\pm0.48$	$20.77^{a}\pm0.45$	$26.60^{d}\pm0.36$	$70.27^{a}\pm1.86$	$54.93^{bc}\pm0.96$
WL_2	$50.93^{abc}\pm0.68$	$7.70^{cd}\pm0.78$	$21.43^{a}\pm1.21$	$26.17^{d}\pm1.65$	$70.27^{a}\pm1.16$	$55.70^{bc}\pm0.26$
WL_3	$48.00^{ab}\pm0.95$	$7.73^{cd}\pm0.06$	$24.67^{bcd}\pm1.76$	$22.77^{abc}\pm1.35$	$71.33^{ab}\pm1.02$	$56.70^{bc}\pm0.95$
WL_4	$47.53^{a}\pm1.70$	$8.70^{d}\pm0.44$	$25.40^{cd}\pm0.36$	$21.87^{abc}\pm0.50$	$73.03^{bc}\pm0.21$	$57.10^{c}\pm2.26$

Values are average of three determinations (mean ± SD). Means with different letters in a column are significantly different at $p < 0.05$. Control = wheat biscuit, WM_1 = 90% wheat + 10% *mabonde*, WM_2 = 85% wheat + 15% *Mabonde*, WM_3 = 80% wheat + 20% *Mabonde*, WM_4 = 70% wheat + 30% *Mabonde*, WL_1 = 90% wheat + 10% *Luvhele*, WL_2 = 85% wheat + 15% *Luvhele*, WL_3 = 80% wheat + 20% *Luvhele*, WL_4 = 70% wheat + 30 % *Luvhele* flours.

Polyphenolic compounds and antioxidant activity of biscuits samples

Polyphenolic compounds and antioxidant activity of composite and control biscuits are summarised in Table 4. There was a significant increase ($p < 0.05$) in total phenolic content (TPC) of the biscuits with increase in UBF incorporation. A higher TPC was observed in *Mabonde* biscuits extracts of 357.88 mg/g at 30% level compared to *Luvhele* and control extracts which had low values of 321.82 at 30% level and 263.97 mg/g at 100% wheat flour, respectively. The increasing range of TPC values is mainly caused by the stage of maturity in which bananas were used to produce flour during the study. Total phenolic content and ascorbic acid are influenced by maturity and harvesting methods, therefore, as TPC decreases, ascorbic acid increases and vice versa (**Seung & Adel, 2000**). **Lim *et al*.** (**2007**) reported that fruits have variety of antioxidants like ascorbic acid, phenolics, vitamin E, and β-carotene and some of them contribute to TPC. The antioxidant capacity of the bananas might be as result of gallocatechin content (**Ramakrishnan *et al*., 2011**). Moreover, thermal processing such as baking increases the antioxidant properties of plant materials, including the extraction of some bound polyphenols from the biscuit, because of the break-down of covalent bonds which causes TPC to increase (**Kowalczewski, *et al*., 2019**; **Lee *et al*., 2003**). **Elhassaneen *et al*.** (**2011**) found similar results where substitution of wheat flour at 5% level with powders from prickly pear and potato peels increased the TPC of the composite biscuits. In terms of the total flavonoids content (TFC), a significant difference at $p < 0.05$ was noted between each cultivar with *Luvhele* biscuits extracts exhibiting higher flavonoid content (659.52 mg/g) proving to be a better source of flavonoids compounds. The values of TFC for *Mabonde* biscuits ranged from 339 to 470 mg/g and they were lower than the control biscuit extracts (477.14 mg/g).

Table 4 Polyphenolic compounds and antioxidants properties of composite biscuits

Treatment	TPC(mg/g)	TFC(mg/g)	DPPH (%)	FRAP (mg/g)
Control	$263.97^{a}±7.69$	$477.14^{c}±11.34$	$50.41^{a}±0.23$	$0.032^{a}±0.002$
WM_1	$280.91^{b}±2.41$	$470.57^{c}±3.78$	$78.51^{g}±0.55$	$0.057^{b}±0.002$
WM_2	$302.12^{d}±2.29$	$419.52^{b}±27.57$	$72.72^{f}±1.06$	$0.062^{bc}±0.002$
WM_3	$342.73^{f}±0.91$	$390.00^{b}±33.29$	$67.64^{e}±1.11$	$0.071^{d}±0.001$
WM_4	$357.88^{g}±1.05$	$339.52^{a}±17.22$	$62.68^{c}±0.09$	$0.076^{d}±0.003$
WL_1	$293.33^{c}±4.10$	$679.52^{d}±0.83$	$77.17^{g}±0.74$	$0.065^{c}±0.003$
WL_2	$319.09^{e}±3.15$	$663.33^{d}±5.77$	$61.77^{c}±1.64$	$0.066^{c}±0.002$
WL_3	$320.00^{e}±2.41$	$659.52^{d}±19.40$	$54.78^{b}±1.33$	$0.083^{e}±0.002$
WL_4	$321.82^{e}±1.58$	$509.05^{c}±17.86$	$50.80^{a}±1.76$	$0.085^{e}±0.001$

Values are average of three determinations (mean ± SD). Means with different letters in a column are significantly different at $p < 0.05$. Control = wheat biscuit. WM_1 = 90% wheat + 10% *Mabonde*, WM_2 = 85% wheat + 15% *Mabonde*, WM_3 = 80% wheat + 20% *Mabonde*, WM_4 = 70% wheat + 30% *Mabonde*, WL_1 = 90% wheat + 10% *Luvhele*, WL_2 = 85% wheat + 15% *Luvhele*, WL_3 = 80% wheat + 20% *Luvhele*, WL_4 = 70% wheat + 30% *Luvhele* flours, TPC = Total phenolic content. TFC = Total flavonoids content. DPPH = 1.1-Diphenyl-2-picrylhydrazyl. FRAP = Ferric ion reducing antioxidant power

The observed TFC variations between *Luvhele* and *Mabonde* biscuits could be because of the development of brown pigments (meladoins) which are the product of non-enzymatic browning which take place during the baking process (**Manzocco et al., 2000**). The decrease in TFC values in added with *Mabonde* flour might be due to the heating temperature used in baking of biscuits samples which decreases the antioxidant activity (**Chlopicka et al., 2011**). The DPPH of biscuits significantly increased at $p < 0.05$ in both cultivars. Biscuits with 10% level of *Mabonde* and *Luvhele* flours had the highest values of 78.51 and 77.1%. The control biscuit had the lowest value of 50.41% and it was not significantly different to 30% *Luvhele* flour inclusion which had 50.80%. The high values of DPPH in composite biscuits might be due to processing steps during baking since they have been found to enhance the antioxidant activity of baked products (**Sharma and Gujral, 2014**; **Baba et al., 2015**). Redox properties of phenolic compounds might also have contributed to the variations in DPPH of biscuits since they have a major role in absorbing and neutralising free radicals (**Loza *et al.*, 2017**). The low value of DPPH in 30% *Luvhele* flour inclusion biscuit might be attributed to the depolymerisation of polyphenols and decarboxylation of phenolic acids during baking process (**Alvarez-Jubete et al., 2010**). Moreover, **Cheung *et al*. (2003)**; **Hurst *et al*. (2006)** indicated that not all baked or cooked products retain the same amount of antioxidant activity. Results of this work are in line with study by **Mabogo *et al.* (2021)** for wheat biscuits supplemented with UBF (*Muomva red* cultivar). The reducing power (FRAP) of the formulated biscuits extracts significantly increased ($p < 0.05$) with incorporation of UBF in both cultivars. High values of 0.076 (*Mabonde* biscuits) and 0.085 (*Luvhele* biscuits) mg/g were observed at 30% level in both cultivars. The lowest value of 0.032% was noted in the control biscuits. The increase in FRAP of biscuits could have been influenced by te different structure of phenolic compounds.

Consumer acceptability of biscuits samples

The biscuits incorporated with UBF were assessed for colour, aroma, taste, crispiness and overall acceptability (Table 5). Sensory properties of biscuits including colour, aroma, taste and crispiness showed significant differences at $p < 0.05$. However, overall acceptability was not significantly different ($p < 0.05$) amongst biscuit samples. Biscuits with higher level of banana flour (20 and 30%) had high scores with regards to colour ranging from 7.67 to 7.82 in *Mabonde* biscuits, 7.13 to 7.50 in *Luvhele* as well as aroma ranging from 7.52 to 7.63 in *Mabonde*, 7.28 to 7.38 in *Luvhele* biscuits. The high score of colour was because that panellists preferred the slightly darker colour of these composite biscuits. The aroma of biscuits significantly increased with UBF incorporation and this might have been influenced by the appetising aroma that exists in banana flour when blended with other ingredients in biscuits making. Similar results were reported by **Loza et al. (2017)** on the functional wheat biscuits supplemented with banana flour (*Musa paradisiaca*) and sesame seeds (Sesamum indicum). The taste of the formulated biscuits generally decreased with the inclusion levels of UBF. This might be associated with high quantity of polyphenols with unpleasant, bitter, and astringent taste in UBF. The mean score for crispiness decreased ranging from 7.70 to 7.60 for *Mabonde* flour added biscuits and from 7.71 to 7.62 (*Luvhele* flour added biscuits) and the values were lower than the control wheat biscuits (7.73). The means score (crispness) for composite biscuits significantly decreased at UBF levels above 10%. This shows that at levels of above 10%, consumer preference on the crispness of biscuits was low. It can be concluded that the biscuits become harder when the UBF levels increase from 10% to 100%. The combination of wheat and banana flour resulted in an increase in hardness of biscuits which contributed in undesirable crispness and this might be due to the modification of gluten content. The inclusion of UBF affected the build-up of gluten matrices in wheat flour. The findings correspond with the results of hardness of composite biscuits shown in Table 2, whereby the hardness increased with the inclusion levels of UBF. These results do not agree with findings by **Alongi et al. (2019)** who observed that the inclusion of apple pomace powder influenced the rankings for fruit and baking flavour of short dough biscuits. The composite biscuits were well accepted at 10% UBF level although the overall acceptability did not differ significantly ($p < 0.05$) with the control biscuits. This means that up to 10% of UBF could be added in bakery products without altering the sensory characteristics of the final products.

Table 5 Sensory evaluation of composite biscuits

Characteristics	Colour	Aroma	Taste	Crispiness	Overall acceptability
Control	$7.58^{e}±1.46$	$4.37^{a}±1.31$	$7.95^{a}±1.68$	$7.73^{b}±1.36$	$7.65^{a}±1.27$
WM_1	$5.63^{a}±1.35$	$5.93^{b}±1.91$	$7.87^{a}±1.60$	$7.70^{b}±1.58$	$7.71^{a}±1.40$
WM_2	$7.63^{e}±1.46$	$7.47^{d}±1.29$	$7.33^{b}±1.60$	$7.65^{a}±1.52$	$7.64^{a}±1,33$
WM_3	$7.67^{e}±1.61$	$7.52^{d}±1.37$	$7.30^{b}±1.51$	$7.64^{a}±1.49$	$7.60^{a}±1.30$
WM_4	$7.82^{e}±1.30$	$7.63^{d}±1.13$	$7.10^{c}±1.42$	$7.60^{a}±1.53$	$7.58^{a}±1.30$
WL_1	$6.48^{b}±1.85$	$6.97^{c}±1.38$	$7.85^{a}±1.72$	$7.71^{b}±1.72$	$7.70^{a}±1.31$
WL_2	$6.95^{c}±1.36$	$7.25^{d}±1.64$	$7.32^{b}±1.68$	$7.65^{a}±1.34$	$7.63^{a}±1.23$
WL_3	$7.13^{d}±1.68$	$7.28^{d}±1.43$	$7.29^{b}±1.66$	$7.63^{a}±1.48$	$7.57^{a}±1.41$
WL_4	$7.50^{e}±1.31$	$7.38^{d}±1.33$	$7.12^{c}±1.34$	$7.62^{a}±1.33$	$7.54^{a}±1.80$

Values are presented as mean ± SD of triplicate measurements. Values with different superscripts in a column are significantly different from each other ($p < 0.05$). Control = wheat biscuit. WM_1 = 90% wheat + 10% *mabonde*, WM_2 = 85% wheat + 15% *Mabonde*, WM_3 = 80% wheat + 20% *Mabonde*, WM_4 = 70% wheat + 30% *Mabonde*, WL_1 = 90% wheat + 10% *Luvhele*, WL_2 = 85% wheat + 15% *Luvhele*, WL_3 = 80% wheat + 20% *Luvhele*, WL_4 = 70% wheat + 30% *Luvhele* flours,

CONCLUSION

The BD of the flour increased as the level of banana flour was added while a decreasing trend in water and oil holding and foaming capacities were observed. Incorporating banana flour to wheat flour resulted in an increased in physical properties such as weight, diameter, thickness and texture while the reduction of spread ratio was noted. A similar fashion of increase was also noted in polyphenols compounds and antioxidant activity of composite biscuits. Addition of banana flour decreased the L*, b* and hue values of the biscuits while a* and colour differences increased. The banana flour formulated biscuits were well preferred by their sensory attributes. This study has shown that composite wheat and banana flour at different proportions can produce acceptable biscuits.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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