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Effect of partial replacement of wheat flour with unripe banana flour on the functional, thermal, and physicochemical characteristics of flour and biscuits

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Abstract

Banana (*Musa* sp.) is a highly consumed fruit, and the fifth most important crop in world export market. It contains nutrients such as dietary fibre, minerals, vitamins, pro-vitamins, and phenolic compounds that are important in lowering the risk of chronic diseases. However, the onset of ripening due to the climacteric nature of the fruit makes banana susceptible to spoilage and short storage period. Therefore, the current work was aimed to evaluate the properties of flour and biscuits formulated with under-ripe *muomva red* banana flour (MRF) as partial replacement (0, 10, 15, 20, and 25%) for wheat flour. Functional, thermal, and physicochemical characteristics of flour and biscuits were determined. The inclusion of MRF improved the functional properties such as bulk density, oil, and water holding capacity of wheat flour. The results showed an increase in all the gelatinisation temperature parameters (T_o, T_p, and T_e) of flour with increase in MRF concentration. The onset temperature (T_o) of flour increased from 70.25 to 109.41°C, peak temperature (T_p) from 72.59 to 116.21°C, and end temperature (T_e) from 91.07 to 123.21°C. However, colour measurements showed that MRF significantly contributed to darker colour (lower L*) of biscuits. The L* values of biscuits from wheat and *muomva red* composite flour at different ratios decreased from 52.63 to 41.43. The a*, b*, and chroma values also decreased as MRF increased. Meanwhile the weight, spread ratio, break force, and fracturability of biscuits increased. The inclusion of MRF significantly improved the bioactive compounds and DPPH values of biscuits. In conclusion, the incorporation of MRF could be an effective way to produce nutritious and acceptable biscuits.

Introduction

Biscuit is one of the most favoured bakery products in human diet because it is convenient, palatable, ready-to-eat, inexpensive, contains high nutritional quality, and has longer shelf-life than other bakery products (Nagi *et al.,* 2012; Mahloko *et al.,* 2019). It is produced by combining ingredients such as flour, milk/water, fat, and sugar. The dough is normally rolled out and then cut into small circles, which is then baked to produce biscuits (Yadav *et al.,* 2012). Biscuit is characterised by having low moisture content, high fats and sugar, and well-developed gluten structure. The high amount of sugar and fat makes gluten more extensible and less elastic (Cauvin and Young, 2006). Biscuit is hard and crunchy due to the low moisture content which is related to the thickness of biscuits.

In recent years, the main aim has been to fortify bakery products such as biscuits, with ingredients that are rich in phenolic compounds, antioxidant activity, dietary fibres, proteins, vitamins, and minerals (Mesías *et al.,* 2016; Čukelj *et al.,* 2017). Banana (*Musa* spp.) is a type of edible fruit commonly cultivated and consumed (Kumar *et al.,* 2012). Anyasi *et al.* (2015) reported that three of the main non-commercial banana cultivars cultivated around the Limpopo Province of South Africa are *luvhele* (*Musa* AAA), *muomva red* (*Musa balbisiana*), and *mabonde* (*Musa* ABB). Non-commercial or indigenous cultivars/varieties are not often cultivated for trade or export, and are usually planted in home gardens for consumption (Anyasi *et al.,* 2013).

During unripe stage, bananas contain large amounts of starch/resistant starch, which may be a good source for new products development. Unripe bananas are also rich in minerals and vitamins available both in pulps and peels (Lima *et al.,* 2000). Since unripe banana flour (UBF) has a high amount of starch content, it is used in the preparation of products such as mayonnaise, breads, pates, pasta, and others (Borges *et al.,* 2009). High quantities of unripe bananas that are rejected due to poor grade or having skin defects are processed into flour for local and export industry

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(Menezes *et al.,* 2011). Recently, the banana flour industry has drawn attention due to the nutritional value of the flour, particularly the high content of resistant starch (approximately 40.9 to 58.5%), dietary fibre (approximately 6.0 to 15.5%), and bioactive compounds such as phenolic acids (Aurore *et al.,* 2009). The unripe banana contains numerous classes with free and bounded phenolics such as anthocyanidins (Bennett *et al.,* 2010).

There are different reports on the use of UBF for manufacturing of composite flour and biscuits (Adeola and Ohizua, 2018; Mahloko *et al.,* 2019). In this regard, using non-commercial bananas such as *muomva red* as an ingredient in composite flour is one of the innovative approaches to improve the nutritional value and quality characteristics of the biscuits. The prospect of using UBF for the manufacturing of ready-to-eat food such as biscuits provides a significant opportunity of incorporating bioactive compounds which are missing nutrients in these products (Aurore *et al.,* 2009). Therefore, the present work sought to determine the effect of incorporating UBF on the functional, thermal, and physicochemical characteristics of flour and biscuits. The results obtained from the present work will contribute to the general use of *muomva red* banana flour in bakery products such as biscuits, bread, and cakes, and boast its commercialisation potential.

Materials and methods

Sample collection and preparation

Unripe non-commercial banana cultivar of *muomva red* (*M. balbisiana*) was obtained from local banana farms. The banana fingers were peeled and cut into 5 mm size, and pre-treated with citric acid at a concentration of 10 g/L, and then allowed to stand for 10 min to prevent oxidative browning. The banana slices were allowed to drain for 2 min. Using forced air oven, the slices were dried at a temperature of 70°C for a period of 12 h.

Banana flour processing

Oven-dried pre-treated banana slices were used to produce UBF through milling (Retsch ZM 200 miller, Haan, Germany), running at a speed of 16,000 *g* until homogenised flour was achieved. The banana flour was termed MRF.

Baking of wheat and muomva red composite biscuits

Biscuits were prepared following the method of Laguna *et al.* (2011) with slight modifications. Brown sugar (23%), margarine (23%), flour (53%), and (1%) baking powder were used. Brown sugar and margarine were used instead of butter and sucrose due to cost and availability. The flour, baking powder, and sugar were mixed thoroughly for 60 s inside a bowl before they were mixed with margarine manually for 2 min. The batter was shaped by a circle cookie cutter before being placed inside the oven at a temperature of 180°C for 8 min. After cooling down, the biscuits were packed in Ziploc bags. Cookie cutter was used to cut the biscuit dough as well as giving the biscuits uniform shape. For flour, five treatments were prepared: control (wheat flour, no addition of MRF), 10% MRF, 15% MRF, 20% MRF, and 25% MRF.

Determination of functional properties of wheat and muomva red composite flour

Bulk density

The method described by Onwuka and Abasiekong (2006) was followed to measure the bulk density of the flour. Approximately 50 g of the flour was weighed into a 100 mL graduated measuring cylinder, and the cylinder was gently tapped 50 times until the contents were tightly packed. The bulk density was calculated as weight of composite flour (g) divided by flour volume.

Water and oil absorption capacities

The method of Sosulski *et al.* (1976) was followed to determine the water and oil absorption capacities of the flour samples. Approximately 1 g of flour sample was weighed in a conical graduated centrifuge tube, and dispersed in 10 mL of distilled water or refined sunflower oil. The mixture was thoroughly shaken for 1 min at room temperature. The sample was then allowed to stand for 30 min before it was centrifuged at 2,000 *g* for 30 min. Water or oil absorption capacity was expressed as percent water or oil bound per gram of the sample.

Swelling power

The swelling power of the flour was determined following the method of Okaka and Potter (1977). Approximately 1 g of flour sample was placed in a graduated 50 mL centrifuge tube (weighed). To obtain a total volume of 10 mL, distilled water was added, and samples in the tubes were shaken manually and carefully for 30 s before being heated at 60°C for a minimum of 30 min. The samples were centrifuged at 3,000 *g* for 30 min after cooling at 30°C. The weight of sediment was recorded.

Water solubility index

The supernatant obtained from the determination of the swelling power was decanted in a pre-weighed evaporation dish, and dried to constant weight in an oven. Water solubility index was obtained using the following equation:

$$WSI= \frac{(Wd+Ds)-Wd}{Wds}$$

where, *Wd* = weight of the dish, *Ds* = dried supernatant, and *Wds* = weight of dried sample.

Thermal analysis of wheat and muomva red composite flour

Differential scanning calorimeter (DSC 4000, PerkinElmer, Shelton, CT, USA) was used to analyse the gelatinisation character of wheat flour and MRF based on the method of Anyasi *et al.* (2015). An amount of 3 g of flour sample was weighed inside a small aluminium pan. A drop of distilled water was added to the pan, and was sealed hermetically using universal crimper press (Perkin Elmer, Shelton, CT, USA). The samples were equilibrated before testing for 1 h. An empty aluminium pan was used as a reference. The sample was heated at a rate of 10°C/min under a temperature of 25 - 120°C. The Pyris software (PerkinElmer, Shelton, CT, USA) was used to calculate the onset temperature (T_o), peak temperature (T_p), end temperature (T_e), and enthalpy.

Physical properties of biscuits

Colour measurement

Colour measurement was analysed using a Hunter Lab colourflex (D65; Reston, VA, USA) calibrated against a white and black tile, and the results were obtained in the form of L*, a*, b*, C*, and Hue angle; where L* = lightness, a* = redness, b* = yellowness, and C = chroma.

Thickness

The thickness of biscuits was measured by stacking ten pieces of biscuits, following the method explained by Ahmed and Hussein (2014). The samples were recorded in triplicates, and the mean values for thickness were reported in mm.

Diameter

The diameter of a single biscuit was measured by arranging ten pieces of biscuits edge to edge. The samples were conducted in triplicates for each sample. The average values were recorded in mm.

Weight

A weighing balance was used to determine the weight of biscuits. Mean values for weight were reported in g.

Spread ratio

The spread ratio was determined by dividing diameter by thickness following the method of Ahmed and Hussein (2014).

Texture

The TA-XT Plus texture analyser was used to measure the hardness and fracturability of the biscuit samples as described by Ahmed and Hussein (2014). The first bite of the biscuits was determined by a texture analyser set to perform single cycle measurements. A speed of 2 mm/s and 5 mm distance were applied. The plots of force against time were analysed for fracturability and breaking force. Break force is a force required to break down biscuits or food material.

Determination of bioactive compounds of biscuits

Determination of total phenolic content

The Folin-Ciocalteu colorimetric method was used to determine the total polyphenols of the biscuits. About 0.2 g of milled biscuits was weighed and mixed with 2 mL of acetone. The mixture was incubated for 1 h (shaking occasionally at 25 ± 2°C). The mixture was then centrifuged at 6,000 rpm for 5 min at 4°C. Next, 109 µL of Folin-Ciocalteu solution was added to 9 µL of the centrifuged sample placed in a microplate. Approximately 180 µL of Na_2CO_3 (7.5% concentration) was added to the mixture. The mixture was covered with aluminium foil before incubating it at 50°C for 5 min. Using a UV spectrophotometer microplate reader (Zenyth 200rt Biochrom, UK), the absorbance of the samples was read at 760 nm. Acetone solvent was used for extraction and gallic acid was used as the standard phenol compound. The results obtained were recorded as gallic acid equivalents (mg/100 g) from a calibration curve (Anyasi *et al.,* 2015).

Determination of total flavonoid content

The total flavonoid content was determined using a colorimetric method. Approximately 0.5 mL extract was deposited into 15 mL conical flask having 4 mL of distilled water and 0.15 mL of $NaNO_2$ (5% sodium nitrate). After 5 min, a solution of 4 mL of distilled water and 5% of $AICI_3$ was added. The solution was allowed to stand for 5 min after the addition of 1 mL of NaOH. Afterwards, the solution was thoroughly mixed, and 1 mL of NaOH was added again before 15 min incubation. The absorbance was measured at 415 nm with a spectrophotometer. All values were recorded as the mean ± standard deviation in triplicates, and expressed as mg/g (Shen *et al.,* 2009).

Determination of antioxidant activity of biscuits
DPPH (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant activity (DPPH assay) of the biscuit samples was assessed using the method of Anyasi *et al.* (2015). Milled biscuit sample (0.2 g) was weighed and mixed with 2 mL of methanol, and incubated for 30 min (25 ± 2°C) before being centrifuged at 6,000 rpm for a minimum of 10 min at 4°C. Different concentrations of the samples (10, 20, 30, 40, and 50 mg/mL) were used to evaluate the IC_{50} of the sample, which is the minimum amount of antioxidant needed to decrease the first DPPH absorbance by 50%. The value of IC_{50} was obtained by plotting disappearance percentage of DPPH as a function of concentration of the sample. A sample of 0.28 mL together with 0.25 mL DPPH were placed in a microplate and covered by aluminium foil before it was incubated for 1 h at 25 ± 2°C. A UV spectrophotometer microplate reader was used to measure the absorbance at 517 nm. Gallic acid was used as a standard, and results were expressed as percentage inhibition of the DPPH radical.

Ferric reducing antioxidant power (FRAP)

The method of Oyaizu (1986) was followed to determine the FRAP of the biscuits. A volume of about 100 µL of acidified methanol extract was adjusted to 1 mL with methanol in a test tube. About 2.5 mL of 1% potassium ferricyanide and 0.2 M phosphate buffer (pH 6.6) were added to the tube, and vigorously shaken. A water bath was used to keep the mixture for 20 min at 50°C. Approximately 2.5 mL of 10% trichloroacetic acid was added to the mixture after incubation, and centrifuged at 5,000 *g* for 10 min. The mixture was allowed to rest for 30 min, then 2.5 mL distilled water was added to the 2.5 mL of the supernatant and 0.5 mL of 0.1% ferric chloride. Extracts were measured at an absorbance of 700 nm, and results were expressed in mg gallic acid equivalents (GAE) per gram of sample.

Statistical analysis

One-way analysis of variance (ANOVA) was used to conduct the statistical analysis (SPSS Version 24) and averages of results for each experiment were differentiated using the Duncan's multiple range test. All measurements were taken in triplicate, and the results were recorded as mean ± standard deviation. The level of significance of the mean values was assigned at $p < 0.05$.

Results and discussion

Functional properties of wheat and muomva red composite flour

Table 1 shows the functional properties of wheat flour (control) and MRF at various concentrations. Bulk density of flour samples ranged from 0.14 to 1.34 g/mL. Control (100% wheat flour) significantly yielded the lowest bulk density. The bulk density of the flour samples generally increased with the increase in MRF concentration. The increase in bulk density is attributed to the hard banana flour starch polymer structure. The structure of starch polymer has been reported to influence bulk density of flour with loose starch polymer contributes to low bulk density (Plaanmi, 1997). Flours with high bulk densities (> 0.7 g/mL) are utilised as thickeners in food products, therefore, MRF could also be used as thickeners.

There was a significant difference between the water absorption capacity (WAC) of control (lowest; 1.25 g/g) and MRF, with the highest value observed in 25% MRF (2.85 g/g). The low WAC in control might be attributed to the loose amylopectin and amylose association in the natural starch granule, and weak forces sustaining the structure of granules (Lorenz and Collins, 2009). Higher WAC values observed in MRF samples could be attributed to high amylose and dietary fibre content in the banana flour. The addition of MRF increased the water

Table 1. Functional properties of wheat and *muomva red* composite flour.

Parameter	Concentration (%)				
	Control	10% MRF	15% MRF	20% MRF	25% MRF
BD (g/mL)	0.14 ± 0.01[a]	1.29 ± 0.02[c]	1.34 ± 0.02[c]	1.22 ± 0.02[b]	1.31 ± 0.12[c]
WAC (g/g)	1.25 ± 0.10[a]	1.88 ± 0.10[b]	2.12 ± 0.12[c]	2.26 ± 0.04[d]	2.85 ± 0.08[e]
OAC (g/g)	0.99 ± 0.03[a]	1.07 ± 0.04[b]	1.23 ± 0.02[c]	1.42 ± 0.04[d]	1.47 ± 0.03[e]
SC (mL)	1.03 ± 0.01[a]	1.14 ± 0.04[b]	1.99 ± 0.03[d]	2.04 ± 0.07[e]	1.88 ± 0.65[c]
WSI (g/g)	5.37 ± 0.05[e]	5.15 ± 0.11[d]	5.03 ± 0.08[c]	4.92 ± 0.19[b]	4.23 ± 0.07[a]

Values are means ± standard deviation ($n = 3$). Mean values in the same row with different superscripts are significantly different from each other ($p < 0.05$). BD = bulk density, WAC = water absorption capacity, OAC = oil absorption capacity, SC = swelling capacity, WSI = water solubility index, Control = 100% wheat flour, and MRF = *muomva red* flour.

binding ability which is good for improving the reconstitution ability and texture characteristics of the dough from both banana and wheat flours (Adebowale *et al.,* 2012). High WAC of MRF samples suggest their potential to be used in the production of foods such as processed cheese, sausages, and bakery products. These results are in line with those by Ade-Omowaye *et al.* (2008) on tigernut and wheat composite flour and bread.

For oil absorption capacity (OAC), there was significant difference between control and MRF samples, ranging from 0.99 to 1.47 g/g. The lowest value was observed in control (0.99 g/g), and the highest value in 25% MRF (1.47 g/g). The increase in OAC of MRF samples could probably be due to the difference in availability of non-polar side chain that might stick hydrocarbon chain of the oil in the flours. High values were attributed to the improved hydrophobic properties of proteins in the banana flour. The proteins available in the flour that are bound physically to fat through capillary action exhibits the OAC. Hydrophobicity is enhanced as these proteins uncover several non-polar amino acids, resulting in the absorption of oil by flours (Badar, 2013). MRF samples in the present work have the potential to be used in the food industry for flavour retention and shelf-life extension, especially in bakery or meat production where fat absorption is needed.

The extent of binding forces in the granule is indicated by its swelling capacity (SC). The SC values differed significantly with the lowest value exhibited by control (1.03 mL), and the highest in 20% MRF (2.04 mL). The variation in SC depends on how much the internal structure of the starch present in the flour is exposed to water action; the internal structure of control was more exposed than that of MRF samples. The low SC of control suggests the presence of stronger bonding forces within the inside of starch granules, and more amylose lipid complex.

The water solubility index (WSI) of the flour samples was significantly different to each other, ranging from 4.23 (25% MRF) to 5.37 g/g (control). Low WSI values might be the result of high resistant starch content of MRF due to its state of unripeness (Tribess *et al.,* 2009). This shows that the MRF samples could have a positive impact on health. The benefits include good colonic health and microflora, diabetes management, and low glycaemic index and blood cholesterol levels (Ashwar *et al.,* 2016). A similar observation was reported by Sharma *et al.* (2017) on rice and banana flour based extruded snacks.

Thermal properties of wheat and muomva red composite flour

The effect of MRF addition on the thermal properties is given in Table 2. Based on Table 2, T_o ranged from 70.25 to 109.41°C, T_p ranged from 72.59 to 116.21°C, and T_e from 91.07 to 123.21°C. The results showed an increase in all the temperatures (T_o, T_p, and T_e) with the increase in MRF concentration. This is because the starch in MRF is more concentrated with resistant starch, therefore it is difficult to destruct (Borges *et al.,* 2009; Anyasi *et al.,* 2015). Moreover, the increase in all temperatures of MRF samples is also attributed to the low damaged starch content of banana flour, which allowed strong bond formation between starch granules and water molecules (Fredriksson *et al.,* 1998). Change in binding energy between starch and water molecules have been shown to contribute to the specific heat of a material (Oladunmoye *et al.,* 2010). The ΔH values of the flours differed significantly with the lowest value observed in control. Total starch content, amylose-amylopectin ratios, and size of the starch granule are factors that explain the higher ΔH value for MRF samples (Bi *et al.,* 2017).

Table 2. Thermal properties of wheat and *muomva red* composite flour.

Thermal property	**Concentration (%)**				
	Control	**10% MRF**	**15% MRF**	**20% MRF**	**25% MRF**
T_o (°C)	70.25 ± 0.95^a	81.29 ± 0.46^b	88.47 ± 0.53^c	102.29 ± 0.40^d	109.41 ± 0.21^e
T_p (°C)	72.59 ± 0.89^a	85.88 ± 0.71^b	101.37 ± 0.52^c	110.13 ± 0.27^d	116.21 ± 0.36^e
T_e (°C)	91.07 ± 1.26^a	103.61 ± 0.62^b	109.05 ± 0.33^c	119.93 ± 0.22^d	123.21 ± 0.22^e
ΔH (j/g)	10.23 ± 0.89^a	12.03 ± 0.71^b	14.65 ± 0.32^c	15.56 ± 0.53^d	17.42 ± 0.47^e

Values are means ± standard deviation ($n = 3$). Mean values in the same row with different superscripts are significantly different from each other ($p < 0.05$). T_o = onset temperature, T_p = peak temperature, T_e = end temperature, Δ H = enthalpy of gelatinisation, Control = 100% wheat flour, and MRF = *muomva red* flour.

Colour measurement of wheat and muomva red composite biscuits

Colour measurement is important in the appearance of food products. It affects acceptability of foods, sensory quality, and the preference by consumers. Upon addition of MRF at different concentrations (Table 3), the L*, a*, b*, C*, and Hue values obtained varied significantly. The L* values of control was 55.60 (highest) as compared to MRF samples. The L* values of biscuits at different ratios decreased from 52.63 (10% MRF) to 41.43 (25% MRF). The decrease in L* value of biscuits could be due to processing of banana flour where slices were oven-dried, giving them a brown colour, thus leading to a yield of darker banana flour. This observation is supported by Falade and Olugbuyi (2010) who reported that oven-drying reduces the whiteness of the flour. The a* values varied significantly between control and MRF samples. The values of a* increased from 3.27 (control) to 4.93 in 25% MRF. The b* values, however, decreased from 25.90 (control) to 14.47 (25% MRF). The C* values of food products increase with the increase in pigment concentration, and decrease when the sample becomes darker (Wrolstad and Smith, 2010). This was also observed in the present work where control had a high value of 26.50, and when the MRF was added, the C* value decreased to 15.06. These results are comparable to those by Wrolstad and Smith (2010) on colour analysis in foods with banana starch.

Physical properties of wheat and muomva red composite biscuits

The effect of the addition of MRF at various concentrations on the physical properties of biscuits is shown in Table 4. There was no significant difference between the diameter of control and MRF at different concentrations, with values ranging from 36.01 to 36.03 mm. This is probably due to the consistency of circle cookie cutter. Biscuits with high diameter and spread ratio are more desirable to consumers because of crispiness (Sarabha and Prabhasankar, 2015).

Similar to the diameter, no significant difference was observed between thicknesses of the biscuits. The higher amount of fibre and lower starch contribute to small dimension of biscuits. Similar results were reported by Chinma *et al.* (2012) on biscuits produced from composite flours.

The weight differed significantly between control and MRF samples. The highest value was recorded in 25% MRF (10.51 g). The high starch content in MRF and less crude fibre amount in control could be the reason for this observation (Chinma *et al.,* 2012). The increase in weight of MRF samples could also be due to high bulk density

Table 3. Colour profile of wheat and *muomva red* composite flour.

Colour property	Concentration (%)				
	Control	10% MRF	15% MRF	20% MRF	25% MRF
L*	55.60 ± 0.20^{e}	52.63 ± 0.05^{d}	49.30 ± 0.36^{c}	47.26 ± 0.57^{b}	41.43 ± 0.57^{a}
a*	5.50 ± 0.61^{e}	3.27 ± 0.12^{a}	4.13 ± 0.32^{b}	4.37 ± 0.58^{c}	4.93 ± 0.11^{d}
b*	25.90 ± 0.10^{e}	17.78 ± 0.58^{c}	18.57 ± 0.12^{d}	16.90 ± 0.10^{b}	14.47 ± 0.06^{a}
C*	26.50 ± 0.10^{e}	18.07 ± 0.56^{c}	19.23 ± 0.42^{d}	17.50 ± 0.10^{b}	15.06 ± 0.06^{a}
Hue	77.6 ± 0.10^{b}	79.2 ± 0.11^{c}	77.6 ± 0.10^{b}	71.5 ± 0.06^{a}	71.2 ± 0.06^{a}

Values are means ± standard deviation ($n = 3$). Mean values in the same row with different superscripts are significantly different from each other ($p < 0.05$). Control = 100% wheat flour, and MRF = *muomva red* flour.

Table 4. Texture profile and physical properties of wheat and *muomva red* composite biscuits.

Physical property	Concentration (%)				
	Control	10% MRF	15% MRF	20% MRF	25% MRF
Diameter (mm)	36.02 ± 0.02^{a}	36.03 ± 0.01^{a}	36.02 ± 0.01^{a}	36.01 ± 0.01^{a}	36.03 ± 0.01^{a}
Thickness (mm)	13.04 ± 0.01^{a}	13.03 ± 0.01^{a}	13.0 ± 0.11^{a}	13.03 ± 0.01^{a}	13.04 ± 0.01^{a}
Weight (g)	8.13 ± 0.21^{a}	9.54 ± 0.31^{b}	9.85 ± 0.08^{c}	10.15 ± 0.22^{d}	10.51 ± 0.12^{e}
Spread ratio	2.65 ± 0.01^{a}	2.64 ± 0.01^{a}	2.63 ± 0.01^{a}	2.63 ± 0.01^{a}	2.61 ± 0.01^{a}
Break force (N)	0.85 ± 0.04^{a}	1.31 ± 0.03^{b}	1.40 ± 0.01^{c}	1.63 ± 0.33^{d}	1.73 ± 0.05^{e}
Fracturability (N)	0.26 ± 0.01^{a}	0.31 ± 0.01^{b}	0.31 ± 0.01^{b}	0.32 ± 0.03^{b}	0.32 ± 0.01^{b}

Values are means ± standard deviation ($n = 3$). Mean values in the same row with different superscripts are significantly different from each other ($p < 0.05$). Control = 100% wheat flour, and MRF = *muomva red* flour.

of MRF.

There was no significant difference between the spread ratio of the biscuits. The slight decrease in the spread ratio of MRF samples with the increase in MRF concentrations could be attributed to low content of protein in banana flour. An increase in the number of hydrophilic sites due to increase in protein amount causes a competition for inadequate free water in the dough of the biscuit, which results in decrease in spread ratio (Nasir *et al.,* 2010). Low spread ratio could also be attributed to the hygroscopic state of MRF which absorbs moisture, thus resulting in the reduction of spread ratio (Chinma *et al.,* 2012).

There was significant difference between the break force of control and MRF samples. The highest break force value was observed in 25% MRF (0.173 N), and the lowest in control. The results indicate that more force was required to break MRF biscuits than control biscuits. This is associated with the highly organised resistant starch and amylopectin in MRF. The breaking force indicates the crispiness of the biscuit samples, with lower breaking force corresponding to higher crispiness. Crispy biscuits are expected to exhibit lower breaking force due to their crispy/crunchy texture, which allows them to attain the break point quicker (Idowu, 2014). This means that MRF biscuits in the present work had less crispy/crunchy texture as compared to control biscuits.

There was a significant difference between fracturability of control and MRF biscuits. The highest fracturability value of 0.32 N was observed in biscuits with 25% MRF, and the lowest value was 0.26 N in control biscuits. The fracturability increased with the increase in MRF concentration. This could be explained by looser matrix formation in these formulations which contained lower levels of proteins. Although MRF has no gluten, it does provide a base for dough formation. Similar results were obtained by Öksüz and Karakaş (2016) on gluten free biscuits containing buckwheat.

Bioactive compounds and antioxidant activity composite biscuits

The total phenolic contents (TPC) of control and MRF samples were significantly different (Table 5). The lowest value was 1.12 mg GAE/100g (control), and the highest 162.73 mg GAE/100g (25% MRF). This could be attributed to the fact that the TPC of baked wheat-banana products increases with the increase in proportions of banana flour (Zuwariah and Aziah, 2009). UBF is rich in TPC, and it contains flavonoids such as catechin, gallocatechin, and tannin. It also contains antioxidant compounds such as dopamine in large amounts (Someya *et al.,* 2002). Phenolic compounds commonly found in edible and non-edible parts of fruits constitute some of the major groups of compounds that act as free radical terminators in humans (Sulaiman *et al.,* 2011). Polyphenols derived from fruits and vegetables are said to be the most important antioxidants.

The total flavonoid contents (TFC) of control and MRF samples was significantly different, ranging from 10.32 to 373.97 mg CEQ/100g. In general, the TFC was higher in MRF biscuits than control biscuits. This is because banana flour has high amount of flavonoids than wheat flour, as reported by Mohapatra *et al.* (2010). Consumption of MRF biscuits full of flavonoids can benefit humans, as these interact with different biological systems, and show antioxidant and hypoglycaemic activities.

There was significant difference between FRAP of control and MRF biscuits. The lowest value was in control (0.01 mg/g). This could be attributed to the low amount of polyphenols in control (Lim *et al.,* 2007). The highest FRAP value was 2.11 in biscuits with 25% MRF, and this could be due to the high amount of polyphenols in banana flour. The higher reducing potential is correlated with a higher amount of polyphenolics.

The DPPH differed significantly between control and MRF biscuits, ranging from 0.01 (control) to 0.42 mg/g (25% MRF). A higher DPPH

Table 5. Bioactive compounds and antioxidant activity of wheat and *muomva red* composite biscuits.

Parameter	Concentration (%)				
	Control	10% MRF	15% MRF	20% MRF	25% MRF
TPC (mg/g)	1.12 ± 0.49^{a}	44.24 ± 0.58^{b}	95.45 ± 1.42^{c}	120.30 ± 0.41^{d}	162.73 ± 0.99^{e}
TFC (mg/g)	10.32 ± 0.31^{a}	385.71 ± 1.42^{d}	376.19 ± 2.18^{c}	290.95 ± 0.81^{b}	373.97 ± 1.11^{c}
FRAP (mg/g)	0.01 ± 0.32^{a}	0.99 ± 0.23^{b}	1.21 ± 0.17^{C}	1.73 ± 0.21^{d}	2.11 ± 0.03^{e}
DPPH (%)	0.01 ± 0.05^{a}	0.16 ± 0.002^{b}	0.23 ± 0.03^{c}	0.31 ± 0.02^{d}	0.42 ± 0.04^{e}

Values are means ± standard deviation (n = 3). Mean values in the same row are significantly different ($p < 0.05$). AA = ascorbic acid, TPC = total phenolic content, TFC = total flavonoids content, control = 100% wheat flour, and MRF = *muomva red* flour.

value is related to stronger antioxidant activity, and a lower value is associated with a weaker antioxidant activity. The inhibition of DPPH follows the same order as the TFC and TPC, *i.e.*, when the concentration of phenolic compounds increases, the DPPH also increases. This observation is supported by Lim *et al.* (2007) who reported that DPPH increases as the amount of fruit increases because of the presence of ascorbic acid and polyphenols.

Conclusion

The inclusion of MRF increased the bulk density, water absorption capacity, oil absorption capacity, and swelling capacity; whereas water solubility index of wheat-banana composite flour decreased. The onset temperature, peak temperature, and end temperature of MRF also increased. The inclusion of MRF also increased the textural and physical properties of the biscuits such as fracturability, weight, break force, and spread ratio. However, the addition of MRF to wheat flour resulted in darker flour, but the bioactive compounds and antioxidant activity of the biscuits increased.

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