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BIOACTIVE COMPONENTS, ENZYMES INHIBITORY AND ANTIOXIDANT ACTIVITIES OF BIOFORTIFIED YELLOW MAIZE (*ZEA MAYS* L.) AND COWPEA (*VIGNA UNGUICULATA* L. WALP) COMPOSITE BISCUITS

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Abstract

This study evaluated the bioactive components, enzymes inhibitory and antioxidant activities of biofortified yellow maize (YM) and cowpea (CP) composite biscuits. Composites of YM and CP, mixed at the ratios of 100:0 (YM); 75:25 (YMCP-1); 50:50 (YMCP-2); 25:75 (YMCP-3) and 0:100 (CP), were used to bake composite biscuits designated YM-B, YMCP-1B, YMCP-2B, YMCP-3B and CP-B, respectively. Refined wheat flour (WT) served as the control biscuit (WT-B). The bioactive components (total carotenoids, total phenolics, tannins, total flavonoids and total saponins), enzymes (pancreatic lipase, α-amylase, α-glucosidase) inhibitory and antioxidant (ABTS^{*+}, DPPH* scavenging and reducing power) activities of the flours and biscuits were determined. Total carotenoids content increased significantly ($p < 0.05$) with increasing proportion of YM, while total phenolics, tannins, total flavonoids and saponins contents, enzymes inhibitory and antioxidant activities increased with increasing proportion of CP in the composite flours and biscuits. Among the composite biscuits, YMCP-3B had the strongest ($p < 0.05$) enzymes inhibitory and antioxidant activities. The composite biscuits, especially YMCP-3B, may serve as functional biscuits for retarding the rate of fatty acids and glucose formation, and mitigating oxidative stress, which represent a clinical strategy for managing obesity and type 2 diabetes.

Keywords: bioactive components, composite biscuits, cowpea, digestive enzymes, yellow maize

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Introduction

Obesity, indicated by a body mass index (BMI) of 30 kg/m^2 or above, affects both adults and children (Alzaman and Ali, 2016; Bae *et al*., 2016). In the year 2015, 107.7 million children and 603.7 million adults had obesity globally (GBD, 2017); making it one of the greatest global health challenges in this century. It is characterized by the accumulation of excess fat in the adipose tissue, due to higher energy intake relative to energy expenditure (Hossain *et al*., 2007). Obesity, especially visceral obesity, is a major risk factor for type 2 diabetes (T2D), with about 80% of patients suffering from T2D being overweight or obese (Gomez-Ambrosi *et al*., 2011; Wander *et al*., 2012). Furthermore, it increases the odds of developing many common diabetic complications, such as cardiovascular diseases, retinopathy and dyslipidaemia in patients with T2D (American Diabetes Association, 2014). Obesity and T2D have oxidative stress as a denominator (Rani *et al*., 2016).

An important clinical approach for treating obesity and T2D is by inhibiting pancreatic lipase, α-amylase and α-glucosidase (Villiger *et al*., 2015; Irondi *et al*., 2018a). Pancreatic lipase, an enzyme component of the pancreatic juice, catalyzes the hydrolysis of dietary triacylglycerol to fatty acids and monoacylglycerols in the intestine (Li *et al*., 2011). Its inhibition slows down the production of fatty acids and their subsequent absorption, thereby serving as an index for testing the efficacy of a potential anti-obesity agent (Sugiyama *et al*., 2007). Similarly, α-amylase and α-glucosidase catalyze the digestion of dietary carbohydrates. First, α-amylase present in the small intestine hydrolyzes the α-1,4 bonds of starch, releasing oligosaccharides and disaccharides. The oligosaccharides and disaccharides are subsequently hydrolyzed by α-glucosidase present in the brush border of the small intestine, producing absorbable monosaccharides, such as glucose and fructose (Tucci *et al*., 2010). Hence, α-amylase and α-glucosidase inhibition is a key clinical approach for managing postprandial hyperglycaemia in T2D, and a well-documented mechanism by which several anti-diabetic agents express their effects (Irondi *et al*., 2018a; Kim *et al*., 2005).

The intake of energy-dense foods, characterized by high fat and sugar contents, promotes the development of obesity and T2D (Martinez-Saez *et al*., 2017). On the other hand, foods with a low sugar and a high fiber contents are beneficial in improving satiety, controlling blood glucose levels and body weight gain (Van *et al*., 2012). Consequently, the food industry has intensified efforts on producing high-fiber, low-fat/low-calorie foods, such as biscuits, in response to the public health concerns associated with the consumption of energy-dense foods in recent time. In doing so, biscuits made from whole grains were identified as good sources of bioactive components (Cukelj *et al*., 2017).

Biscuits are baked food products that are well-liked for their ready-to-eat nature, high nutritive value, and availability in various sizes and shapes at an affordable price. Usually, biscuits are formulated to be rich in fat and sugar, which makes consumers that are calorie-conscious to keep them off (Aggarwal *et al*., 2016). In addition to being an energy-dense snack, biscuits are deficient in the bioactive

components of grain, including phytochemicals and dietary fiber that are beneficial to health due to the use of refined ingredients in baking them (Fardet, 2010). In an effort to produce functional biscuits for the calorie-conscious people and those suffering from obesity, T2D and other non-communicable diseases, the major ingredients of the biscuits are modified. Such modification includes use of artificial sweeteners, fat replacers and replacement of part or whole of the wheat flour with whole composite or multigrain flour (Aggarwal *et al*., 2016). Whole grains are rich sources of bioactive components, such as dietary fiber, polyphenolics and saponins that play important role in protecting against obesity and T2D (Irondi *et al*., 2019a).

Biofortified yellow maize (*Zea mays* L.) is an important dietary source of biologically active compounds, including polyphenolic compounds, carotenoids, anthocyanins and vitamin C (Alamu *et al*., 2018; Beta and Hwang, 2018). Health benefits such as antioxidant, antidiabetic and anti-obesity activities (Irondi *et al*., 2019a; Žilić *et al*., 2012) have been attributed to these bioactive components that are found in yellow maize. Similarly, cowpea (*Vigna unguiculata* L. Walp), a drought- and heat-tolerant grain legume, is a rich dietary source of protein, calories, vitamins and essential minerals for humans, especially in the semi-arid parts of the world (Sreerama *et al*., 2012; Awika and Duodu, 2017). It is also an important source of bioactive components such as peptides (Segura-Campos *et al*., 2011) and polyphenolic compounds (Irondi *et al*., 2019b) that have some health benefits.

The health benefits derivable from biofortified yellow maize, cowpea and their products can be optimized when they are consumed as composites rather than as individual food due to the synergy among their bioactive compounds. Hence, this study evaluated the bioactive components, enzymes inhibitory and antioxidant activities of biofortified yellow maize and cowpea composite biscuits.

Materials and methods

Chemicals and reagents

Alpha-amylase, *Bacillus stearothermophillus* α-glucosidase, porcine pancreatic lipase, acarbose, orlistat, *p*-nitrophenyl butyrate, trolox, quercetin, L-ascorbic acid, 2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic (ABTS), 2,2-diphenylpicrylhydrazyl (DPPH), diosgenin, and gallic acid were Sigma (St. Louis, USA) products. All other chemicals used in the experiments were analytical grades.

Samples collection and preparation

Samples (1 kg each) of biofortified yellow maize (provitamin A yellow maize) and cowpea (IT10K-837-1) were procured from the Institute of Agricultural Research and Training, Ibadan, Nigeria, and the Department of Crop Production, Kwara State University, Malete, Nigeria, respectively. Subsequently, the samples were sorted and 200 g portion of each was ground into whole meal flour, which was

packed hermetically in sample containers and stored at -4°C during the experiments.

Preparation of composite flours

The whole meal flours of biofortified yellow maize (YM) and cowpea (CP) were mixed at the ratios of 100:0; 75:25; 50:50; 25:75; 0:100 to obtain yellow maize and cowpea composite flours designated YM, YMCP-1, YMCP-2, YMCP-3 and CP, respectively. Refined wheat flour (WT) served as a control flour.

Formulation and baking of biscuits

The method described by Saha *et al*. (2011) was adopted to formulate the biscuits, with a slight modification. Sugar-free composite biscuits were formulated from the YM and CP composite flours (YM, YMCP-1, YMCP-2, YMCP-3 and CP) and were coded YM-B, YMCP-1B, YMCP-2B, YMCP-3B and CP-B, respectively. Refined wheat flour (WT) was used for the control biscuit (WT-B). Biscuit dough was prepared according to the following formula: flour 100 g, shortening 30 g, sodium chloride 0.6 g, sodium bicarbonate 0.3 g, ammonium bicarbonate 0.6 g, water 20 mL. The flour and shortening agent were thoroughly mixed for 5 minutes, using a kitchen mixing apparatus. Subsequently, dough water containing sodium bicarbonate, ammonium bicarbonate, sodium chloride and milk powder, as specified in the formula, was added to the mixture of the flour and shortening agent, and mixed thoroughly to obtain a uniform dough. Thereafter, the dough of each flour was kneaded with a rolling pin to obtain a uniform thickness. Thereafter, biscuits were cut into regular shapes with a cutter (diameter: 43 mm) and baked on a tray covered with aluminium foil at 210°C for 10 minutes. After baking, the biscuits (Figure 1) were allowed to cool for 30 minutes, and stored in hermetic plastic containers for further analysis.

Figure 1. Biscuits samples: A-cowpea biscuit (CP-B); B-25:75 (% w/w) yellow maize and cowpea composite biscuit (YMCP-3B); C-50:50 (% w/w) yellow maize and cowpea composite biscuit (YMCP-2B); D-75:25 (% w/w) yellow maize and cowpea composite biscuit (YMCP-1B); E-yellow maize biscuit (YM-B); F-refined wheat flour biscuit (WT-B)

Preparation of flour and biscuit extracts

Extracts of flours and pulverized biscuit samples were prepared by soaking 2 g of each sample in 20 mL of absolute methanol overnight. Thereafter, the mixture was centrifuged at 4000 rpm for 5 minutes, and the supernatant was collected by filtering through a filter paper (Whatman No. 2). The filtrate was concentrated at 45°C with the aid of a rotary evaporator, following which the extract was reconstituted with 6 mL of methanol (Engida *et al*., 2013).

Determination of bioactive constituents (total carotenoids, total phenolics, total flavonoids, tannins and total saponins)

Total carotenoid content of the samples was quantified as per the method described by Rodriguez-Amaya (1999). The method reported by Singleton *et al*. (1999) was followed to determine the total content of phenolics in the samples, and the results were presented in milligram gallic acid equivalent per gram of sample (that is, mg GAE/g). The level of total flavonoids in the samples was quantified based on the method reported by Meda *et al*. (2005), and the results were presented in milligram quercetin equivalent per gram of sample (mg QE/g). The tannins level of the samples, presented in milligram tannic acid equivalent per gram of sample (that is, mg TAE/g) was analyzed by adopting the method reported by Amorim *et al*. (2008). For the quantification of total saponins level of the samples, the method described by Makkar *et al*. (2007) was adopted, and the results were presented in milligram diosgenin equivalent per gram of sample (that is, mg DE/g).

Enzymes inhibitory assays

Determination of pancreatic lipase inhibitory activity

The pancreatic lipase inhibitory activity of samples was determined according to the protocol reported by Eom *et al*. (2013), in which orlistat was used as a reference inhibitor of pancreatic lipase. The enzyme solution was prepared by mixing 30 µL of pancreatic lipase (10 units, in morpholinepropane sulphonic acid, 10 mM, and EDTA, 1 mM of pH 6.8) with 850 µL of Tris buffer (containing Tris-HC1, 100 mM, and $CaCl_2$, 5 mM; pH 7.0). Thereafter, a mixture of 100 µL of different dilutions of the sample extract (or orlistat) and 880 µL of the enzyme solution was subjected to incubation for 10 minutes at 37ºC. Subsequently, 20 µL of the substrate, comprising *p*-nitrophenyl butyrate (10 mM) in dimethyl formamide, was dispensed into the mixture, and the hydrolytic reaction proceeded for 20 minutes at 37ºC. Finally, the absorbance of the *p*-nitrophenol formed by the hydrolytic reaction was read at 405 nm, and the inhibition (%) of pancreatic lipase by the extract was calculated.

Determination of α-amylase inhibitory activity

The capacity of the samples to inhibit α-amylase was determined following the protocol described by Kwon *et al*. (2008), using acarbose as a standard inhibitor. A mixture of equal volume (500 µL) of varied concentrations of the extract of sample and sodium phosphate buffer solution (0.02 M; pH 6.9, containing NaCl, 0.006 M), containing 0.5 mg/mL of α-amylase was subjected to incubation for 10 minutes at 37°C. Afterwards, 500 µL of the substrate solution (1 g soluble starch dissolved in

0.02 M sodium phosphate buffer) was dispensed into the reaction mixture, and it was subjected to incubation for 15 minutes at 37°C. Starch hydrolysis was terminated by adding 1.0 mL of DNSA reagent (comprising 3, 5-dinitrosalicylic acid, 1%, and sodium potassium tartrate, 12%, in 0.4 M NaOH), followed by 5 minutes of incubation in a water bath at 100°C. The reaction mixture was cooled to room temperature and diluted with 10 mL of water (distilled). Finally, the absorbance reading was measured at 540 nm, and the inhibition (%) of α-amylase activity by the extract was calculated.

Analysis of α-glucosidase inhibitory activity

The ability of the samples to inhibit the activity of α-glucosidase was determined following the protocol reported by Kim *et al*. (2005), in which acarbose served as a standard inhibitor. A mixture, containing α-glucosidase (50 µL) and different concentrations of extract (50 µL), was subjected to incubation for 10 minutes at 37°C. Thereafter, 100 µL of 3 mM *p*-nitrophenylglucopyranoside (PNPG) dissolved in phosphate buffer (20 mM, pH 6.9) was dispensed into the reaction mixture to initiate the hydrolytic reaction. The hydrolytic reaction proceeded for 20 minutes at 37°C, after which a volume of 2 mL of Na_2CO_3 (0.1 M) was added to terminate it. Next, the absorbance of the *p*-nitrophenol formed by the hydrolysis of PNPG was read at 400 nm, and the percentage inhibition of α-glucosidase by the extract was calculated.

Antioxidant activity assays

Determination of 2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) scavenging activity

The $ABTS^{*+}$ scavenging activity of samples, expressed as trolox equivalent antioxidant capacity (TEAC), was analyzed as per the protocol reported by Re *et al*. (1999). To prepare the working reagent of $ABTS^{*+}$, a mixture of equal amount (v:v) of aqueous solutions of 2.45 mM $K_2S_2O_8$ and 7 mM $ABTS^{*+}$ was subjected to incubation at room temperature in a dark condition for 16 hours. The absorbance reading (0.70 ± 0.02) of the working $ABTS^{*+}$ reagent at 734 nm was acheived by diluting it with ethanol, 95%. Thereafter, 2.0 mL of the $ABTS^{*+}$ reagent was mixed with 0.2 mL of the sample extract, and the mixture was incubated in a dark condition at room temperature for 15 minutes. The absorbance of the sample was read at 734 nm, and the $ABTS^{*+}$ scavenging activity was subsequently calculated from a calibration curve prepared using trolox.

Determination of 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^{*}$) scavenging activity

The $DPPH^{*}$ scavenging activity of samples was determined based on the procedure reported by Cervato *et al*. (2000). Ascorbic acid was used as a standard antioxidant. A total of 1.0 mL of varied extract concentrations (or ascorbic acid) was mixed with 3.0 mL of $DPPH^{*}$ solution (60 µM), and the mixture was incubated in the dark condition for 30 minutes at room temperature. Afterwards, the absorbance reading was taken at 517 nm, and the ability of the sample extract to scavenge $DPPH^{*}$ was calculated.

Determination of reducing power

The reducing power of samples was analyzed based on the procedure reported by Oyaizu (1986). In brief, 2.5 mL of sample extract, 2.5 mL of 200 mM sodium phosphate buffer, pH 6.6, and 2.5 mL of 1% potassium ferricyanide were mixed and subjected to incubation for 20 minutes at 50ºC. Afterwards, 2.5 mL of 10% trichloroacetic acid was dispensed into the reaction mixture, which was later divided into 2.5 mL aliquots in different test tubes. The content of each test tube was diluted with 2.5 mL of distilled H_2O, followed by addition of 1 mL of ferric chloride solution (0.1%). Subsequently, the absorbance reading was taken at 700 nm, and the reducing power of the samples was calculated based on gallic acid calibration curve.

Data analysis

One-way analysis of variance (ANOVA) was performed on the results of three independent determinations. Mean values were compared by least significant difference (LSD) test at $p < 0.05$, using version 17 of SPSS statistical software. The IC_{50} (that is, the concentration of each sample that caused 50% inhibition of enzyme activity and the SC_{50} (that is, the concentration of each sample that scavenged DPPH* by 50%) were calculated using Graphpad Prism®, version 4.0 (Sandiego, CA).

Results and discussion

Bioactive constituents

Table 1 presents the bioactive constituents of the yellow maize (YM) and cowpea (CP) composite flours and biscuits. YM had the highest ($p < 0.05$) total carotenoids content, while CP had the highest ($p < 0.05$) total phenolics, tannins, total flavonoids and saponins contents. The total carotenoids content of the YM obtained in this study (20.57±0.42 µg/g) agrees closely with the concentration of total carotenoids (20.4 ± 0.2 µg/g) reported by Taleon *et al*. (2017) in biofortified orange maize variety. In contrast, the total phenolics content of yellow maize (300.25 mg/100 g, equivalent to 3.00 mg/g) reported by Oboh *et al*. (2010) is higher than the total phenolics content of the YM (2.08±0.03 mg/g) obtained in this study. Also, the total phenolics and flavonoids of the CP in this study (9.20±0.06 and 3.92±0.08 mg/g, respectively) are lower than the total phenolics and flavonoids contents (12.16 ± 0.27 and 7.24 ± 0. 20 mg/g, respectively) reported by Sreerama *et al*. (2012).

The level of total carotenoids increased significantly ($p < 0.05$) with increasing proportion of YM, while the levels of total phenolics, tannins, total flavonoids and saponins increased with increasing proportion of CP in the composite flours and biscuits. Thus, YMCP-1 (composed of 75% YM and 25% CP) and its corresponding biscuit, YMCP-1B, had a significantly ($p < 0.05$) higher level of total carotenoids, while YMCP-3 (comprising 25% YM and 75% CP) and its counterpart biscuit, YMCP-3B, had significantly ($p < 0.05$) higher levels of total phenolics, tannins, total flavonoids and saponins, than the other composite flours

and biscuits. These trends suggest that YM was the major source of carotenoids, while CP was the major source of polyphenolics (total phenolics, tannins, total flavonoids) and saponins in the composite flours and biscuits. However, refined wheat flour (WT) and its corresponding biscuit (WT-B) had the lowest levels of all the bioactive components.

Table 1. Bioactive constituents of yellow maize and cowpea composite flours and biscuits

Samples	Total carotenoids (µg/g)	Total phenolics (mg GAE/g)	Tannins (mg TAE/g)	Total flavonoids (mg QE/g)	Total saponins (mg DE/g)
			Flour		
YM	20.57±0.42^{a}	2.08±0.03^{e}	1.98±0.01^{e}	0.93±0.03^{e}	0.92±0.02^{e}
YMCP-1	16.03±0.06^{b}	2.87±0.04^{d}	2.39±0.02^{d}	1.08±0.02^{d}	1.58±0.13^{d}
YMCP-2	9.92±0.21^{c}	3.26±0.06^{c}	2.93±0.04^{c}	1.89±0.04^{c}	2.71±0.06^{c}
YMCP-3	4.00±0.25^{d}	4.07±0.07^{b}	3.26±0.01^{b}	2.65±0.03^{b}	3.74±0.11^{b}
CP	0.94±0.04^{e}	9.20±0.06^{a}	5.71±0.04^{a}	3.92±0.08^{a}	6.19±0.12^{a}
WT	0.21±0.01^{f}	0.63±0.04^{f}	0.53±0.01^{f}	0.15±0.01^{f}	0.28±0.01^{f}
			Biscuits		
YM-B	13.94±0.19^{a}	1.61±0.03^{e}	1.48±0.11^{d}	0.67±0.03^{e}	0.71±0.01^{e}
YMCP-1B	11.08±0.03^{b}	2.15±0.11^{d}	1.69±0.14^{d}	0.85±0.01^{d}	1.23±0.13^{d}
YMCP-2B	6.41±0.20^{c}	2.35±0.12^{c}	2.09±0.01^{c}	1.19±0.12^{c}	2.11±0.11^{c}
YMCP-3B	2.80±0.17^{d}	3.08±0.08^{b}	2.56±0.01^{b}	2.00±0.03^{b}	3.01±0.01^{b}
CP-B	0.52±0.02^{e}	7.00±0.06^{a}	4.38±0.11^{a}	3.00±0.02^{a}	5.04±0.11^{a}
WT-B	0.32±0.01^{e}	0.50±0.02^{f}	0.42±0.01^{e}	0.10±0.00^{f}	0.21±0.01^{f}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$). YM, 100% yellow maize flour; YMCP-1, 75:25 (% w/w) yellow maize and cowpea composite flour; YMCP-2, 50:50 (% w/w) yellow maize and cowpea composite flour; YMCP-3, 25:75 (% w/w) yellow maize and cowpea composite flour; CP, 100% cowpea flour; WT, 100% refined wheat flour; YM-B, yellow maize biscuit; YMCP-1B, 75:25 (% w/w) yellow maize and cowpea composite biscuit; YMCP-2B, 50:50 (% w/w) yellow maize and cowpea composite biscuit; YMCP-3B, 25:75 (% w/w) yellow maize and cowpea composite biscuit; CP-B, cowpea biscuit; WT-B, refined wheat flour biscuit

Carotenoids are well-known for their health benefits including vitamin A activity (Elemosho *et al*., 2020), protection against chronic diseases, for example, cardiovascular diseases (Gammone *et al*., 2017) and antioxidant activity, which has been identified as the main mechanism underpinning their health benefits (Seifried *et al*., 2007). In addition, Sugiura *et al*. (2015) reported that consumption of carotenoids-rich diets may confer protection against the development of T2D. On the other hand, polyphenolics such as tannins and flavonoids are notable for their health benefits, including antidiabetic and antioxidant (Bai *et al*., 2017), anti-hypertensive and anti-obesity Irondi *et al*., 2018b) activities. Similarly, saponins

were reported to possess anti-diabetic (Lu *et al*., 2016), antioxidant (Guo *et al*., 2018) and anti-obesity (Chen *et al*., 2017) activities.

The levels of all the bioactive constituents, except total carotenoids, decreased in the composite biscuits relative to their corresponding flours (Table 1). This decrease may be attributed to the thermal degradation and oxidation of the bioactive constituents induced by heat (Irondi *et al*., 2019a; Rawson *et al*., 2013) during baking. For the phenolic compounds, their oxidation induced by heat is known to promote the formation of Maillard reaction products in food products (Lin *et al*., 2016).

Enzymes inhibitory activity

The results of enzymes (pancreatic lipase, α-amylase and α-glucosidase) inhibitory activity of the yellow maize and cowpea composite flours and biscuits are presented in Table 2 in terms of their IC_{50} values. Among the flours, CP had the lowest IC_{50} values, indicating the strongest inhibitory activity towards the tested enzymes, while WT had the highest IC_{50} value (the weakest inhibitory effect). Relative to the IC_{50} values reported for red sorghum variety against α-amylase, α-glucosidase and pancreatic lipase (16.93 ± 1.08, 10.78 ± 0.63 and 12.72 ± 1.13 µg/mL, respectively) (Irondi *et al*., 2019a), the IC_{50} values of the YM used in this study against the same enzymes (237.12 ± 2.60, 157.18 ± 1.05 and 138.02 ± 1.77 µg/mL, respectively) are higher. Similarly, the IC_{50} values of the CP variety (IT10K-837-1) used in this study against α-amylase, α-glucosidase and pancreatic lipase (147.34 ± 0.80, 97.17±1.20 and 62.93 ± 0.49 µg/mL, respectively) are generally higher than the values earlier reported for Ife Brown, another variety of cowpea, against the same enzymes (132.91 ± 5.37, 76.74 ± 3.02 and 53.08 ± 4.13 µg/mL, respectively) (Irondi *et al*., 2019b). The variations in the IC_{50} values obtained in this study against the tested enzymes and those previously reported, in the case of cowpea, may be attributed to differences in the sample extraction methods, genotype and environmental factors (Mpofu *et al*., 2006).

Consistently, among the composite flours and their counterpart biscuits, the IC_{50} values decreased with increasing proportion of CP, such that YMCP-3 and YMCP-3B had the least IC_{50} values (the strongest inhibitory activity) towards the tested enzymes. Furthermore, the IC_{50} values of the biscuits towards the tested enzymes increased relative to their corresponding flours, indicating a decreasing inhibitory activity. This may be attributed to the observed decrease in the levels of bioactive constituents in the biscuits, in comparison to their corresponding flours (Table 1). The bioactive constituents analyzed in the flours and biscuits, including total phenolics, tannins, total flavonoids (phenolic compounds) and saponins, have been shown to inhibit digestive enzymes, including pancreatic lipase, α-amylase and α-glucosidase (Irondi *et al*., 2019a; Liu and Xu, 2015). Phenolic compounds, for instance, have a high affinity for proteins through hydrogen and hydrophobic interactions; a property that enables them to inhibit digestives enzymes by protein denaturation (Villiger *et al*., 2015). Consequently, the thermal degradation and heat-induced oxidation of the bioactive constituents was accompanied by a decrease in their enzymes inhibitory activity.

Table 2. IC_{50} values of yellow maize and cowpea composite flours and biscuits extracts on α-amylase, α-glucosidase and pancreatic lipase activity.

Samples	α-amylase (µg/mL)	α-glucosidase (µg/mL)	Pancreatic lipase (µg/mL)
	Flours		
YM	237.12±2.60^{b}	157.18±1.05^{b}	138.02±1.77^{b}
YMCP-1	208.36±1.61^{c}	135.98±1.82^{c}	106.75±1.41^{c}
YMCP-2	171.45±1.09^{d}	112.20±1.06^{d}	80.72±0.40^{d}
YMCP-3	160.14±0.71^{de}	105.17±0.62^{de}	71.39±0.94^{e}
CP	147.34±0.80^{e}	97.17±1.20^{e}	62.93±0.49^{f}
WT	1353.47±16.94^{a}	891.66±11.16^{a}	343.50±3.66^{a}
	Biscuits		
YM-B	274.69±2.99^{b}	181.69±2.35^{b}	166.85±2.72^{b}
YMCP-1B	241.62±1.98^{c}	152.52±2.88^{c}	124.19±1.93^{c}
YMCP-2B	199.17±1.58^{d}	137.41±1.92^{d}	96.02±1.87^{d}
YMCP-3B	186.66±2.94^{e}	121.39±1.41^{e}	83.38±1.75^{e}
CP-B	171.44±1.90^{f}	111.27±2.07^{f}	72.74±2.27^{f}
WT-B	1505.49±3.92^{a}	1020.50±4.24^{a}	392.09±3.46^{a}
Acarbose (µg/mL)	11.46 ± 0.95^{f}	19.37 ± 1.04^{f}	-
Orlistat (µg/mL)	-	-	1.14 ± 0.04^{g}

Results areaverage values ± standard deviations of triplicate analyses. Values with different superscript alphabets along the same column, differ significantly at $p < 0.05$. YM, 100% yellow maize flour; YMCP-1, 75:25 (% w/w) yellow maize and cowpea composite flour; YMCP-2, 50:50 (% w/w) yellow maize and cowpea composite flour; YMCP-3, 25:75 (% w/w) yellow maize and cowpea composite flour; CP, 100% cowpea flour; WT, 100% refined wheat flour; YM-B, yellow maize biscuit; YMCP-1B, 75:25 (% w/w) yellow maize and cowpea composite biscuit; YMCP-2B, 50:50 (% w/w) yellow maize and cowpea composite biscuit; YMCP-3B, 25:75 (% w/w) yellow maize and cowpea composite biscuit; CP-B, cowpea biscuit; WT-B, refined wheat flour biscuit; IC_{50}: concentration of extract that inhibited enzyme activity by 50%.

The pattern of inhibition of the tested enzymes indicates that both the flours and biscuits had the strongest inhibitory effect on pancreatic lipase, followed by α-glucosidase and then α-amylase. This pattern may have an important implication in the management of obesity and T2D. First, by inhibiting pancreatic lipase, the flours and biscuits may retard the rate of production and the subsequent accumulation of fatty acids, which represents an important clinical approach for treating obesity (Irondi *et al*., 2018b). Moreover, since obesity is a major predisposing factor to T2D (Gomez-Ambrosi *et al*., 2011), a stronger inhibition of pancreatic lipase is desirable to forestall obesity-induced diabetogenesis.

Relative to acarbose, an oral hypoglycaemic drug that inhibits α-amylase and α-glucosidase, the flours and biscuits inhibited α-glucosidase more than α-amylase. This inhibition pattern is in agreement with the trend of inhibition of these two enzymes reported by previous studies (Irondi *et al*., 2014; Figueiredo-Gonzalez *et*

al., 2015). This ability of the flours and biscuits to inhibit α-glucosidase more than α-amylase suggests that the side effects that characterize the clinical use of acarbose, such as flatulence and abdominal distention, which stem from acarbose's stronger inhibitory effect on α-amylase than on α-glucosidase (Dalar and Konczak, 2013, may not occur in the case of the flours and biscuits.

Table 3. Antioxidant activity of yellow maize and cowpea composite flours and biscuits extracts.

Samples	ABTS*+ scavenging ability (µmol TEAC/g)	DPPH* SC_{50} (µg/mL)	Reducing power (mg GAE/g)
	Flours		
YM	294.81±2.23^{e}	52.83±0.74^{b}	7.92±0.26^{e}
YMCP-1	316.56±1.11^{d}	47.59±0.48^{c}	13.15±0.38^{d}
YMCP-2	339.61±2.17^{c}	45.31±0.57^{c}	21.22±0.25^{c}
YMCP-3	353.77±3.34^{b}	40.49±0.54^{d}	29.02±0.23^{b}
CP	367.49±3.32^{a}	33.89±0.35^{e}	39.31±0.57^{a}
WT	174.58±1.10^{f}	645.27±2.69^{a}	2.16±0.01^{f}
	Biscuits		
YM-B	327.68±3.20^{e}	43.17±0.39^{b}	8.43±0.14^{e}
YMCP-1B	357.54±1.58^{d}	38.55±0.34^{c}	14.38±0.30^{d}
YMCP-2B	378.36±1.02^{c}	36.16±0.47^{d}	23.96±0.21^{c}
YMCP-3B	393.72±2.63^{b}	32.01±0.17^{e}	33.07±0.13^{b}
CP-B	411.09±1.61^{a}	27.99±0.30^{f}	46.22±0.35^{a}
WT-B	193.24±1.20^{f}	528.12±0.62^{a}	2.78±0.20^{f}
Ascorbic acid	-	7.52 ± 0.81^{f}	-

Results are means ± standard deviations (SD) of triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$). YM, 100% yellow maize flour; YMCP-1, 75:25 (% w/w) yellow maize and cowpea composite flour; YMCP-2, 50:50 (w/w) yellow maize and cowpea composite flour; YMCP-3, 25:75 (% w/w) yellow maize and cowpea composite flour; CP, 100% cowpea flour; WT, 100% refined wheat flour; YM-B, yellow maize biscuit; YMCP-1B, 75:25 (% w/w) yellow maize and cowpea composite biscuit; YMCP-2B, 50:50 (% w/w) yellow maize and cowpea composite biscuit; YMCP-3B, 25:75 (% w/w) yellow maize and cowpea composite biscuit; CP-B, cowpea biscuit; WT-B, refined wheat flour biscuit; SC_{50}: extract concentration that scavenged 50% of DPPH*

Antioxidant activity

Table 3 presents the antioxidant activity of the yellow maize and cowpea composite flours and biscuits, as tested using free radicals (ABTS*+ and DPPH*) scavenging assays and reducing power. As with the enzymes inhibitory results (Table 2), CP had the strongest ($p < 0.05$) ABTS*+ and DPPH* scavenging ability and the highest ($p < 0.05$) reducing power, while WT had the weakest ABTS*+ and DPPH* scavenging ability and the least reducing power. Compared to the DPPH* SC_{50} range (15.4 - 40.2 mg/mL) in orange maize hybrids recently reported by

Alamu *et al*. (2021), the YM used in this study had a lower DPPH* SC_{50} of 52.83 µg/mL (0.05 mg/mL), indicating a stronger DPPH* scavenging activity. Similarly, the DPPH* SC_{50} of the CP in this study (33.89 µg/mL) is lower than the DPPH* IC_{50} (48.2 ± 1.9 µg/mL) earlier reported for cowpea flour by Sreerama *et al*. (2012). It well-known that a lower DPPH* SC_{50} value represents a stronger capacity of a sample to scavenge DPPH* (Irondi *et al*., 2019c).

The ABTS^{*+} and DPPH* scavenging ability, and reducing power of the composite flours and their corresponding biscuits increased as the proportion of CP in the flours and biscuits increased, such that YMCP-3 and its counterpart biscuit, YMCP-3B, had the strongest ABTS^{*+} and DPPH* scavenging ability and the highest reducing power among the composite flours and biscuits, respectively.

The ABTS^{*+} and DPPH* scavenging ability, and reducing power of the biscuits were consistently higher than those of their corresponding flours. This is contrary to the trend observed in the enzymes inhibitory activity, in which the biscuits had weaker inhibitory effects on the tested enzymes (Table 2). The higher antioxidant activity of the biscuits relative to their corresponding flours may be attributed to the Maillard reaction products that may have been formed during baking. Maillard reaction products such as melanoidins and reductones, formed at the expense of polyphenolic compounds in food matrices during thermal treatment, exhibit antioxidant activity (Irondi *et al*., 2019b; Samaras *et al*., 2005). Thus, the biscuits, when eaten, may have enhanced capacity to mitigate the formation of free radicals and reactive oxygen species, thereby preventing and/or extenuating oxidative stress, obesity and T2D. This is supported by an earlier report by Irondi *et al*. (2018) that boosting the antioxidant capacity of the cell is vital for the treatment of various metabolic diseases.

Conclusions

The level of total carotenoids increased as the proportion of biofortified yellow maize increased, while the levels of total phenolics, tannins, total flavonoids and saponins, enzymes inhibitory and antioxidant activities increased as the proportion of cowpea increased in the composite flours and biscuits. Among the composite flours and biscuits, YMCP-3 (comprising 25% yellow maize and 75% cowpea) and its corresponding biscuit, YMCP-3B, had the strongest enzymes inhibitory and antioxidant activities. Hence, the composite biscuits, especially YMCP-3B, may be beneficial in retarding the rate of production of fatty acids and glucose formation, and mitigating oxidative stress, which are important clinical strategies for managing obesity and T2D.

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Conflict of interest statement

The authors do not have any conflict of interest to declare.

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