Influence of Sourdough Fermentation on Amino Acids Composition, Phenolic Profile, and Antioxidant Properties of Sorghum Biscuits

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ABSTRACT: Biscuits were produced from sorghum with and without the addition of sourdough. The influence of sourdough fermentation on the amino acids composition, phenolic profile, and antioxidant properties of the biscuits were evaluated. Phenolic compounds of the biscuits were identified and quantified using gas chromatography/mass spectrometer. The total phenol contents (TPC), total flavonoid contents (TFC), ferric reducing antioxidant properties (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) (ABTS) scavenging ability, 1,1-diphenyl-2-picryl-hydrazy (DPPH) scavenging ability, and nitric oxide (NO) scavenging ability of the biscuits were determined. Addition of sourdough increased the total essential amino acids, total non essential amino acids, hydrophobic, and aromatic amino acids contents of the biscuits. Essential amino acid index, biological value, and predicted protein efficiency were higher in biscuits with sourdough than in biscuits without sourdough. Six phenolic compounds were identified and quantified in the biscuits. Ferulic acid was the most prominent phenolic compound, followed by chlorogenic acid. TPC, TFC, FRAP, ABTS, DPPH, and NO scavenging abilities increased significantly with the addition of sourdough. Sorghum biscuits with sourdough could be useful in dietary interventions to prevent protein-energy malnutrition. Similarly, the presence of bioactive phenolic compounds and their antioxidant efficacy suggest health benefits in the management of oxidative stress and degenerative diseases.

Keywords: sourdough, biscuits, amino acids, phenolic profile, antioxidant properties

INTRODUCTION

In recent decades, the consumption of natural, tastier, and healthier foods has become a priority to consumers. Sourdough fermentation has been employed in the manufacture of baked products such as breads, cakes, crackers, and biscuits (1,2). The health promoting abilities of products from sourdough fermentation might be attributed to their exceptional gastronomic qualities. Through fermentation, the nutritional constituents and bioactive properties of food matrices were modified by the activities of the inherent enzymes and metabolic activities of the microorganisms (3). This has resulted in new compounds with health-modulating potentials. A previous report showed the synthesis of vitamin B including folate, riboflavin, and B_{12} during sourdough fermentation from non-vitamin precursors by certain bacteria in the substrate (4). Similarly, the synthesis of some amino acids and their derivatives during fermentation has been reported by Becerra-Tomás et al. (5). Some proteins and exopolysaccharides, which are produced during food fermentation could serve as antioxidants and possess hypocholesterolemic activities (6,7). Sourdough fermentation also improves texture and palatability of whole grain, fiber-rich, and/or gluten free-products (8).

Biscuits are one of the confectionary food products consumed as snacks by all ages. They are ready to eat, convenient, and inexpensive food products (9). Sourdough biscuits from gluten free sources such as sorghum would be an inexpensive carrier of the aforementioned health benefits, making it a promising functional food. To further explore the health benefits of sourdough, the present study evaluated the amino acids composition, identified, and quantified the phenolic bioactive compounds present and the antioxidant properties of the sorghum biscuits with and without sourdough (BWS and BWOS, respectively).

MATERIALS AND METHODS

Materials

White sorghum grains were obtained from the Erekesan Market in Akure, Nigeria. The sorghum grains were cleaned manually by winnowing and sorted by hand-picking to remove damaged seeds and dirt. The whole sorghum grains were milled using a laboratory hammer mill fitted with a 500 μm opening screen to produce relatively coarse whole grain flour, which was packed and stored at 4°C prior to usage.

Defatted soy flour (DFS) was obtained from Rom Oil Mills (Ibadan, Nigeria). Margarine and granulated sugar were purchased from the Erekesan Market. All other chemicals and reagents used were of analytical grades and water was glass distilled.

Sourdough preparation

Traditional sourdough lactic acid starter culture was prepared from the white sorghum flour using the back sloping method as described by Taylor and Taylor (10). About 235 g of sorghum flour was mixed with 300 mL of distilled water in a ratio of 1:1.3 (w/v) and incubated at 25°C. The pH and titratable acidity (TTA) were monitored daily until a pH of 3.8 and TTA of 2.60 were achieved. After, 20 mL of the sourdough was introduced into a fresh mix and incubated again (back slopping), and the process was repeated until use.

Sourdough biscuits preparation

Sourdough biscuits were prepared as described by Omoba et al. (2). Biscuits (±10.4 g each) were prepared from the white sorghum whole grain flour and DFS, with and without sourdough. By weight, the sorghum-soya biscuit formulations comprised of 100 parts whole grain flour, with addition, on flour basis of 40% DFS, 35% sugar, 50% baking margarine, and water at different levels. For the sourdough biscuits, 50% of the flour portion formed part of the sourdough with water and 50% were added during mixing. Biscuits were baked at 180°C for 20±5 min. The cooled biscuits were vacuum-packed in polyethylene bags and stored at 4°C.

Determination of amino acids profile and protein quality of biscuits

Sample preparation for amino acids analysis: 2.5 g of each biscuit sample was weighed into the extraction thimble, and fat was extracted with a chloroform/methanol (2:1, v/v) mixture using a Soxhlet apparatus for 5~6 h as described by the AOAC (11).

Hydrolysis of samples: About 30 mg of each of the defatted biscuits was weighed into glass ampoules. Seven milliliters of 6 mol/L HCl was added and oxygen expelled by passing nitrogen gas into the sample. The glass ampoules were sealed with a Bunsen flame and put into an oven at 105±5°C for 22 h. Each ampoule was allowed to cool; the content was filtered to remove humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in a plastic specimen bottle kept in the deep freezer.

Amino acid analysis was determined by ion exchange chromatography (12) using the technicon sequential multisample (TSM) amino acid analyser (Technicon Instruments Corp., Tarrytown, NY, USA). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 mL/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder from the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two. Norleucine was used as the internal standard.

Tryptophan: The tryptophan content was determined in a separate analysis. The weighed samples were placed in polypropylene tubes and after the addition of the internal standard (norleucine), they were hydrolyzed in 4.67 mol/L KOH containing 1% (w/v) thiodiglycol for 18 h at 110°C. After hydrolysis, KOH was neutralized with 2.4 mol/L perchloric acid, and the supernatant was adjusted to pH 3.0 with acetic acid. A 20 μL aliquot of the hydrolyzed sample was subjected to derivatization as described above. The solution of the amino acid standard was supplemented with tryptophan. Quality assurance for the tryptophan determination was obtained by demonstrating that the method yielded the correct number of tryptophan residues for egg white lysozyme. Tryptophan analysis was performed using a Waters C18 reversed phase column (3.9×150 mm) (Waters Corporation, Milford, MA, USA), and the solvents and gradient conditions were as described by Hariharan et al. (13). Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine, which results from the alkaline hydrolysis of arginine.

Evaluation of protein quality

The nutritional qualities of the protein in sourdough biscuits were determined based on the obtained amino acid profiles. The proportion of total essential amino acids (TEAA) to the total amino acids (TAA) of the protein was calculated using the method of Chavan et al. (14) and essential amino acids index (EAAI) was calculated according to the method of Labuda et al. (15). The biological value (BV) was calculated according to Oser (16) as cited by Mune-Mune et al. (17). The predicted protein efficiency ratio (P-PER) was estimated according to the regression equations developed by Alsmeyer et al. (18) and cited by Mune-Mune et al. (17).

Quantification of phenolic profile of sourdough biscuits using gas chromatography/mass spectrometer (GC/MS)

The extraction was done by adding 40 mL of 62.5% aqueous methanol containing BHT (1 g/L) to 0.5 g of biscuit sample to which 10 mL of 6 M HCl was added. The mixture was stirred, and nitrogen was bubbled for $40 \sim 60$ s into each sample. The extraction mixture was then sonicated for 15 min and refluxed in a water bath at $90^{\circ}C$ for 2 h. The mixture was then extracted with 30 mL (3×10 mL) ethyl acetate. The organic layer was collected, reduced to 10 mL by rotary evaporation ($37^{\circ}C$), and centrifuged for 10 min. Anhydrous Na_2SO_4 was then added to remove moisture, and 100 μL of the organic layer were derivatized after evaporation of the solvent under a nitrogen stream. For the silylation procedure, a mixture of trimethylchlorosilane (100 μL) and N,O-bis(trimethylsilyl)trifluoroacetamide (200 μL) were added and vortexed in screw cap glass tubes (priory deactivated with 5% dimethyldichlorosilane in toluene, and rinsed two times with toluene and three times with methanol), and consecutively placed in a water bath at $80^{\circ}C$ for 45 min. From the silylated mixture, 1 μL was directly analyzed by GC/MS. To prevent enzymic oxidation, extraction of the polyphenols from plants with boiling alcohol is essential and was adopted routinely (19). For the same reason analysis was carried out in the dark and under a nitrogen atmosphere.

The silylated samples were injected into a GC/MS system consisting of a Fisons GC 8000 Series (ThermoQuest Italia Spa, Milan, Italy), model 8060 gas chromatograph coupled with a Fisons MD 800 mass spectrometer in the electron impact mode with the electron energy set at 70 eV and the mass 0.32 mm, i.d., range at m/z $25 \sim 700$. A capillary column, Low-Bleed CP-Sil 8 CB-MS (30 m×0.25 μm film thickness of coated material, Agilent Technologies, Inc., Santa Clara, CA, USA) was used. The injector was set at $280^{\circ}C$ and the detector at $290^{\circ}C$. GC was used in the splitless mode with 1 min splitless time. The temperature program was as follows: from $70^{\circ}C$ to $135^{\circ}C$ with $2^{\circ}C$/min, hold for 10 min; from $135^{\circ}C$ to $220^{\circ}C$ with $4^{\circ}C$/min, hold for 10 min; from $220^{\circ}C$ to $270^{\circ}C$ with $3.5^{\circ}C$/min, and then hold for 20 min. A post run of 10 min at $70^{\circ}C$ was sufficient for the next injection. The flow rate of the carrier gas (helium) was maintained at 1.9 mL/min. Identification of compounds was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from the Wiley and NIST libraries. Each determination was carried out in duplicate.

Antioxidant activities of sourdough biscuits

Preparation of biscuits extracts: The extracts of biscuit samples were obtained as described by Bloor (20). A half gram from each of the milled biscuit samples was extracted with 20 mL of methanol : water (6:4, v/v). The mixture was centrifuged and the supernatant was adjusted to 25 mL. An aliquot was used for the quantification of total phenolic and antioxidant activity.

Determination of total phenol content (TPC) of sourdough biscuits: The TPC of the biscuit samples was determined using the Folin-Ciocatlteu method, as described by Singleton et al. (21). Aliquots of 0.5 mL of each extract were added to 0.5 mL of Folin-Ciocalteu reagent, followed by the addition of 0.5 mL of an aqueous 20% solution of sodium carbonate. The mixture was stirred and allowed to stand for 30 min. The absorbance at 765 nm was measured using a model UV/VIS 1201 spectrophotometer (Shimadzu, Kyoto, Japan). A blank sample consisting of water and reagents was used as a reference. Gallic acid was used as a standard, and the results were expressed as mg gallic acid equivalents (GAE)/per g.

Determination of total flavonoid content (TFC) of sourdough biscuits: The TFC in white sorghum sourdough biscuit was determined by a colorimetric assay based on the procedure described by Park et al. (22). One thousand μL of biscuit extract was added into a test tube. Then 0.1 mL CH_3COOK and 0.1 mL of 10% $Al(NO_3)_3$ in 4.3 mL ethanol solution was added, and the samples were vortexed. The vortexed sample was kept at room temperature for 40 min. The absorbance measurements were recorded at 415 nm. The distilled water was used either as a blank or as control instead of sample. Quercetin was used for comparison. The absorbance measurements of the sample containing 20, 40, 60, and 80 μg quercetin were recorded, and the standard graph was drawn. The results were reported as mg quercetin equivalents (QE) per g extract. The total flavonoid content of the sample was determined in duplicates.

Determination of ferric reducing antioxidant power (FRAP) of sourdough biscuits: The FRAP assay was conducted as described by Benzie and Strain (23) with slight modifications. The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (24). An appropriate dilution of the aliquot extract (2.5 mL) was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at $50^{\circ}C$ for 20 min, and 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 mins, and 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm.

Determination of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging ability of sourdough biscuits: The ABTS scavenging ability of the extracts was determined according to the method described by Re et al. (25). The ABTS was generated by incubating equal vol-

umes of 7 mmol/L ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/L) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.70 ± 0.02 with 95% ethanol. Then, a 0.2 mL of the appropriate dilution of the extract was added to 2.0 mL ABTS solution, and the absorbance was measured at 734 nm after 15 min. The ABTS scavenging ability of the extracts was subsequently calculated and expressed as Trolox equivalents (TE).

Estimation of 1,1-diphenyl-2-picryl-hydrazy (DPPH) free radical scavenging ability: The DPPH scavenging ability of the extracts was determined as described by Cervato et al. (26). Briefly, an appropriate dilution of the extract (1 mL) was mixed with 3 mL of 60 mol/L DPPH; the mixture was left in the dark for 30 min before the absorbance was measured at 517 nm. The decrease in absorbance of DPPH by the addition of extract in relation to the reference test was used to calculate the percentage scavenging ability following the equation:

$$\% \text{ Scavenging ability} = \frac{A_{517} \text{ reference} - A_{517} \text{ sample}}{A_{517} \text{ control}} \times 100$$

Data analysis
The results of triplicate measurements of the experiments were expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the mean, and the post hoc treatment was performed using Duncan's multiple test. Significance was accepted at $P \leq 0.05$. The EC_{50} (extract concentration causing 50% enzyme/antioxidant activity) was determined using nonlinear regression analysis.

RESULTS AND DISCUSSION

Amino acid composition of BWS and BWOS
Table 1 shows the amino acids composition of BWS and BWOS. Addition of sourdough resulted in an increase in both the TEAA and TNEAA contents of the biscuits. The values of TEAA increased from 189.00 mg/g in BWOS to 203.30 mg/g in BWS. Similarly, the TNEAA increased from 511.30 mg/g in BWOS to 536.40 mg/g in BWS. The TAA contents increased from 700.30 mg/g in BWOS to 739.70 mg/g in BWS. Sourdough fermentation was observed to cause an increase in the free amino acids, which might be attributed to proteolysis caused by the flour enzymes, lactic acid bacteria (LAB), as well as the microbial enzymes in the flour. The increase is revealed by the higher values observed in the amounts of specific amino acids, such as the basic amino acids, sulphur-containing amino acids, aromatic amino acids, and hydrophobic amino acids. Proteolytic actions are dependent on the fermentation conditions, especially temperature. Previous

reports have shown that LAB are precise in their needs for amino acids and could therefore spawn activities vital to obtaining the required peptides and amino acids (27). This observation corroborates the report of Thiele et al. (28) that LAB fermentation of dough improves the amino acids profile in sourdough bread. Food of high-quality protein should have essential amino acids in ratios proportionate with human requirements. This was affirmed by comparing the amino acid contents with the FAO reference pattern for age group (children $1\sim2$ years) as shown in Table 1. The TEAA of the BWS compares favourably with the amino acids reference (29).

The hydrophobic and aromatic amino acid contents of BWS (283.60 and 101.50 mg/g, respectively) were higher than those of BWOS (267.40 and 94.60 mg/g, respectively). Hydrophobic and aromatic amino acids assist in radical scavenging and metal chelating activities. Amino acids with aromatic side groups are assumed to contribute to the strong radical scavenging activities of peptides. Also, the ability of histidine (imidazole group), trypto-

Table 1. Amino acid composition of sorghum biscuits with and without sourdough (unit: mg/g protein)

Amino acids	BWOS	BWS	Amino acid reference FAO/WHO/UNU
Cystine	13.30	13.30	
Methionine	15.50	17.10	25 (26)
Tyrosine	24.10	27.50	
Phenylalanine	40.80	42.60	47 (46)
Threonine	25.50	30.00	27 (27)
Valine	38.00	40.30	32 (42)
Leucine	56.90	60.40	55 (61)
Lysine	32.10	33.40	51 (52)
Isoleucine	28.50	30.80	25 (31)
Tryptophan	8.00	8.40	7 (7.4)
Aspartic acid	102.00	106.70	
Alanine	31.90	34.10	
Glutamic acid	163.50	168.10	
Glycine	46.60	48.00	
Proline	34.50	36.60	
Serine	35.40	41.60	
Arginine	75.70	78.30	
Histidine	21.70	23.00	
Total essential amino acid (TEAA)	189.00	203.30	287 (312.4)
Total non essential amino acid (TNEAA)	511.30	536.40	
Total amino acid (TAA)	700.30	739.70	
TEAA/TAA	0.27	0.28	
Hydrophobic amino acid	267.40	283.60	
Aromatic amino acid	94.60	101.50	
Acidic amino acid	265.50	274.80	
Sulphur-containing amino acid	28.80	30.40	

BWOS, biscuit without sourdough; BWS, biscuit with sourdough. Aromatic amino acid, phenylalanine, tryptophan, histidine, and tyrosine; Acidic amino acid, aspartic acid and glutamic acid.

phan (indolic group), and tyrosine (phenolic group) to donate hydrogen have been accredited to the special groups they have in their side chain. Aromatic amino acids (tryptophan and phenylalanine) have the ability to give protons easily to electron deficient radicals while at the same time maintaining their stabilities via resonance structures (30,31).

Addition of sourdough increased the sulphur-containing amino acids from 28.80 mg/g in BWOS to 30.40 mg/g in sourdough biscuits. Sulphur-containing amino acids (methionine and cystine) have the capacity to offer their sulfur hydrogen; hence, these amino acids are considered effective radical scavengers (32). LAB however, might therefore have the capacity to synthesize antioxidant peptides during the sourdough fermentation of cereal flours.

Protein quality evaluation of BWOS and BWS
Protein quality evaluation of BWOS and BWS is presented in Table 2. The EAAI % of the biscuits based on whole egg and essential amino acids reference pattern were 89.09% for BWOS and 92.22% for BWS. The value obtained for the BWS was higher than the 90% amino acids reference (29). An EAAI $\geq$90% for BWS could be referred to as good and EAAI of approximately 80% is regarded as useful for BWOS.

The BV obtained were 144.82 (BWS) and 145.47 (BWOS). The values obtained were higher than the 70 reported by FAO/WHO/UNU (29). The BV helps to predict the nitrogen retention of the consumer and thereby indicates the utilizable fraction of a protein. It therefore implies that protein of both biscuits would be properly utilized by the body.

The P-PER obtained were 2.35 and 2.54 for BWOS and BWS, respectively. The P-PER is one of the quality parameters used for protein evaluation. A good quality protein must have value of 2.5 or more (12). This implies that a sourdough biscuit has a good protein quality when compared to the reference of 2.5 (12).

Bioactive phenolic compounds in BWOS and BWS
Table 3 shows the bioactive phenolic compounds in BWOS and BWS. Six bioactive compounds were identified and quantified in the BWS and BWOS using GC/MS. The bioactive compounds were hydroxybenzoic, hydroxycinnamic, flavanols, ferulic acid, chlorogenic acid, and quercetin. Hydroxybenzoic, hydroxycinnamic, flavanols, and ferulic acid are the prominent compounds. The bioactive phenolic profile represents a valuable source of health-promoting compounds, belonging to flavonoids, phenolic acids, and lignans. The antidiabetic activity of flavonoids and phenolic acids has been reported (33,34). The antidiabetic activity of flavonoids depends on the chemical criterion (C-2-C-3 double bond and ketonic group at C-4 position on ring B), which is fundamental for the bioactivity of polyphenol compounds (35).

Bioactive phenolic compounds in BWOS were higher than in BWS. The effect of sourdough fermentation on bioactive phenolic compounds (increase or decrease) is dependent on factors such as on the nature of the molecules and the type of sourdough process (36). The decrease observed in this study is similar to the observation reported by Koistenen et al. (37), indicating a decrease in phenolic acids present in rye and wheat sourdough. This observation might be attributed to the ability of the various LAB strains to metabolize the main phenolic acids (38). Similarly, the decrease as detected by the GC/MS method used might be as a result of the change between formation of simple phenolics by degradation of complex polymeric phenolic compounds and the total degradation of simple phenolics with possibilities of re-formation of more complex biphenolics and flavonoid moieties.

In BWOS, hydroxybenzoic was 19.65 mg/100 g, hydroxycinnamic was 36.36 mg/100 g, flavanol was 49.36 mg/100 g, and ferulic acid was 228 mg/100 g. In BWS, hydroxybenzoic was 12.57 mg/100 g, hydroxycinnamic was 26.66 mg/100 g, flavanol was 42.54 mg/100 g, and ferulic acid was 203.00 mg/100 g. This implies that addition of sourdough reduced phenolic compounds in the biscuits. LAB metabolizes phenolic acids by decarboxylation and/or reduction, and the reduction observed in hydroxycinnamic might be attributed to the action of re-

Table 2. Nutritional qualities of white sorghum biscuits with and without sourdough

Protein quality	BWOS	BWS	Amino acid reference FAO/WHO/UNU
Essential amino acid index	89.09	92.22	90
Biological value	144.82	145.47	70
Predicted protein efficiency ratio	2.35	2.54	2.5

BWOS, biscuit without sourdough; BWS, biscuit with sourdough.

Table 3. The phenolic profile of sorghum with and without sourdough (unit: mg/100 g)

Samples	BWOS	BWS
Hydroxybenzoic	19.65±0.10[a]	12.57±0.49[b]
Hydroxycinnamic	36.36±0.05[a]	26.66±0.45[b]
Flavanols	49.36±0.10[a]	42.54±0.10[b]
Ferulic acid	228.00±0.10[a]	203.00±0.10[b]
Chlorogenic acid	57.43±0.01[a]	34.45±0.02[b]
Quercetin	23.36±0.10[a]	10.26±0.10[b]

BWOS, biscuit without sourdough; BWS, biscuit with sourdough.
Mean±standard deviation of triplicate.
Mean values with the same letters (a,b) within the same row differ significantly ($P<0.05$).

Table 4. Antioxidant properties of sorghum biscuits without and with sourdough

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	FRAP (mg AAE/g)	ABTS (μmol/TE/100 g)	DPPH (mg/mL)	NO (mg/mL)
Commercial biscuit	2.32±0.09[c]	3.00±0.40[c]	0.79±0.30[b]	54.63±0.45[b]	88.76±0.34[a]	18.75±0.24[a]
BWOS	3.62±0.17[b]	3.46±0.13[b]	0.78±0.44[c]	42.01±0.25[c]	63.23±0.16[c]	14.58±0.44[c]
BWS	4.38±0.18[a]	4.75±0.21[a]	0.82±0.60[a]	60.31±0.42[a]	86.36±0.12[b]	16.66±0.43[b]

BWOS, biscuit without sourdough; BWS, biscuit with sourdough.
TPC, total phenol content; GAE, gallic acid equivalents; TFC, total flavonoid content; QE, quercetin equivalents; FRAP, ferric reducing antioxidant properties; AAE, ascorbic acid equivalent; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic) scavenging ability; TE, Trolox equivalents; DPPH, 1,1-diphenyl-2-picryl-hydrazy scavenging ability; NO, nitric oxide scavenging ability.
Mean±standard deviation of triplicate.
Mean values with the same letters (a-c) within the same column differ significantly ($P<0.05$).

ductase, which hydrogenates the double bonds. However, the degradation of phenolic compounds by LAB might have given rise to compounds influencing the aroma of the biscuits as well as compounds with increased antioxidant activity (2). Hydroxycinnamic acids are phenolic phytochemicals. They include groups of polyphenols such as caffeic acid, ferulic acid, chlorogenic acid, and isoferulic acid, as well as coumaric acid. They are notable for their valuable effect, owing to their chemopreventive activities against carcinogenesis and mutagenesis, which is related to their antioxidant activities (39).

Chlorogenic acid (CGA) in BWOS and BWS were 57.43 and 34.45 mg/100 g, respectively. CGA is one of the major polyphenol compounds present in the human diet. It is a group of phenolic secondary metabolites produced by certain plant species. CGA is an ester produced from cinnamic acid and quinic acid. It exhibits many biological properties, including antibacterial, antioxidant, and anticarcinogenic activities, particularly hypoglycemic and hypolipidemic effects (40,41).

Quercetin in the BWOS and BWS were 23.36 and 10.26 mg/100 g, respectively. Quercetin is one of the major bioflavonoids present in some plant materials. It is known for its anti-inflammatory, antihypertensive, vasodilator properties, antiobesity, antihypercholesterolemic, and antiatherosclerotic activities (42). Worthy of note is the ability of quercetin to inhibit inflammatory enzymes cyclooxygenase and lipooxygenase thereby decreasing inflammatory mediators such as prostaglandins and leukotrienes (43). Quercetin is the most commonly consumed flavonol in the human diet.

Antioxidant properties of BWOS or BWS
Table 4 reveals the antioxidant properties of BWOS and BWS. The TPC observed in BWS and BWOS were higher than that of the commercial biscuits. The TPC of biscuits increased from 3.62 mg GAE/g in BWOS to 4.38 mg GAE/g in BWS. The increase in the TPC might be attributed to enzymatic reactions within the substrates, resulting in the secretion of more phenolic compounds as end products. During sourdough fermentation, the presence of LAB triggers pH reduction, which results in the activation of enzymes capable of hydrolyzing complex polyphenols and producing simpler and active polyphenols (44). Similarly, Katina (3) reported that amylases, proteases, and xylanases derived from microbes and grains during sourdough fermentation are capable of modifying the grain composition and releasing phenolics.

The total flavonoid contents of sorghum biscuits increased with the incorporation of sourdough as values increased from 3.46 mg QE/g (BWOS) to 4.75 mg QE/g (BWS). Similarly, FRAP increased with the incorporation of sourdough from 0.78 mg BWOS to 0.82 mg ascorbic acid equivalents/g with sourdough. DPPH and NO scavenging abilities also followed similar trend with the addition of sourdough as other antioxidant properties, although the values obtained for DPPH (86.36 mg/mL) and NO (16.66 mg/mL) were lower than the values obtained for commercial biscuits. The activities of sourdough LAB during sourdough fermentation resulting in the structural breakdown of the cereals cell walls and enzymatic breakdown of matrix compounds might be attributed to the improved concentration of antioxidant compounds. This agrees with the report of Coda et al. (45) that sourdough LAB have the capacity to release peptides with antioxidant activity through the proteolysis of native cereal proteins. According to Chen et al. (46), a strong correlation has been established between antioxidant activity and amino acids composition, conformation, and hydrophobicity.

Table 5. Effective concentration (EC$_{50}$) of sorghum biscuits with and without sourdough (unit: mg/mL)

Sample	DPPH	NO
Commercial biscuit	37.32±0.11[c]	0.35±0.07[c]
BWOS	40.36±0.12[a]	0.51±0.32[b]
BWS	38.44±0.23[b]	0.61±0.54[a]

DPPH, 1,1-diphenyl-2-picryl-hydrazy; NO, nitric oxide; BWOS, biscuit without sourdough; BWS, biscuit with sourdough.
Mean±standard deviation of triplicate.
Mean values with the same letters (a-c) within the same column differ significantly ($P<0.05$).

EC$_{50}$ of some antioxidant properties in sorghum sourdough biscuits

The effective concentration at 50% (EC$_{50}$) of some antioxidant properties in white sorghum sourdough biscuits such as DPPH and NO scavenging capacities are presented in Table 5. The EC$_{50}$ of DPPH scavenging capacity ranged from 37.32 to 40.36 mg/mL. The BWOS had the highest EC$_{50}$ value (40.36 mg/mL), while the commercial biscuits had the lowest value (37.32 mg/mL). No significant difference ($P>0.05$) was observed between the commercial biscuits and sourdough biscuits. The higher the EC$_{50}$ values, the lower the effectiveness in antioxidant properties. This implies that the commercial and sourdough biscuits are effective in DPPH scavenging activities, than the BWOS. The EC$_{50}$ of NO scavenging capacity ranged from 0.35 to 0.61 mg/mL. The commercial biscuits have the lowest EC$_{50}$ value (0.35 mg/mL), while sourdough biscuits had the highest EC$_{50}$ value (0.61 mg/mL). The commercial biscuits and BWOS have better NO scavenging activities than BWS.

In conclusion, addition of sourdough in sorghum biscuit production resulted in biscuits with improved amino acids composition, as well as increased amounts of specific amino acids such as sulphur-containing amino acids, hydrophobic, and aromatic amino acids with radical scavenging and metal chelating activities. The biscuits have improved nutritional indices (EAAI %, BV, and P-PER), compared with the BWOS. Such products may have significant potential for use as protein-rich supplement food in semi-arid tropical countries to prevent protein energy malnutrition (PEM). Ferulic and chlorogenic acids are the most prominent bioactive phenolic compounds present in the BWS and BWOS. Sourdough biscuits had increased antioxidant properties compared to BWOS and the commercial biscuits. The regular consumption of biscuits, especially sourdough sorghum biscuits can have beneficial effect on human health with respect to preventing PEM and for the management of degenerative diseases.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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