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Research Article

Compositional and Functional Analysis of Red Sorghum Bran, Roselle Calyx, and Avocado Leaf Flour Blends

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ABSTRACT

Typical flour blends produced from Red sorghum bran, Roselle calyx and Avocado Leaf were examined for their antioxidant, amino acid and phenolic components. The antioxidant activity revealed that phenol decreased significantly and FRAP insignificantly from 58.03 to 51.28 mg GAEqv/g and 57.47%-52.27%. The most abundant amino acid was Arginine which increased significantly from 231%-260.5% while Tryptophan was the limiting amino acid which decreased significantly from 34.54%-23.64% according to the amino acid score. The Aspartic acid increased significantly from 8.65 to 10.92 g/100 g while glutamic acid and Tryptophan decreased significantly from 11.74 to 9.45 g/100 g and 0.38 to 0.26 g/100 g. Arginine-Lysine ratio increased significantly from 0.67 to 0.80 g/100 g. This shows that the flour blends can be used as supplement mixture for poor lysine food mixture like cereals. The anti-nutrients were within the safe levels. Catechin, caffeic, quercetin, chlorogenic acid and isoquercitrin were most abundant among the phenolic components. The lightness, yellowness and hue strength increased with increase in zobo calyx and decrease in Avocado leaf flour. It can be concluded that nutritious health promoting infused beverages can be produced from the enriched flour which may play protective roles against cardiovascular, neuro-degenerative and age related diseases.

Keywords: Flour; Blends; Zobo; Avocado

INTRODUCTION

Beverages are liquid substances consumed for their thirst-quenching properties and calorie value. There is a growing demand for pure natural herbal beverage especially those without the addition of chemicals and sugar. Tea is currently the most widely consumed beverage in the world and therefore ranks as an important world food product [1]. It is generally consumed for its attractive aroma and taste as well as the unique place it holds in the culture of many societies. Traditionally, tea is consumed to improve blood flow, improve resistance to diseases and eliminate toxins [2]. Sorghum (Sorghum bicolor (L.) Muench), the fifth most important cereal grown in the world after maize, rice, wheat and barley which is resistant to semiarid climates, gluten free, and a good source of phytochemical compounds that have been associated with antioxidant, anti-inflammatory, and anti-proliferative capacities [3,4]. Pharmacological research has also reported the effects of sorghum and its extracts on cardio vascular disease prevention and glycemic control. Almost all kinds of phenolic are found in sorghum and much more are present in their outer layer (bran) [5]. The leaves of P. Americana are reported to possess anti-inflammatory, analgesic, antimicrobial, antiviral, antihypertensive, antihyperglycemic, antiulcer, anticonvulsant, larvicidal antihepatotoxic, vasorelaxant, toxicological activities [5-7]. The genus Hibiscus (family Malvaceae) includes more than 300 species of annual or perennial herbs or shrubs and commonly known as Roselle or red sorrel calyx. Infusions of zobo calyces are traditionally used for their diuretic, choleretic, febrifugal and hypotensive effects, decreasing the viscosity of the blood and stimulating intestinal peristalsis, recommended as a hypotensive. It is also useful in the treatment for cardiac, nerve diseases, cancer and liver toxicity. Besides its importance as a food or traditional medicine in the countries of its geographic origin, it is used worldwide as an important ingredient in industrially produced teas and beverages [8]. The research work therefore deemed it fit to evaluate the antioxidant, phenolic components, amino acids and anti-nutritional constituents of formulated flour blends from underutilized red sorghum bran, zobo calyx and Avocado leaf. The diagrams of red sorghum bran, roselle and avocado leaf are shown in Figures 1-3 [9].

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Figure 1: Sorghum stem sheath.

Figure 2: Roselle leaf.

Figure 3: Aocado leaf.

MATERIALS AND METHODS

Source of materials

Avocado leaves were plucked from Federal College of Agriculture and Research Farm in Akure, Nigeria. All wilting and visibly diseased plant materials were manually removed. Sorghum red bran and Roselle calyx were bought from Oja-Oba market, Akure, Ondo State. The plant materials were authenticated by Crop, Soil and Pest Management Department, Federal University of Technology, Akure.

Methods

All plant materials were carefully inspected and all foreign materials removed. Diseased and damaged leaves were discarded manually just after the collection of fresh leaves. Sorghum red bran and Roselle calyx were picked, sorted and extraneous materials removed. The Avocado leaf was washed under running water to remove extraneous materials and was air dried by spreading on sterile clean green net in a well-ventilated room to prevent loss of nutrients through exposure to high temperature for 4 days. After drying, the leave samples were milled to a coarse form using an attrition mill. The machine was washed before and after milling of each sample. The sieved samples were stored in glass bottles with tight lids and labeled.

Drying of the leaf powders

Sorghum red bran, Avocado leaf and Roselle calyx powders were further dried at 50°C in a cabinet dryer (model 85, no 64 Shandom, UK) for 30 minutes to reduce moisture content to 10% to enhance increased shelf life. The leaf powders were stored separately in clean air tight containers, protected from light and humidity, and kept in a refrigerator to maintain a temperature below 24°C (75.2°F).

Mixing of the blended samples

Each of the leaves sample was weighed into a Kenwood blender for proper mixing in the ratios as shown in Table 1 to obtain seven samples. They were bagged in non-drip tea bags after operating the mixer for three minutes for each blend using a stay material and sealing machine. Each tea bag contained approximately 3 g of the blended samples. The tea bags were stored in glass bottles with tight lids and labeled for analyses.

Analysis

Amino acid profile analysis of red sorghum bran-roselle calyx-avocado flour blends: Amino acid composition was determined according to Blackburn, using S433 Amino Acid Analyzer (SYKEM, Germany).

Determination of bioactive compound in red sorghum bran-roselle-avocado flour blends: Bioactive compounds examined include: total carotenoid, total flavonoids and anthocyanin [10].

Determination of antioxidant properties of red sorghum bran-roselle calyx-avocado leaf flour blend and drink samples: Antioxidant properties investigated include: the hydroxyl radical-scavenging activity was determined according to a previous method by, ABTS assay, Metal chelating activity, DPPH, total phenol, FRAP.

Determination of antinutritional constituents of red sorghum bran-roselle calyx-avocado flour blends: Red Sorghum bran-Roselle-Avocado leaf flour blends was examined for the following antinutritional components: alkaloids Awah and Verla, phytates content Tannin saponins and oxalate.

Statistical analysis

All determinations were carried out in triplicates. Descriptive statistics, analysis of variance (ANOVA) and Duncan Multiple Range Test were used to interpret the results obtained, and the level of significance was set at $p \leq 0.05$.

Table 1: Anti-nutrient content of Enriched red Sorghum bran samples

Samples	Tannin (mg/100 g)	Phytate (mg/100 g)	Oxalate (mg/100 g)	Saponin (%)	Alkaloids (%)
H100	0.93 ± 0.02b	1.56 ± 0.20a	1.17 ± 0.03b	1.45 ± 0.05a	0.74 ± 0.08bc
R100Z0A0	0.80 ± 0.15b	1.66 ± 0.10a	1.12 ± 0.08ab	1.10 ± 0.40a	0.25 ± 0.10bc
R0Z0A100	0.53 ± 0.17bc	0.26 ± 0.10b	2.26 ± 0.05a	1.33 ± 0.17a	2.26 ± 0.01a
R0Z100A0	2.73 ± 0.07a	0.34 ± 0.18b	0.37 ± 0.03bc	0.82 ± 0.18ab	1.84 ± 0.03ab
R50Z45A5	1.67 ± 0.03ab	1.00 ± 0.15ab	0.84 ± 0.09b	0.99 ± 0.11ab	1.06 ± 0.05b
R50Z42.5A7.5	1.62 ± 0.05ab	1.00 ± 0.13ab	0.88 ± 0.07b	1.00 ± 0.11ab	1.08 ± 0.04b
R50Z40A10	1.56 ± 0.10ab	1.00 ± 0.12ab	0.94 ± 0.10c	1.10 ± 0.40a	1.10 ± 0.02b

Note: Values with different alphabet along the same column are significantly different from each other ($p \leq 0.05$).
H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf flour; R0Z100A0 (0% Red sorghum bran+100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf flour;): R50Z42.5A7.5 (50% Red sorghum bran+42.5% zobo calyx +7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour.

RESULTS AND DISCUSSION

Selection criteria for determining optimal substitution level of avocado leaf and roselle flour and the best outfit samples

Central composite result was used to determine optimal substitution level of Avocado leaf and Roselle calyx according to the modified method of Ijarotimi and Keshinro [11]. In this study, only nutritional criteria (DPPH) were used for the ranking system based on the relative importance of the criteria. The three flour blends of: Red sorghum bran, Avocado leaf and Roselle calyx flour from preliminary study that were very high in DPPH activity were picked to objectively determine the choice of best substitution level along with three controls and a major reference sample. The seven samples were then subjected to further investigation.

Antinutrients content of the enriched red sorghum bran samples

Tannin content of the enriched samples ranged from 1.56 mg/100 g (R50Z40A10) to 1.67 mg/100 g (R50Z45A5) while that of the control (H100) was 0.93 mg/100 g (Table 1). Tannin content of red sorghum bran enriched samples were not significantly different ($p \leq 0.05$) from each other but significantly different from that of the control with lower value. Tannin content of the enriched product was higher than 1.03 mg/100 g reported by Fowomola et al. for mango flour, 9.26 mg/100 g reported for raw tigernut flour and (0.25%) obtained for Persea americana (Mill.) leaf flour and (0.97%) obtained for Xylopia aethiopica leaf flour by Gbadamosi et al. [12,13].

Phytate content of control sample (H100) was 1.56 mg/100 g while phytate content obtained for all the enriched red sorghum bran sample was 1.00 mg/100 g which was higher than (0.52%) obtained for Persea americana (Mill.) leaf flour and (0.09%) obtained for Xylopia aethiopica leaf flour by Gbadamosi et al. [13]. There was significant decrease ($p \leq 0.05$) in phytate content of all the enriched samples when compared with the control (1.56 mg/100 g). The result implied that phytate content of enriched product was reduced as a result of processing (such as sieving and drying). Enujuigha (2006) also reported that substitution of Maize with African oil bean seed flour in the production of ogi flour yielded products with lowered phytate content than 100% maize flour. Whether or not high levels of consumption of phytate containing foods will result in mineral deficiency depend on its effect on the bioavailabilty of such minerals which include Ca, Fe, and Zn. The value obtained in this study was higher than 0.21-0.98 mg/100 g reported for ogi flour with different substitution level of African oil bean seed flour, but lower than 15.31-32.9 mg/100 g reported by Ijarotimi et al. for infant flour formula made from germinated popcorn, Bambara groundnut and African locust bean [11]. Phytate markedly decrease Ca, Fe and Zn bioavailability, and Phytate to nutrient molar ratio has been proposed as an indicator of this mineral bioavailability. The critical molar ratio of 0.2, 0.4 and 1.5 has been reported for phytate: Ca, Fe and Zinc, respectively.

Saponin content of control sample (H100) was 1.45 mg/100 g while phytate content obtained for enriched red sorghum bran ranged from 0.99 mg/100 g for (R50Z45A5) to 1.10 mg/100 g for (R50Z40A10). There was significant increase ($p \leq 0.05$) in saponin content of all the enriched samples when compared with the control (1.45 mg/100 g). Saponin content of the enriched products (0.99 to 1.10) mg/100 g were lower than the values reported for some important legumes like soybeans flour (5.6%) and chickpea flour (3.6%) by Koratkar and (0.61%) obtained for Persea americana (Mill.) leaf flour and (0.34%) obtained for Xylopia aethiopica leaf flour by Gbadamosi et al. [13].

Bioactive compound of the enriched red sorghum bran samples

Generally all the bioactive components increased with the addition of Avocado leaf and decrease in zobo calyx flour. Total carotenoid content of the enriched samples ranged from 2.84 µg/g for R50Z45A5 to 2.94 µg/g for R50Z40A10 and that of the control (H100) was 3.40 (Table 2). The carotenoid content of the enriched samples (2.84 to 2.94) µg/g were lower than (16.80 to 46) µg/g obtained for Ogi-Moringa seed flour reported by Anwar. The enriched samples are generally low in carotenoid, however can be fortified with carotenoid based fruit flour like mango, guava and pawpaw.

Flavonoid contents of the enriched samples are presented in Table 2. Flavonoid content discovered in the control sample (H100) was 0.61% while that of the enriched flour samples ranged from (2.40%-2.46%). The flavonoid content of the enriched flour (2.40%-2.46%) is higher than (0.0031%-0.0076%) for Ogi-Moringa oleifera seed flour reported by Anwar, (0.03%-0.08%) for Kariya defatted flour obtained by Gbadamosi et al. but lower than (3.443%) for Hibiscus sabdarrifa calyx flour obtained by Chinatu et al. and (776.7%) obtained for Persea americana (Mill.) leaf flour, (431.7%) obtained for Xylopia aethiopica leaf flour by Gbadamosi et al. and (7.36%-10.24%) obtained for sorrel seed flour by Ayo-Omogie et al. [13-16]. Flavonoid has antioxidants properties. It has been shown to be highly effective scanvengers of most types of oxidizing molecules, including singlet oxygen and various free radicals.

Table 2: Bioactive compounds of Enriched red Sorghum bran samples

Sample	Total carotenoid (µg/g)	Anthocyanin (mg/g)	Total Flavonoid (%)
H100	3.40 ± 0.05ab	86.32 ± 0.43b	0.61 ± 0.30bc
R100Z0A0	4.01 ± 0.01a	98.59 ± 0.21a	1.54 ± 0.25b
R0Z0A100	3.58 ± 0.03a	90.99 ± 0.37ab	2.78 ± 0.12ab
R0Z100A0	1.45 ± 0.07bc	81.87 ± 0.50bc	3.44 ± 0.10a
R50Z45A5	2.84 ± 0.14b	90.88 ± 0.38ab	2.46 ± 0.34b
R50Z42.5A7.5	2.89 ± 0.09b	91.06 ± 0.30ab	2.44 ± 0.36b
R50Z40A10	2.94 ± 0.04b	91.28 ± 0.10ab	2.40 ± 0.40b

Note: Values are means ± standard deviation of three determination (n=3).

Values with different alphabet along the same column are significantly different from each other ($p \leq 0.05$) according to Duncan multiple comparism. H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf) flour; R0Z100A0 (0% Red sorghum bran+ 100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf)flour;): R50Z42.5A7.5 (50% Red sorghum bran+42.5% zobo calyx+7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour.

Total anthocyanins content of the enriched samples ranged from 90.88 mg/100 g for R50Z45A5 to 91.28 mg/100 g for R50Z40A10 and that of the control (H100) was 86.32 mg/100 g (Table 2). Total anthocyanins for the enriched samples (90.88 to 91.28) mg/100 g was lower than (493.5-118.2) mg/100 g for roselle-fruit flour blends obtained by Mgaya et al. and (2.8%) obtained for Persea americana (Mill.) leaf flour and (6.2%) obtained for Xylopia aethiopica leaf flour by Gbadamosi et al. [13,17].

Amino acid profile of red Sorghum bran-Roselle calyx-avocado leaf flour blends

Table 3 shows the amino acid composition of Sorghum red bran-Roselle Calyx-Avocado leaf flour blend samples. Lysine and leucine generally had the highest amounts in the enriched flour samples as compared to other essential amino acids and compared favourably with the reference, while for the non-essential amino acids, higher values were obtained for arginine, aspartic and glutamic acids in the enriched flour sample. This compared well with previous reports that leucine, lysine, arginine, phenylalanine, valine and glutamic acid are abundant amino acids in sorrel seed flour [18]. The most limiting essential amino acid was Tryptophan. Reverse is the case with Methionine being the most limiting essential amino acid in Sorrel seed flour reported by previous workers [19]. Similar results have been reported in other plant seeds flour also [20]. Also, the lysine content of the enriched flour samples which were higher than the FAO/WHO (1991) human requirement may make this flour useful as a supplement food mixture for poor lysine food sources such as cereals used as weaning foods and a major staple in developing countries. Hence, it may contribute significantly to lysine content of complementary foods when combined with cereals (which are poor in lysine) and thus be useful for combating PEM in young children since values in this flour are higher than FAO/WHO (1991) requirements for infants, pre-school and school age children. The Arg/Lys ratios of the enriched flour (0.73-0.74) g/100 g compared well with (0.73-0.96) g/100 g reported by Ayo-Omogie et al. but lower than that of soybean (1.40) and higher than that for casein (0.44) [16,19]. Malomo reported that high ratio of Arg/Lys in the diet produces beneficial hypocholesterolemic effects that may improve cardiovascular health and help in regulation of hypertension [21].

Table 3: Amino acid profile of Sorghum red bran-Roselle Calyx -Avocado leaf flour blends (g/100 g).

Samples	H100	R100Z0A0	R0Z0A100	R0Z100A0	R50Z45A5	R50Z42.5A7.5	R50Z40A10	Adult	Children
NEAA Glycine	4.17b	3.53e	5.14a	4.10c	3.87d	3.89d	3.92d		
Alanine	3.60e	3.68d	4.27a	3.95b	3.83c	3.84c	3.85c		
Serine	4.13a	2.96e	3.89b	3.40c	3.20d	3.21d	3.23d		
Proline	3.20e	3.78d	3.00e	4.20a	3.93b	3.90b	3.87b		
Aspartate	11.02a	9.68e	10.03d	10.79b	10.20c	10.18c	10.16c	-	
Cysteine	2.30a	1.15d	0.70e	1.50b	1.29c	1.27c	1.25c		
Glutamic	16.61a	9.27g	15.78b	11.04c	10.39f	10.51e	10.63d		
Tyrosine	3.63b	2.99d	4.98a	3.35b	3.25c	3.29c	3.33c		
Arginine	7.45a	4.50e	4.92c	5.20b	4.84d	4.83d	4.82d		
Total NEAA	56.11a	41.54f	52.71b	47.53c	44.80e	44.92e	45.06d		
EAA Phenylalani	4.83b	4.02e	5.26a	4.49c	4.29d	4.31d	4.33d	2.50	6.90
Histidine	2.31b	1.75d	3.44a	1.86c	1.88c	1.92c	1.96c	1.00	1.00
Methionine	1.27b	0.88d	1.93a	1.01c	0.99c	1.01c	1.04c	1.50	2.70
Valine	3.57d	4.21c	5.83a	4.57b	4.45b	4.48b	4.52c	2.60	3.80

Tryptophan	0.54b	0.26d	0.57a	0.29c	0.29c	0.30c	0.30c	0.40	1.25
Threonine	3.60a	2.89e	3.33b	3.16c	3.03d	3.04d	3.04d	1.50	3.70
Isoleucine	3.23c	2.99e	3.64a	3.34b	3.18d	3.19d	3.20d	2.00	3.10
Lysine	6.48c	6.49c	5.54d	6.84a	6.60b	6.57b	6.54b	3.00	6.40
Leucine	5.85a	3.71e	5.66b	4.38c	4.11d	4.14d	4.17d	3.90	7.30
TAA	87.79a	68.74e	87.91a	77.97b	73.62d	73.88d	74.16c	-	
TEAA	31.68b	27.20f	35.20a	29.94d	28.82e	28.96d	29.10c	18.40	36.15
TEAA/TAA	0.36d	0.39b	0.40a	0.38c	0.39b	0.39b	0.39b	-	
TSAA	3.57a	2.03d	2.63b	2.51c	2.28e	2.28e	2.29e		
ArAA	9.00b	7.27e	10.81a	8.13c	7.83d	7.90d	7.96d		

Note: H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf) flour; R0Z100A0 (0% Red sorghum bran+100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf)flour;): R50Z42.5A7.5 (50% Red sorghum bran+42.5% zobo calyx +7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour; Total essential amino acid (TEAA), Total non-essential amino acid (TNEAA), Sulphur containing amino acid ((HAA=Ala, Val, Iso, Leu, Try, Phe, Trp, Pro, Met, Cys), Positively charged amino acid (PCAA=His, Lys), Negatively charged amin Total Aromatic amino acids (ΣArAA=Phe, Try, Tyr), Branched chain amino acids (tBCAAs=Leu., Iso., Val), Essential amino acid index (EAAI).

Total amino acid content of enriched red sorghum bran sample ranged from (73.62 to74.16 g/100 g) was lower than 848.74 to 929.49 g/100 g reported by Oyarekua et al. for Corn-Ogi [22]. TEAA obtained for the enriched samples (28.82-29.10 g/100 g) was lower than 373.2 g/100 g reported by Oyarekua et al. for Corn ogi and 566 g/100 g of egg reference protein. The ArAA (7.83-7.96 g/100 g) falls below the range suggested for infant protein (68-118 g/100 g), however the enriched flour can be fortified with a protein rich source [22]. The ratio of essential amino acids to the total amino acids in the enriched flour was 0.39 which was lower than (0.5) for egg protein but compared well with 0.39 recommended for infant's food protein, far above 0.26 and 0.11 recommended for children and for adults food protein respectively. Total sulphur amino acid (TSAA) obtained in this study (2.28-2.29 g/100 g) was lower than (58 g/100 g) recommended for infants. Leucine (4.11 to 4.17 g/100 g) and Lysine (6.54 to 6.60 g/100 g) were the most abundant essential amino acids present in the enriched flour samples. Glutamate (10.39-11.04) g/100 g, Arginine (4.82-4.84) g/100 g, and Aspartate (10.16-10.20) g/100 g were the most abundant non essential amino acids present in the enriched products.

Antioxidant activity of sorghum red bran-roselle calyx-avocado leaf infused drinks: The DPPH radical scavenging activity of the red sorghum bran-Roselle calyx-Avocado leaf infused drinks is shown in Table 2. The DPPH radical scavenging activity of the enriched samples ranged from 71.57% for R50Z40A10 to 73.44% for R50Z45A5 which was lower than that of the control (H100) was 82.10% (Table 4). The DPPH radical scavenging activity of the enriched samples (71.57%-82.10%) aqueous extract was higher than (16.51%-68.35%) obtained for some local drinks by Oboh et al. (70.18%; 62.55% and 63.90%) reported by Oboh et al. and Al-Hashimi et al. for roselle calyces aqueous extract respectively [23,24]. For all the flour samples and standard (ascorbic acid), the DPPH activity ranged from (32.73%-90.00%) at different concentrations. The DPPH radical scavenging activity decreased significantly ($p<0.5$) with increased Avocado leaf and decreased cocentration of Zobo calyx to red sorghum bran flour. This could be attributed to the differential DPPH of the three base materials which are red sorghum bran, Zobo calyx and Avocado leaf flours. The DPPH results showed that red sorghum bran and Zobo calyx contained high phenolic compounds exhibited by high antioxidant activity when determined by DPPH assays. The DPPH radical scavenging activity of the standard (Ascorbic acid) (90.00%) was significantly higher ($p<0.05$) than that of the control (H100) (82.10).

Table 4: Antioxidant activities of Sorghum red bran-Roselle calyx-Avocado leaf infused drinks.

Samples	(mg GAE/ml) Phenol	(%) DPPH	(%) Fe Chelation	(mMol/100ml)	(%) OH-radical	(mg GAE/ml) FRAP
H100	75.30 ± 0.06ab	82.10 ± 0.02a	44.00 ± 0.48abc	0.65 ± 0.02ab	52.51 ± 0.58bc	73.06 ± 0.23ab
R100Z0A0	73.92 ± 0.10b	80.44 ± 0.04ab	52.07 ± 0.22b	0.53 ± 0.04b	62.12 ± 0.38ab	65.65 ± 0.30b
R0Z0A100	81.96 ± 0.04a	32.73 ± 0.15c	58.03 ± 0.12a	0.74 ± 0.01a	66.17 ± 0.25a	75.19 ± 0.12a
R0Z100A0	14.45 ± 0.25cd	70.18 ± 0.13bc	48.31 ± 0.38bc	0.52 ± 0.05b	50.30 ± 0.60abc	27.83 ± 0.47c
R50Z45A5	47.56 ± 0.20c	73.44 ± 0.06b	50.88 ± 0.32b	0.53 ± 0.04b	57.01 ± 0.80b	49.11 ± 0.35bc
R50Z42.5A7.5	49.25 ± 0.13bc	72.51 ± 0.09bc	50.92 ± 0.28b	0.54 ± 0.03b	57.40 ± 0.43b	50.99 ± 0.52abc
R50Z40A10 Ascorbic Acid	50.94 ± 0.16abc	71.57 ± 0.10abc	51.16 ± 0.04b	0.55 ± 0.02b	57.70 ± 0.15b	51.48 ± 0.05abc
EDTA	-	90.00 ± 0.05	87.00 ± 0.02	-	-	-

Note: Values are means ± standard deviation of three determination (n=3).
Values with different alphabet along the same column are significantly different from each other ($p \leq 0.05$) according to Duncan multiple comparism. H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf) flour; R0Z100A0 (0% Red sorghum bran+100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf)flour;): R50Z42.5A7.5 (50% Red sorghum bran+ 42.5% zobo calyx+7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour

The Metal Chelating (MC) activity of red sorghum bran-Roselle calyx-Avocado leaf infused drinks and standard (EDTA) are shown in Table 2. The Metal Chelating (MC) activity of the enriched samples ranged from 50.88% for R50Z45A5 to 51.16% for R50Z40A10 which was higher than that of the control (H100) was 44.00% (Table 4). The metal chelating activity of the enriched samples (50.88%-51.16%) aqueous extract was lower than 73.97% reported by Al-Hashimi et al. for roselle calyxes' aqueous extract [24]. The high metal chelating activity of the enriched samples could enhance ability of tissues to reduce rate of deteriorative metal-catalyzed lipid oxidation. The metal chelating activity of all the samples increased significantly (p<0.5) with increased concentration of Avocado leaf and decreased concentration of Zobo calyx flour. The metal chelating activity of all the extracts revealed their antioxidant potency [25]. The Metal Chelating activity of the standard (EDTA) was significantly higher (87.00%) at (p<0.5) than that of the control (H100) (44.00%).

The Ferric Reducing Antioxidant power (FRAP) of red sorghum bran-Roselle calyx-Avocado leaf infused drinks are shown in Table 2. The FRAP of the enriched samples ranged from 49.11 mgGAE/ml for R50Z45A5 to 51.48 for R50Z40A10 which was lower than that of the control (H100) was 73.06 mg GAE/ml (Table 4). The Ferric Reducing Antioxidant of the enriched samples (49.11 to 51.48) mg GAE/ml aqueous extract was lower than (0.14 to 32.43) mg GAE/ml reported by obtained for some local drinks by Oboh et al. indicated that red sorghum bran is a well-known natural antioxidant and excellent reducing agent [23].

Antioxidant activity of sorghum red bran-roselle calyx-avocado leaf flour blends: The phenolic content of red sorghum bran-Roselle calyx-Avocado leaf infused drinks is shown in Table 2. The phenolic content of the enriched samples ranged from 49.58 mg GAE/g for R50Z45A5 to 52.97 for R50Z40A10 which was lower than that of the control (H100) was 78.42 mg GAE/g (Table 2). The phenolic content of the enriched samples (49.58 to 52.97) mg GAE/g was higher than (7.36 to 10.24) mg GAE/g obtained for sorrel seed flour by Ayo-Omogie et al. [16]. Total phenolic content (TPC) in red sorghum bran (75.60) mg GAE/g and Avocado leaf (84.42) mg GAE/g were high because of their bioactive compositions.

The ABTS radical scavenging activity of the red sorghum bran-Roselle calyx-Avocado flour blends is shown in Table 2. The ABTS radical scavenging activity of the enriched samples ranged from 57.64% for R50Z45A5 to 58.98% for R50Z40A10 which was lower than that of the control (H100) was 60.11 (Table 2). The ABTS radical scavenging activity of the enriched samples (57.64%-58.98%) aqueous extract was higher than (22.3%-37.9%) obtained for four guava genotypes flour by Kriengsak et al. The ABTS radical scavenging activity of the enriched bran decreased insignificantly (p<0.5) with increased Avocado leaf and decreased cocentration of Zobo calyx to red sorghum bran flour.

The OH radical scavenging activity of the red sorghum bran-Roselle calyx-Avocado leaf flour blends is shown in Table 2. The OH radical scavenging activity of the enriched samples ranged from 59.75% for R50Z40A10 to 60.44% for R50Z45A5 which was higher than that of the control (H100) was 54.65% (Table 2). The OH radical scavenging activity of the enriched samples (59.75%-60.44%) was higher than (18.32%-27.28%) obtained for three varieties of sorghum bran flour by Yingying et al. [26]. The OH radical scavenging activity decreased insignificantly (p>0.5) with increased Avocado leaf and decreased concentration of Zobo calyx to red sorghum bran flour (Table 5).

Table 5: Antioxidant activities of Sorghum red bran-Roselle Calyx-Avocado leaf flour blends.

Samples	(mg GAE/g) Phenol	(%) DPPH	(%) Fe Chelation	ABTS (%)	(%) OH-radical	(mg GAE/g) FRAP
H100	78.42 ± 0.28ab	85.67 ± 0.08a	45.34 ± 0.66abc	60.11 ± 0.20ab	54.65 ± 0.30abc	75.35 ± 0.23ab
R100Z0A0	75.60 ± 0.32b	82.48 ± 0.10ab	55.88 ± 0.22ab	62.00 ± 0.15a	65.00 ± 0.10ab	68.20 ± 0.25b
R0Z0A100	84.42 ± 0.18a	35.19 ± 0.61c	60.36 ± 0.12a	46.20 ± 0.42abc	69.17c ± 0.05a	80.45 ± 0.12a
R0Z100A0	16.81 ± 0.60cd	75.42 ± 0.58bc	51.48 ± 0.52bc	55.05 ± 0.30bc	52.63 ± 0.32c	28.57 ± 0.77c
R50Z45A5	49.58 ± 0.52c	76.94 ± 0.20b	54.13 ± 0.47b	58.98 ± 0.22b	60.44 ± 0.12b	50.98 ± 0.30bc
R50Z42.5A7.5	51.28 ± 0.48abc	75.93 ± 0.07bc	54.35 ± 0.25b	58.87 ± 0.13b	59.52 ± 0.28bc	52.27 ± 0.45c
R50Z40A10 Ascorbic acid	52.97 ± 0.43bc	74.92 ± 0.08abc	54.57 ± 0.03b	57.64 ± 0.36b	59.75 ± 0.05bc	53.57 ± 0.34abc
EDTA	-	90.00 ± 0.05	87.00 ± 0.02	-	-	-

Note: Values are means ± standard deviation of three determination (n=3).
Values with different alphabet along the same column are significantly different from each other (p ≤ 0.05) according to Duncan multiple comparism.
H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf) flour; R0Z100A0 (0% Red sorghum bran+100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf)flour;): R50Z42.5A7.5 (50% Red sorghum bran+42.5% zobo calyx+7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour.

Phenolic profile of Sorghum red bran-Roselle Calyx-Avocado leaf flour blends

The increase in p-coumaric acid ranged from 7.2 mg/100 g for R50Z45A5 to 7.4 mg/100 g for R50Z0A10 compared with the control (H100) 6.39 mg/100 g (Table 3). The p-coumaric acid value (7.2 mg/100 g to 7.4 mg/100 g) of the enriched flour was higher than (0.34 to 0.64) mg/100 g obtained for three varieties of sorghum bran by Yingying (Table 6) [26]. The increase could be a result of the release of bound phenolic compounds under the acidic condition because a significant proportion of phenolic compounds are present in bound form in cereals [27,28].

The decrease in caffeic acid ranged from to 309.96 mg/100 g for R50Z45A5 to 302.38 mg/100 g for R50Z0A10 compared with the control (H100) 620 mg/100 g (Table 3). The caffeic acid value (302.38 mg/100 g to 309.96 mg/100 g) of the enriched flour was however higher than (148 to 172) mg/100 g obtained for red sorghum bran extract by Jeremiah et al. [29,30].

Table 6: Phenolic profile of Sorghum red bran-Roselle Calyx-Avocado leaf flour blends (mg/100 g).

Samples	H100	R100Z0A0	R0Z0A100	R0Z100A0	R50Z45A5	R50Z42.5A7.5	R50Z40A10
Phloretic	0.0046	0.0082	0.00174	0.0043	0.0070	0.00198	0.00206
Vanillic	0.0030	0.00256	0.00258	0.00184	0.00916	0.00134	0.00178
P-Hydroxy Benezoic	0.00293	1.0161	0.0090	0.00664	0.00299	0.00152	0.00117
Cinnannic	0.00120	0.0084	0.0050	0.00309	0.00367	0.00597	0.00219
Protocatechuc	0.00301	0.00265	0.00115	0.00467	0.00178	0.00147	0.00109
p- coumaric	6.39	8.48228	9.3518	5.564	7.2000	7.3100	7.4000
o-coumaric	0.00827	0.00363	0.00212	0.00177	0.00706	0.004	0.00172
Apigenin	0.00202	0.00261	0.00527	0.00104	0.00176	0.00978	0.00450
Gallic acid	0.227	0.924	0.205	0.00532	0.00682	0.0010	0.00119
Kaempferol	4.3765	3.025	4.130	5.48	4.19	4.15	4.12
Catechin	71.89	80.80	94.73	91.11	86.13	86.22	86.31
Naringenin	0.00114	0.00141	0.00201	0.00703	0.00234	0.00513	0.00370
Ferulic acid	0.0722	0.00192	0.00217	0.00667	0.00291	0.00332	0.00682
Syringic acid	0.208	1.3534	0.2901	0.00690	0.00965	0.00968	0.00360
Naringin acid	0.483	1.4714	0.00616	0.00786	0.00896	0.00881	0.00193
Ellargic	0.00228	0.00247	0.00269	0.00159	0.00181	0.00222	0.00378
Piperic	0.00149	0.00919	0.00500	0.00500	0.00500	0.00104	0.00104
Luteolin	0.00614	0.00153	0.00997	0.00997	0.00997	0.00372	0.00372
Caffeic	620	108.86	374.60	526.23	309.96	306.17	302.38
Sinapinic	0.00572	0.00144	0.00593	0.00176	0.00286	0.00255	0.00136
Epicatechin	0.845	3.0595	0.00952	0.0029	0.00377	0.00367	0.00120
Epigallocatechin	0.00988	1.6318	0.00421	0.0046	0.00300	0.00387	0.00437
Kaemppferol-3-0-glucoside	0.10313	0.269	0.00904	0.00401	0.00511	0.00358	0.00224
Quercetin	6.553	8.11	5.518	17.33	12.13	11.83	11.54
Isorhamnetin	0.00324	0.00172	0.00224	0.0019	0.00197	0.00142	0.00119
Myricetin	3.08047	3.47	2.5824	1.18	2.40	2.43	2.47
3-0-caffeoylquinic	0.0697	0.00309	0.00372	0.0014	0.00386	0.00171	0.00204
Hespertetin	30.901	15.90	37.18	52.97	33.65	33.25	32.86
Kaempferol-3-0-nitroside	0.00163	0.00153	0.00121	0.00118	0.00227	0.00443	0.00339
Chlorogenic acid	79.654	49.50	82.382	84.88	67.07	67.00	66.95
Chicoric acid	0.0036	0.00549	0.00294	0.0064	0.00925	0.00598	0.00126
Quercitrin	0.00123	0.00916	0.00942	0.00123	0.00296	0.00434	0.00527
Isoquercitrin	28.295	22.50	26.112	39.101	30.16	29.83	29.50
Hesperidin	5.19	11.70	15.83	13.854	12.87	12.93	12.97
Rutin	19.587	41.10	15.854	12.90	27.15	27.22	27.30
Quercetin3,4-diglucoside	0.00167	0.0029	0.00152	0.00442	0.00275	0.00501	0.00645

Note: Values are means ± standard deviation of three determination (n=3), Values with different alphabet along the same column are significantly different from each other ($p \leq 0.05$) according to Duncan multiple comparism. H100 (Commercial herbal tea flour); R100Z0A0 (100% Red sorghum bran+0% zobo calyx+0% Avocado leaf): R0Z0A100 (0% Red sorghum bran+0% zobo calyx+100% Avocado leaf) flour; R0Z100A0 (0% Red sorghum bran+100% zobo calyx+0% Avocado leaf)flour; R50Z45A5 (50% Red sorghum bran+45% zobo calyx+5% Avocado leaf) flour: R50Z42.5A7.5 (50% Red sorghum bran+42.5% zobo calyx+7.5% Avocado leaf) flour; R50Z40A10 (50% Red sorghum bran+40% zobo calyx+10% Avocado leaf) flour.

CONCLUSION

The present study established that the enriched flour blends produced from Red sorghum bran, Roselle calyx and Avocado leaf were very rich in essential nutrients like amino acids, bioactive compounds, phenolic components and antioxidant properties but low in anti-nutritional factors.

It can be concluded that nutritious health promoting infused beverages can be produced from the enriched flour particularly R50Z40A10, which may be suitable as a functional food ingredient and play protective roles against cardiovascular, neuro-degenerative and age-related diseases compared to commercial sugar laden-fizzy carbonated beverages which are detrimental to human health.

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