

Nutritional Characteristics and Antioxidant Activity of Dough Meal Flour Blends from Acha (*Digitaria exilis*) Tigernut (*Cyperus esculentus*) and Sorrel (*Hibiscus sabdariffa*, Linn.) Seed Protein Isolate

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ABSTRACT: This study evaluated the nutritional quality and antioxidant activity of dough meal from acha-tigernut flour blends enriched with sorrel protein isolate. Flour samples were prepared from acha (ACF) and tigernut (TNF) while protein was isolated from sorrel seed (SPI). The flour blends were optimized using Response Surface Methodology design expert 8.3.0.1(trial version) to generate eleven runs. The flour blends were developed into dough meal and evaluated for proximate and sensory attributes, using a commercial flour sample (Cerolina) as control (CNL). Two blends were selected based on their protein, fibre contents and sensory attributes. The dough meal flour blends, i.e., ATS (5% SPI, 45% TNF and 50% ACF); TNS (5%SPI, 42.64% TNF and 52.36% ACF); and CNT (a commercial flour) were evaluated for nutritional quality and antioxidant activity. The formulated dough meal flour samples contained appreciable amount of protein, energy value and antioxidant activity.

Keywords: Protein, Functional Dough Meal, Antioxidants, Tigernut and Acha.

I. INTRODUCTION

Dough meals are regular meals taken in Nigeria and other African countries. Dough is a paste made out of different cereal (grains) or leguminous crop by mixing flour with a small amount of water and/or other liquid, boiled to gelatinization to form a semi solid food product. In Nigeria, the common dough meals are eba, made from gari (cassava), fufu (also from cassava) and amala, made from milled dried yam or plantain flour. The need to enhance the nutritional availability of the staple food and its functionality has brought about the use of Acha, Tigernut and Sorrel Seed Protein Isolate. Acha (*Digitaria exilis*), is commonly called hungry rice or fonio and is considered as one of the most nutritious grains; its seeds contain 8.79% protein and may be up to 11.89% in some black fonio samples (Temple and Bassa, 1991). The grains are rich in amino acids; leucine (9.8%), methionine (5.6%) and valine (5.8%) (Temple and Bassa 1991) and cysteine which are vital to human health but deficient in today's major cereals. Acha grains contain substantial minerals (mostly iron, calcium and phosphorus) about 5% dry matter. Acha could be mixed with other cereal flours to make cookies, such as candy and fermented beverages. The grains could also be a substitute for semovita and other wheat products such as spaghetti and other pastas. Tigernut (*Cyperus esculentus*) is an underutilized sedge that has been reported to be of help in reducing the risk of colon cancer, due to the high content of soluble glucose (Belewu and Abodurin, 2006). The nut is rich in energy content (starch, fat, sugars

and protein), mineral (phosphorus, potassium), vitamins E, and C (Belewu and Abodurin, 2006). Sorrel seed is a valuable source of protein, calorie, fat, and substantial amount of fiber and essential micronutrients (Akanbi *et al.*, 2009). The seeds are a good source of lipid soluble antioxidants, particularly γ -tocopherol (Mohammed, 2007). The nutrient composition of *H. sabdariffa* seed varies due to different varieties, genetic, environmental, ecology and harvest conditions of the plant (Atta *et al.*, 2013). This study aimed at formulating and determining the nutritional quality and antioxidant activity of dough meals from underutilized locally available food materials.

II. MATERIALS AND METHODS

A. Source of Raw Materials

Acha (*Digitaria exilis*), tigernut (*Cyperus esculentus*) yellow variety and Sorrel seeds (*Hibiscus sabdariffa*) were purchased from a local market in Sokoto, Sokoto State, Nigeria, respectively.

B. Flour Sample Preparations

(a). Tigernut Tuber Flour Processing

Tigernut tubers was processed into flour using the method of (Adejuyitan *et al.*, 2011) with little modification. Yellow Tigernut tubers were sorted, winnowed, severally washed with distilled water, drained, oven dried at 60° C for 10 h using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, UK), milled using Kenwood laboratory blender (BLX52 model, UK) and sieved using a 60 mm mesh sieve (British Standard) to obtain tiger nut flour. The flour was packed in a plastic container, sealed and stored at room temperature (27° C) until analysis.

(b). Acha Flour Processing

The Acha flour was processed using the method described by Olapade and Oluwole (2013) with little modification. The Acha were sorted to remove stones and foreign materials and washed with clean tap water. The acha grains were drained and dried at 60° C for 8 h, using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, UK), followed by milling using a laboratory mill (Laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK). The flour was sieved (60 mm) and packaged in polyethylene bags and stored at room temperature ($\approx 27^{\circ}\text{C}$) until analysis.

(c). Preparation of Sorrel Seed Flour and Protein Isolate

The sorrel seed was cleaned by removing dust, stones, plant debris, washed and then dried using hot air oven (Model 349/2, Corning Limited) (- 4° C). The seeds were dehulled by adopting the method of Ayo-Omogie and Osanbikan, (2019) with little modification. Thereafter, the dehulled seeds was milled using (Laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) in to fine flour and sieved through a 60 mm mesh screen. Thereafter, the powder was defatted using the soxhlet extractor for 8 hrs. The oil-free flour was desolventized and stored in desiccator at room temperature (27 °C) for subsequent analysis. Sorrel seed protein isolates (SSPI) was obtained from the defatted flour as reported by El- Tinay *et al.*, (1988). The defatted flour was dispersed in distilled water at ratio of 1:10; the pH was adjusted to 10 with 1 M NaOH and stirred for 3 h at room temperature. The suspension was centrifuged at 4000 rpm for 20 min. The residues were re-extracted for the second time as described above. The supernatant was combined and protein was precipitated by adjusting to pH 3.5 with 1 M HCl before centrifugation at 4000 rpm for 20 min. The protein isolate (precipitate) was washed twice with distilled water then re-suspended in distilled protein isolates) was stored in desiccator at room temperature for subsequent analysis.

(d). Formulation of Composite Flour Samples

The acha flour, tiger nut flour and sorrel seed protein isolate were evaluated for their proximate composition. Based on the results obtained for (protein and crude fibre), blend ratios were obtained using Response Surface Methodology (RSM). Thereafter, the following food combination were obtained, that is, ATS (SPI+TNF+ACF: 5.00+45.00+50.00), TNS (SPI+TNF+ACF: 5.00+42.64+52.36) and CNT (a commercial dough meal flour as control).

C. Chemical Analyses

(a). Determination of Chemical Composition of Flour Blends

Proximate compositions, that is, moisture content, ash, crude fiber, crude fat and crude protein content of experimental food samples were determined using the standard methods (AOAC, 2012). Carbohydrate content was determined by difference as follows:

Carbohydrate (%) = 100 - (%Moisture + %Fat + %Ash + %Crude fibre + %Crude protein)

(b). Determination of Energy Value

Energy value was determined using an indirect calculation method. The three groups of nutrients, which provide the body with energy, are carbohydrates, fats and proteins. One gram of carbohydrate (C) was assumed to give 4.2 kCal. Energy; one gram of fat (F) 9.0 kcal energy and one gram of protein (P) 4.2 kCal. The energy values for one gram of the three groups of nutrients, which provides the body with energy, were calculated by using specific values of Atwater factors for protein, fat, and total carbohydrate (Iombor *et al.*, 2009).

Calculation.

$Energy\ value = (4.2) + (F \times 9.0) + (C \times 4.2)$ in kCal/100 g of sample

Where; P = Protein content (%).

F = Fat content (%).

C = Available total carbohydrate (%).

(c). Mineral Composition Determination

The mineral elements (i.e. Ca, Mg, Fe, Cu and Zn) in the formulated flour samples were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). Sodium and potassium were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as the standards (AOAC, 2012). Phosphorus was determined using Vanado-molybdate method (AOAC, 2012). The mineral molar ratios (Na/K & Ca/P) and phytate/mineral molar ratios (Phytate/Zn, Ca/Phytate and [Ca]/[Phytate]/[Zn]) were calculated as described by Ferguson *et al.* (1988).

(d). Determination of Antioxidative Potential of the Dough Meal Flour from Acha, Tigernut and Sorrel Seed Isolate

The Total flavonoid content was determined by Aluminum chloride colorimetric assay. The total phenolic content of the extract was also estimated. The scavenging effect of the samples on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was measured according to the method of Aluko and Monu (2003). The ABTS scavenging ability of the extract was determined according to the method described by Re *et al.* (1999). The ferric reducing power of the protein fraction samples was determined according to the modified method of Zhang *et al.* (2008).

D. Statistical Analysis

Data were subjected to analysis of variance using SPSS (IBM version. 20.0, SPSS Inc., Quarry Bay, Hong Kong) and presented as means (SEM). Comparison between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $p < 0.05$ were considered as statistically significant as described by Yalta and Talha (2008).

III. RESULTS AND DISCUSSION

A. Nutritional Composition and Energy Value of Dough Meal Samples generated from Response Surface Methodology Mixture of Acha, Tigernut and Sorrel Protein Isolate

The proximate (g/100 g), energy value (Kcal/100 g) and mineral composition (mg/g) of dough meal flour samples generated from response surface methodology mixture of acha, tigernut and sorrel protein isolate flour blends and the control sample (CNL) are presented in Table 3.1. The moisture content (MC) of the flour samples indicates that ATS (7.55) had the lowest value, while TNA (9.24) had the highest, and the values were within the control sample (7.58). Interestingly, these values are significantly ($p < 0.05$) lower than 10.00 g/100 g recommended by FAO (2003) for flour samples. The low moisture recorded in this present study indicates low water activity, which is desirable in flour products. Study has reported that low MC in flour sample inhibits the proliferation of microorganisms, hence, enhancing the keeping quality and storage life of the flour (Ayo-Omogie, 2020). The protein content of ATS (22.24) had the highest value, followed by TNA (17.25), and were lower than the control (CNL) (24.43). The variation between the protein contents of the flour samples in this present study and that of control could be due to the raw materials and processing techniques. Comparatively, the protein content obtained in the experimental food samples falls within 12.01 - 18.70 g/100 g reported for wheat-cassava bread samples enriched with 5-15% inclusion of sorrel seed protein isolate (Sanni *et al.*, 2020); and 13.67 to 19.18 g/100g obtained for cracker biscuit blends with acha reported by Olagunju *et al.* (2018). These findings imply that both sorrel seed protein isolate and acha may have significantly contributed to the protein content of the blended flour samples. The energy value of the formulated flour samples ranged from 413.86 in TNA to 425.82 kcal/100 g in ATS, respectively, and the values were within the recommended value of 400 - 425 kcal/ 100 g for children and adults (FAO, 2003). The high-energy value of experimental flour samples could be attributed to high fat content in tigernut, carbohydrate in acha and protein content in sorrel protein isolate (Olagunju *et al.*, 2018; Ayo-Omogie and Osanbikan, 2019). Nutritionally, the protein content and energy value of these formulated dough meal flours may be adequate for physiological needs of children and adults.

The essential minerals (mg/g) like iron, zinc, phosphorous and calcium were present in appreciable amount with values ranging from 80.27 to 90.74, 120.82 to 124.62, 8.73 to 8.81 and 2199.97 to 2216.97, respectively, and were comparable to values obtained for the control sample. Iron and calcium are essential minerals for blood and bone formation, and their presence in the formulated dough meal flour samples may prevent anaemia and deformation of bone of the consumers. The Na/K molar ratios of the dough meal flour samples varied from 0.02 to 0.23, and the values are lower than recommended value of < 1.0 (WHO, 2012 a & b). This indicates that the dough meal flour samples contain high amount of potassium, which is in line with the previous reports that in plant-based food potassium is usually present in abundance (WHO, 2012b); and that regular intakes of this mineral exhibit some health benefits (WHO, 2012b).

Table 1: Nutrient (g/100g), Energy value (kcal/100 g) and Mineral Composition (mg/g) of dough meal flour from Acha, Tigernut and Sorrel Protein Isolate

Sample	ATS	TNA	CNL
Proximate (g/100g)			
Moisture	7.55±0.82 ^b	9.24±0.89 ^a	7.58±1.09 ^b
Crude Fibre	2.98±0.55 ^b	5.04±0.26 ^a	1.18±0.09 ^c
Crude Protein	22.24±0.76 ^b	17.25±0.57 ^c	24.43±0.09 ^a
Total Ash	2.89±0.09 ^a	3.01±0.10 ^a	1.17±0.09 ^b
Crude Fat	9.82±0.33 ^a	9.22±0.31 ^a	1.04±0.04 ^b
Carbohydrate	62.12±2.17 ^c	65.47±2.18 ^b	72.15±2.44 ^a
Energy value	425.82±1.20 ^a	413.86±2.35 ^b	395.68±1.03 ^c
Minerals (mg/g)			
Magnesium	4214.1±6.84 ^c	5002.24±3.86 ^a	4773.22±4.54 ^b
Iron	80.27±1.31 ^c	90.74±1.87 ^a	86.99±2.55 ^b
Zinc	120.82±2.76 ^b	124.62±2.86 ^a	124.68±2.98 ^a
Copper	44.83±1.06 ^b	44.98±1.61 ^b	46.91±1.02 ^a
Phosphorus	8.80±0.06 ^a	8.73±0.06 ^b	8.81±0.06 ^a
Potassium	2020.58±12.81 ^c	19859.51±26.55 ^b	20070.4±18.48 ^a
Sodium	463.22±2.95 ^a	446.62±2.84 ^b	461.83±1.96 ^a
Calcium	2199.97±4.04 ^b	2183.05±3.91 ^b	2216.97±4.12 ^a
Na/K	0.229±0.01 ^a	0.022±0.01 ^b	0.023±0.01 ^b
Ca/P	249.71±1.41 ^a	250.06±2.56 ^a	251.64±4.12 ^a

Means (±SD) with different alphabetical superscripts in the same row are significantly different at P<0.05
Key:

CNL: Cerolina (Control sample)

ATS: Sorrel protein isolate + Tigernut + Acha flour: 5.00 + 45.00 + 50.00)

TNA: Sorrel protein isolate + Tigernut + Acha flour: 5.00 + 42.64 + 52.36)

B. Antioxidant Potential of Acha, Tigernut and Sorrel Protein Isolate Dough Meal Samples

The antioxidant property of dough meal flour samples from acha, tigernut and sorrel protein isolate are presented in Table 3.2. The antioxidant activity of ATS was significantly ($p \leq 0.05$) higher in total flavonoid (34.02 µg Rutin/mg), phenolic (40.03 µg GAE/mg), DPPH (14.03%), ABTS (78.6%) and FRAP (1.06 mg/g) than in TNA (33.25 µg Rutin/mg, 20.01 µg GAE/mg, 14.01%, 77.81% & 0.89 mg/g, respectively). The finding from the study showed that antioxidant activity of experimental flour samples was significantly ($p < 0.05$) higher than control sample. This implies that formulated dough meal flour samples have antioxidant activities, and may be suitable for the prevention of oxidative stress and other degenerative diseases. This finding agrees with the reports that plant-based foods exhibits high antioxidant activities because of high total phenolic content (Badejo *et al.*, 2020). Phenolic compounds present in almost all foods of plant origin have beneficial effects, which include reducing the risk of diabetes, cancer, cardiovascular diseases and allergens (Rasouli *et al.*, 2017). Similarly, this finding agrees with the reports of Oluwajuyitan and Ijarotimi (2019) and Badejo *et al.* (2020) who reported on antioxidant activities of functional dough meals and beverages enriched with tiger nut. Hence, high antioxidant activities of ATS observed in the present study could be attributed to the inclusion of tigernut.

Table 2: Antioxidant Activity of dough meal flour from Acha, Tigernut and Sorrel Protein Isolate

Samples	ATS	TNA	CNL	*STD
Total Flavonoid (μg Rutin/mg)	34.02 \pm 0.34 ^a	33.25 \pm 0.16 ^b	33.25 \pm 0.24 ^b	-
Total Phenol (μg GAE/mg)	40.03 \pm 0.18 ^a	20.01 \pm 0.15 ^b	20.00 \pm 0.12 ^b	-
DPPH (%)	14.03 \pm 0.06 ^a	14.01 \pm 0.03 ^a	12.41 \pm 0.04 ^b	29.43
ABTS (%)	78.60 \pm 0.19 ^b	77.81 \pm 0.10 ^b	80.09 \pm 0.21 ^{ab}	95.38
FRAP (mg/g)	1.06 \pm 0.02 ^a	0.89 \pm 0.03 ^c	0.91 \pm 0.01 ^b	1.195

Means (\pm SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$. *STD: Trolox (ABTS), Ascorbic acid (DPPH & FRAP)

Key: ATS: (Sorrel protein isolate + Tigernut + Acha flour: 5.00 + 45.00 + 50.00);
TNA: (Sorrel protein isolate + Tigernut + Acha flour: 5.00 + 42.64 + 52.36);
CNL: Cerolina (Control sample).

IV. CONCLUSION

The study established that the formulated dough meal samples made from flour blends of acha, tigernut and protein isolated from sorrel seeds contained appreciable amounts of protein and energy values. The experimental dough meal samples, especially ATS (5% Sorrel protein isolate, 45% Tigernut, 50% Acha), exhibited good free radical scavenging abilities. Based on the finding from this study, sample ATS (Sorrel protein isolate + Tigernut + Acha flour: 5.00 + 45.00 + 50.00) is recommended for preparation of dough meal for adequate nutritional benefit.

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