



# Nutritional, Phytochemical, Functional and Antioxidant Properties of Acha, Chia and Soycake Flour Blends

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## Authors' contributions

This work was carried out in collaboration among all authors. Author OCO conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, contributed reagents, materials, analysis tools or data, wrote the paper. Authors OSO, AIO and SAG and DTI conceived designed the experiments, wrote the paper. All authors read and approved the final manuscript.

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## ABSTRACT

Functional flour blends are been developed with nutritional and health promoting benefits to fit in to the third agenda of Sustainable Development Goal (SDG) 2030. A blend of fermented acha (FAF), defatted chia (DCF), and defatted soycake flours (DSF flour were blended to harness the proficient potential of the blends. Response surface methodology (RSM) was used to generate fourteen runs using the the protein and the crude fibre of the flour blends. The three blends that the protein ranges from 15-20% and fibre from 3-5% were selected and the composite flours were analyzed for

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proximate composition, minerals, phytochemical composition, functional properties, and antioxidant properties. Results indicated that protein content increased with higher DSF proportions ranging from 15.37% in ACSF13 (80% of fermented acha, 10% of defatted chia seed, 10% soycake) to 19.51% in ACSF9 (45% of Fermented acha, 10% of defatted chia seed, 45% soycake). Energy content also rose from 335.4 kcal to 391.4 kcal in ACSF13 to ACSF1 (57% of fermented acha, 22% of defatted chia seed, 21% Soycake). ACSF1 is the blend with the highest fibre (5.06%) and ACSF9 possess the highest ash content of (4.13%). The blends were rich in potassium, calcium, zinc, iron and magnesium with phytochemical, phytate- mineral ratio and oxalate mineral ratio within recommended levels. Sample ACSF1 had the highest bulk density while ACSF9 possess both the highest swelling capacity and water absorption. The total phenolic and flavonoid content of the flour blends ranged from 273.55 to 149mg GAE/g and 66.71 to 31.60mgQE/g with sample ACSF13 having the highest phenolic content (273.55GAE/g) and flavonoid content of 66.71mgQE/g. The increase in the Soycake flour is an indications to improvement on the nutritional values of the flour blends.

**Keywords:** *Fermented acha; defatted chia; defatted Soycake; resource surface methodology; phytochemical and antioxidants.*

## 1. INTRODUCTION

Healthy nutrition is essential for maintaining a successful and healthy life, food consumed plays a pivotal role in our daily activities and overall health. Food originates from both animal and plant sources, plant-based foods and functional foods are extensively consumed due to their bioactive substances and associated health benefits [1]. The increasing prevalence of cardiovascular diseases (CVDs), high blood pressure, obesity, diabetes, and related health issues, often linked to sedentary lifestyles and poor dietary habits, has heightened interest in adopting healthier lifestyles. Diets that promote health span, characterized by low glycemic index, high dietary fiber, high omega-3 fatty acids, low saturated fat and cholesterol, and rich in antioxidants and essential nutrients, are increasingly recommended [2]. The World Health Organization (WHO) advocates for low glycemic index diets to prevent diabetes and control blood sugar levels, thus reducing the risk of heart disease and other chronic conditions [3,4].

Composite flours offer a means to improve the nutritional and functional properties of foods. The proportional composition of different flours determines the nutritional quality of the resulting products. Using Response Surface Methodology (RSM), this study formulated flour blends from fermented acha (*Digitaria exilis*), defatted chia (*Salvia hispanica L.*), and defatted soycake flours.

Acha commonly known as fonio, is a low-glycemic index grain rich in starch, protein, dietary fiber, and polyphenols, making it suitable

for managing diabetes and related diseases [5]. The annual production of fonio exceeds 600,000 tonnes (FAOSTAT, 2020). Fermentation enhances the bioavailability of nutrients and phytochemicals, making fermented foods an integral part of many traditional diets with recognized health benefits [6].

Soybean (*Glycine max*), a legume high in lysine and protein, complements cereal-based products to enhance their nutritional profile [7]. Defatted soybeans, rich in protein, are used to increase the protein content of flour blends [8].

Chia seeds (*Salvia hispanica*), rich in protein, dietary fiber, omega-3 fatty acids, and antioxidants, are valuable for improving cardiovascular health and reducing oxidative stress [9,10,11]. Chia flour's nutritional properties make it an excellent addition to composite flours, enhancing their health benefits.

This study aims to formulate and evaluate the nutritional composition, phytochemical, functional and antioxidant properties of flour blends for developing low-glycemic products suitable for individuals with diabetes and related health conditions, combining the benefits of acha, soybean, and chia seeds.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Materials

Acha grains, soycake, and chia seeds were purchased from Jos, Plateau State, and Lagos State respectively. All reagents used in this study were of analytical grade.

## 2.2 Experimental Design

The flour sample of fermented acha (FAF), defatted chia (DCF), and defatted soycake (DSF) flours blends were blended with independent variables of acha (60-70%), Soycake (15-25%), chia (7-15%) with reference to 15-20g/day protein and 3-5g/day fibre (recommended intake) using optimal mixture design Methodology. 13.0 version to obtain fourteen runs (Table 2). Thereafter, the dependent variables such as protein composition and fibre content were used to select the best three samples; Acha Chia Soy Cake Flour 1(57:22:21), Acha Chia Soy Cake Flour9 (45:10:45) and ACSCF13.

## 2.3 Processing of Foods Material into Flour

### 2.3.1 Fermented Acha flour (FAF)

Fermented acha grain were processed into flour following the method of [12] with slight modification. Acha grains were washed, destoned, and left in water for two hours, after which were drained, wet milled and left on standing to ferment at room temperature for 72 hours before being oven dried at 50°C with laboratory oven (model no DHG 9101-LSA) for 12 hours. The dried sample were milled to a fine flour and sieved through a 63 µl mesh sieve, packed in a plastic container and stored at room temperature until ready for use.

### 2.3.2 Chia seed flour

The chia seed was processed using the method described by [13]. The chia seed was washed with distilled water to remove adhering dirt, pulverized using electric blender, thereafter defatted the oil using Soxhlet extraction and removed the defatted flour and allowed it to dry in normal atmospheric condition and milled and sieve through a 63µl mesh sieve and was packed in a plastic container and stored at room temperature until ready for use.

### 2.3.3 Processing of Defatted soybean flour

Defatted soybean flour was produced using the method described by [7] with slight modifications. The defatted soybean was oven dried (model no DHG 9101-LSA) at 50°C for 24hr and milled using a professional mill and sieved through a 63 µl mesh sieve to obtain a fine homogenized flour and stored at room temperature until ready for use.

## 2.4 Formulation of Acha-chia-Soycake Composite Flours

The composite flours containing varying proportion of fermented acha flour (FAF), defatted chia seed flour (DCSF) and Soycake flour (SCF) were prepared based on the combination obtained from experimental design as shown in Table 1. The mixture was carefully homogenized to obtain Acha-Chia-Soycake flour (ACSF). The formulation ratio is shown in Table 1. The proximate composition of the flour with protein ranged between 15-20% and 3-5% was used for further investigation as shown in Table 1.

## 2.5 Determination of Proximate Analysis

### 2.5.1 Proximate composition of Acha-Chia-Soycake flour blends

The proximate composition of processed fonio flour was determined using the methods outlined by the Association of Official Analytical Chemists [14]. Carbohydrate content was calculated by difference using the equation below:

$$\% \text{ Carbohydrate} = 100(\% \text{ ash} + \% \text{ crude protein} + \% \text{ fat} + \% \text{ crude fibre} + \% \text{ moisture})$$

Energy content was calculated using the values of crude protein, lipid, and carbohydrate obtained in this study:

$$\text{Energy (Kcal/100 g)} = (\% \text{ Crude protein} \times 4) + (\% \text{ carbohydrate} \times 4) + (\% \text{ CF} \times 9).$$

### 2.5.2 Mineral content determination of Acha-Chia-Soycake flour blends

The mineral content of the flour blends was determined following [14] guidelines. Potassium was measured using Flame Photometry, and phosphorus was determined using the Vanado-Molybdate method. Iron, magnesium, and zinc were analyzed using Atomic Absorption Spectrophotometry as described by [14].

### 2.5.3 Determination of phytochemical composition of flour blends

Tannin concentration was measured using [14] method. Saponin was analyzed using the method by [15]. Phytate and oxalate was determined using the spectrophotometric method of [14].

**Table 1. Experimental design, protein content and crude fibre of the blends**

Runs	Fermented Acha	Chia seed	Soycake	Protein	Fibre
1*	56.667	21.667	21.667	15.37±0.81	5.06±0.02
2	45.000	45.000	10.000	20.85±0.09	8.47±0.02
3	21.667	21.667	56.667	25.15±1.07	6.85±0.03
4	21.667	56.667	21.667	33.05±0.58	11.77±0.02
5	10.000	10.000	80.000	26.12±0.36	6.73±0.02
6	10.000	80.000	10.000	13.05±0.09	18.52±0.01
7	10.000	45.000	10.0000	32.53±0.05	9.12±0.02
8	10.000	80.00	10.000	29.64±0.03	16.66±0.03
9*	45.000	10.000	45.000	19.51±0.20	3.43±0.03
10	33.333	33.333	33.3333	24.72±0.69	7.91±0.01
11	80.000	10.000	10.000	7.54±0.02	2.51±0.01
12	45.0000	45.000	10.00	21.40±0.15	7.72±0.02
13*	80.0000	10.000	10.000	17.79±0.88	3.22±0.01
14	10.000	10.000	80.000	21.91±0.19	5.01±0.01

#### 2.5.4 Phytate-mineral molar ratio (Bioavailability of minerals)

The moles of phytate and minerals were calculated by dividing the weight of phytate and minerals by their respective molecular weights, as described by [16]. The phytate-mineral molar ratio was calculated by dividing the moles of phytate by the moles of each mineral. The Na/K and Ca/P ratios were calculated following [17].

#### 2.5.5 Determination of functional properties of flour blends

The bulk density of the flour blends was measured using a modified method from [18]. Water absorption capacity and foaming capacity were determined following [19]. The swelling index was assessed using the method described by [20].

#### 2.5.6 Determination of total phenolic and total flavonoid content in Acha, Chia, and Soycake flour blends

##### 2.5.6.1 Total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method [21]. Phenolic compounds were quantified as Gallic Acid Equivalents (GAE) per gram of sample.

##### 2.5.6.2 Total flavonoid content (TFC)

The total flavonoid content was assessed using the method by [22]. Flavonoids were quantified as Quercetin Equivalents (QE) per gram of sample.

#### 2.5.7 Determination of antioxidant properties

##### 2.5.7.1 Ferric reducing antioxidant power (FRAP)

FRAP was determined using a modified method by [23]. Antioxidant power was expressed as milligrams of Ascorbic Acid Equivalent per gram (mg AAE/g) of the sample.

##### 2.5.7.2 DPPH radical scavenging activity (DRSA)

The DPPH radical scavenging activity was measured following the method of [24], as described by [25], and expressed as a percentage.

##### 2.5.7.3 Chelation of metal ions

The chelation potential of the flour blends was assessed using the method [26] with slight modification. The results were expressed as a percentage.

##### 2.5.7.4 Hydroxyl radical scavenging

The capacity to scavenge hydroxyl radicals was determined using the methodology by [27].

#### 2.5.8 Statistical analysis

Data were generated in triplicates and analyzed using SPSS Version 21 and GraphPad Prism 8.01 (GraphPad Software Inc., CA, and USA). Mean values were compared using the New Duncan Multiple Range Test (NDMRT), with p-values < 0.05 considered statistically significant.

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Composition of Acha-Chia-Soycake Flours

The proximate composition, of acha-chia-Soycake flours are presented in Table 2. The moisture content ranged from 4.21% to 4.52%, with ACSF1 having the highest moisture content at 4.52%, likely due to the high percentage of chia flour. Moisture content in ACSF flour blends showed significant differences ( $p < 0.05$ ). These values are below the recommended 10% moisture content for storage stability [28], indicating that the composite flour will have an extended shelf life if stored properly. Crude ash content varied from 2.40% to 4.13%, with ACSF9 showing the highest ash content, suggesting it could be a rich source of mineral salts. Ash content represents the inorganic compounds in food that aid in the breakdown of proteins, fats, and carbohydrates [29]. Crude fat content ranged from 5.59% to 10.11%, with higher fat contents in chia and soy cake compared to acha. This variation is due to the differences in oil content among the flours. The high fat content observed aligns with [30] who reported high fat content in his pasta formulation, and [31], who found significant fat content in his flour blends. Crude protein content ranged from 15.37% to 19.51%, indicating that chia and soy cake are excellent protein sources. This is consistent with [30], who reported chia's high protein content ranging from 15-25%. Chia and soy cake had the highest protein contents of 25% and 45%, respectively, compared to acha. The increment may likely due to their proportion in the blends. Crude fiber content ranged from 3.22% to 5.06%, suggesting that acha-chia-soycake blends can serve as good dietary fiber sources. ACSF1 had the highest fiber content at 5.06%. Carbohydrate content ranged from 53.49% to 62.83%, indicating that both acha and chia are rich carbohydrate sources. Carbohydrates are essential for providing energy and supporting growth and development in both infants and adults. The energy values ranged from 335.45 kcal/100 g to 391.37 kcal/100 g.

#### 3.2 Mineral Composition of Acha-Chia-Soycake Flours

The mineral composition of the acha-chia-soycake flours is detailed in Table 3. ACSF1 exhibited the highest concentrations of calcium (4.55 mg/100 g), zinc (2.90 mg/100 g), potassium (11 mg/100 g), sodium (8.25 mg/100 g), and iron (2.35 mg/100 g). Conversely, ACSF9 had the

highest levels of potassium (12.00 mg/100 g), magnesium (5.70 mg/100 g), and phosphorus (1.2 mg/100 g). These results affirm that chia is a rich source of minerals, as the sample with a higher proportion of chia (ACSF1) had the highest mineral values. Minerals are crucial for the human body, playing vital roles in cellular enzyme activity, nerve responses, muscle contraction, and blood clotting [28]. Potassium, in particular, is essential for regulating body fluid balance and transmitting nerve impulses [32]. A high intake of potassium is linked to lowering blood pressure because it helps reduce sodium intake. Diets low in sodium but high in potassium and magnesium are associated with reduced rates of cardiovascular diseases [33]. This study emphasizes the potential health benefits of acha-chia-soycake flour blends, particularly their mineral content, which can contribute to better health results when incorporated into diets.

In this study, the Na/K values in the three samples examined are all less than one (1). This implies that the presence of potassium is higher relative to sodium and this generally considered beneficial for health as it suggests that acha, chia and soy cake flour blend consumption has the potentials to support effective kidney function, reduce risk of hypertension, cardiovascular disease, and obesity [28]. The values obtained for Ca/P ratio of all the flour blends which were observed to be above the recommended range of (1-2) suggests a higher presence of calcium which by implication reveal flour blends with potentials to improved bone health, teeth health as well as effective cellular functioning.

#### 3.3 The Phytochemical Composition of Acha-Chia-Soycake Flour

The phytochemical composition for the Acha-Chia-Soycake flour is display in Table 2. The phytates in ACSF1, ACSF9 and ACSF13 possess a low phytochemical with a significant difference while ACSF9 has the highest phytate content but not significantly different. Phytic acid is considered as one of the main difficulties for human health and nutrition [34]. The phytate reported in all the formulated flour samples are below the critical value and this shows that the phytate will not be able to bind the minerals.

Oxalate has been reported to have an effect similar to that of phytate [35]. It binds to calcium, making it unavailable for absorption by the body, which can result in the formation of kidney stones. The impact on calcium availability

becomes significant only when the oxalate-to-calcium ratio exceeds one [35]. In this report, the oxalate levels were not significantly different; however, ACSF13, which contains the highest percentage of fonio, had the highest oxalate content with no negative effect. The tannin reported in this study were also very low with a significant difference ( $p < 0.05$ ) in all the formulated sample, ACSF13 was found to be the one with the lowest tannin retention in all the formulated sample. High levels of phytochemicals such as phytate, oxalate, and tannin in food are undesirable because they form complexes with minerals and proteins, making these nutrients unavailable to the body [28].

### 3.4 Molarity Ratio and Bioavailability of Minerals

The result of molar ratio and bioavailability of minerals is shown on Table 6. Bioavailability denotes the proportion of a mineral that can be absorbed in a metabolically active form. The phytate: Ca molar ratio, phytate: Fe molar ratio and phytate: Zn, molar ratios in ACSF1, ACSF9 and ACSF13 below are less than the critical value of 0.24, <1.0, and <15-18 which shows the good bioavailability of calcium, iron and zinc in the formulated flour of blends of acha-chia-soycake flour blends. In the formulated flour blends, the bioavailability is the ratio of a mineral's total amount that may be absorbed in a form that is metabolically active [36,35]. The calculated values of the molar ratios were compared with the previous reported critical values [36]. Phytate specifically impacts zinc and iron, as deficiencies in these minerals have been associated with high phytate consumption. All the sample possess a low phytic acid. These make the minerals in the formulated sample available for use most especially the calcium zinc and iron (ACSF1, ACSF9 and ACSF13)

### 3.5 Oxalate to Calcium Ratio (Oxalate: Ca)

The Table 6 show the results of oxalate to calcium molar ratio. The oxalate-to-calcium oxalate: Ca molar ratio is calculated and found to be within the critical values (<1). This indicates that oxalate does not adversely affect the bioavailability of dietary calcium in all the samples formulated.

### 3.6 Functional Properties of Acha chia Soycake Flours

The functional properties of flours play a crucial role in the development of unique products.

These properties influence the behavior of ingredients during complex food processes such as processing and storage. Table 4 displays the results for the functional properties of acha-chia-soycake flour blend samples. The water and oil absorption capacities of these blends increased with higher levels of soycake flour supplementation. Water absorption capacity (WAC) refers to the amount of water (moisture) absorbed by food or flour to attain the desired consistency and produce a high-quality food product. The highest water absorption value was observed for ACSF1 (86.39%), while the lowest was for ACSF9 (32.40%). The high-water absorption capacity of samples with a higher percentage of soycake indicates a greater affinity for water, which is essential in bakery products as it helps maintain moisture content and prevent staling. The increased water absorption capacity with added soycake flour can be attributed to the presence of different hydrophilic carbohydrates and higher protein content in soycake [37]. The increased oil absorption property of the blend flour with higher soycake flour proportion may be due to variations in the presence of nonpolar side chains, suggesting more hydrophobic interaction sites in the soycake flour. Higher oil absorption capacity is desirable for flavor retention and better mouth feel [37]. High bulk density in flours indicates their suitability for use in food preparations. Conversely, low bulk density is advantageous for creating complementary foods [38]. Bulk density is important during mixing, transport, storage, and packaging, and the bulk density of the blend flours increased but without significant differences. These results are similar to those of [39], where the addition of quinoa flour increased the bulk density of the blend. Foaming capacity is crucial to evaluate the potential of flour to act as a leavening agent, providing improved texture, consistency, and appearance of foods. Bakery products require ingredients with excellent foaming capacity [40]. ACSF9 possess the foaming capacity which may be attributed to the high proportion of soycake.

### 3.7 Polyphenol Compounds and Antioxidant Activities of Acha, Chia, and Soycake flour blends

#### 3.7.1 The total phenolic compounds (TPC) of Acha-Chia-Soycake

Fig. 1a show the result of acha –chia-soycake flour blends. The total phenolic content of the flour blends ranged from 273.55 to 149mg GAE/g (Fig. 1a) and the total flavonoid ranged from 66.71 to 31.60 mgQE/g (Fig. 1b) with

**Table 2. Proximate composition of Acha Chia and Soycake flour blends (%)**

Sample	Moisture	Protein	Fat	Ash	Fiber	CHO	Energy (kcal)
ACSF1	4.52±0.20 <sup>a</sup>	15.37±0.81 <sup>c</sup>	8.73±0.07 <sup>b</sup>	2.40±0.485	5.06±0.02 <sup>a</sup>	62.83±0.19 <sup>a</sup>	391.37±1.07 <sup>a</sup>
ACSF9	3.62±0.06 <sup>c</sup>	19.51±0.20 <sup>a</sup>	10.11±0.25 <sup>a</sup>	4.13±0.11 <sup>b</sup>	3.43±0.03 <sup>b</sup>	49.71±0.20 <sup>c</sup>	367.87±0.65 <sup>b</sup>
ACSF13	4.21±0.01 <sup>b</sup>	17.79±0.88 <sup>b</sup>	5.59±0.58 <sup>c</sup>	2.70±0.33 <sup>b</sup>	3.22±0.01 <sup>c</sup>	53.49±0.62 <sup>b</sup>	335.43±2.08 <sup>c</sup>

Means ( $\pm$ SD) with different alphabetical superscripts in the same row are significantly different at  $P < 0.05$

ACSF1: 57% of Fermented acha, 22% of defatted chia seed, 21% soycake flour blends;

ACSF9: 45% of fermented acha, 10% of defatted Chia seed, 45% Soycakeflour blends;

ACSF13: 80% of fermented acha, 10% of defatted chia seed, 10% Soycake flour blends

**Table 3. Mineral composition and molarity ratio of Acha Chia and Soycake flour blends (mg/100 g)**

Samples	Na	K	Ca	P	Zn	Fe	Mg	Na/k	Ca/p
ACSF1	825±0.15 <sup>a</sup>	1100±0.00 <sup>c</sup>	455±0.02 <sup>a</sup>	88±0.00 <sup>b</sup>	290±4.54 <sup>a</sup>	235±0.00 <sup>a</sup>	526±0.01 <sup>b</sup>	0.75±0.00 <sup>a</sup>	5.17±0.00 <sup>a</sup>
ACSF9	772±0.23 <sup>b</sup>	1200±0.00 <sup>a</sup>	411±0.19 <sup>b</sup>	120±0.00 <sup>a</sup>	78±0.91 <sup>b</sup>	63±0.74 <sup>b</sup>	570±0.74 <sup>a</sup>	0.64±0.19 <sup>c</sup>	3.4±0.02
ACSF13	803±0.00 <sup>c</sup>	1137±0.06 <sup>b</sup>	411±0.13 <sup>c</sup>	85±0.00 <sup>c</sup>	58±0.23 <sup>c</sup>	60±0.50 <sup>c</sup>	515±0.19 <sup>c</sup>	0.71±0.006 <sup>b</sup>	4.8±0.016 <sup>b</sup>

ACSF1: 57% of Fermented acha, 22% of defatted chia seed, 21% soycake flour blends;

ACSF9: 45% of fermented acha, 10% of defatted Chia seed, 45% Soycakeflour blends;

ACSF13: 80% of fermented acha, 10% of defatted chia seed, 10% Soycake flour blends

Means ( $\pm$ SD) with different alphabetical superscripts in the same row are significantly different at  $P < 0.05$

**Table 4. Phytochemical composition of Acha, Chia and Soycake flour blends (mg/100g)**

Samples	Oxalate	Tannin	Phytates	Saponnin	Trypsin inhibitors
ACSFS1	8.86 ± 0.76 <sup>c</sup>	4.27± 0.14 <sup>a</sup>	10.69 ± 0.12 <sup>a</sup>	75.46± 0.56 <sup>b</sup>	39.13 ± 0.2 <sup>a</sup>
ACSF9	11.85± 0.15 <sup>b</sup>	3.60 ± 0.03 <sup>b</sup>	10.69 ± 0.30 <sup>a</sup>	13.00± 0.99 <sup>a</sup>	35.66 ± 0.11 <sup>b</sup>
ACSF13	15.82 ± 0.51 <sup>a</sup>	2.30± 0.22 <sup>c</sup>	10.69± 0.2a <sup>c</sup>	76.00 ± 1.18 <sup>a</sup>	27.00 ± 0.22 <sup>c</sup>

ACSF1: 57% of Fermented acha, 22% of defatted chia seed, 21% soycake flour blends;

ACSF9: 45% of fermented acha, 10% of defatted Chia seed, 45% Soycakeflour blends;

ACSF13: 80% of fermented acha, 10% of defatted chia seed, 10% Soycake flour blends;

Means ( $\pm$ SD) with different alphabetical superscripts in the same row are significantly different at  $P < 0.05$

**Table 5. Molarity ratio and bioavailability of minerals of Acha, Chia and Soycake flour blends**

Samples	Phytate (mmol/100 g)	Ca (mmol/100 g)	Zn (mmol/100 g)	Fe (mmol/100 g)	Phytate: Ca molar ratio	Phytate: Zn molar ratio	Phytate: Fe molar ratio
ACSF1	0.16	11	4.46	4	0.015	0.04	0.04
ACSF9	0.16	10	1.2	1	0.016	0.13	0.16
ACSF13	0.16	11	1	1	0.015	0.16	0.16
CV					0.24	<1.0	<15

ACSF1:57% of Fermented acha, 22% of defatted chia seed, 21% soycake flour blends;  
 ACSF9:45% of fermented acha, 10% of deffatted Chia seed,45% Soycakeflour blends;  
 ACSF13: 80% of fermented acha, 10% of deffatted chia seed, 10% Soycake flour blends;

**Table 6. Oxalate molarity ratio of Acha, Chia and Soycake flour blends (mg/g)**

Samples	Calcium (mol/100 g)	Oxalate (mol/100 g)	Oxalate: Ca molar ratio
ACSF1	11	0.10	0.01
ACSF9	10	0.134	0.01
ACSF13	11	0.180	0.02
Critical value		<1	

ACSF1:57% of Fermented acha, 22% of defatted chia seed, 21% soycake flour blends;  
 ACSF9:45% of fermented acha, 10% of deffatted Chia seed,45% Soycakeflour blends;  
 ACSF13: 80% of fermented acha, 10% of deffatted chia seed, 10% Soycake flour blends

**Table 7. Functional properties of Acha chia and soycake flour blends**

Sample	Bulk density (Kg/m3)	Swelling capacity (%)	Water absorption (%)	Oil absorption (%)	Foaming capacity (%)	Emulsification(%)
ACSF1	0.70±0.03	54.65±3.51	32.40± 0.00	21.23±0.03	16.39±0.00	8.00 ± 0.00
ACSF9	0.67±0.00	64.70±0.00	86.39±0.01	31.85±0.05	18.87±0.00	28.57 ± 0.00
ACSF13	0.63±0.00	53.84 0.00	43.20±0.00	29.68±0.00	8.15±0.05	37.50 ± 0.00

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

ACSF1= Acha (57) chia (22) Soycake (21) flour.  
 ACSF9= Acha (45) Chia (10) Soycake (45), Flour,  
 ACSF13=Acha (80) chia (10) Soycake (10)



sample ACSF13 having the highest phenolic content (273.55 GAE/g) and flavonoid content of 66.71 mgQE/g. This result shows that the formulated blends possess antioxidant activity and has the potential to scavenge free radicals and inhibit oxidative stress related diseases. The result shows a significant difference in the three flour blends. The antioxidative properties of dietary phytochemicals from plant sources are more effectively linked to their additive and synergistic effects compared to those from dietary supplements.

Four different assays were used to evaluate the antioxidant capacity of the flour blends: the 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DDPH) assay, the hydroxyl (OH) assay, Fe<sup>2+</sup> chelation

and the Ferric Reducing Antioxidant Power (FRAP) assay. The DPPH quantity is based on the reducing ability of antioxidants toward this reagent. The assay evaluates the reduction of the deep purple color of DPPH after it reacts with the antioxidant. A higher percentage of remaining DPPH after the reaction indicates greater antioxidant activity in the flour blends. The DDPH ranged from 65.98% to 29.96% (Fig. 2c). The DDPH reduce with the higher percentage of chia added. ACSF13 has the highest percentage of DDPH. This report was in agreement with [31] on the blends from cassava, maize and soybeans. The FRAP assay measures the reducing power of the samples by determining the reduction of ferric 2, 4, 6-tripyridyl-S-triazine (TPTZ) to a blue-colored solution at 595 nm. The FRAP content of

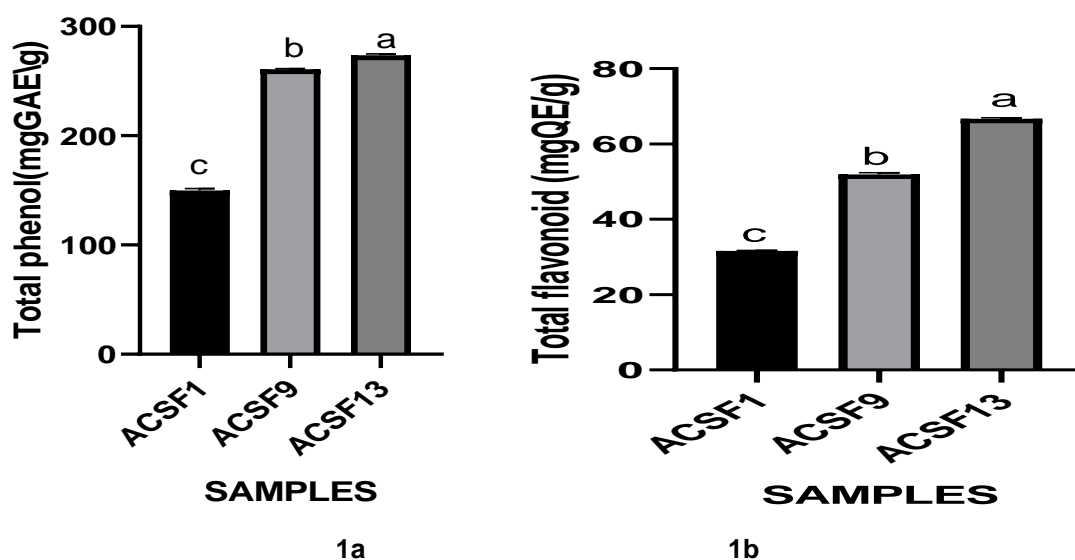
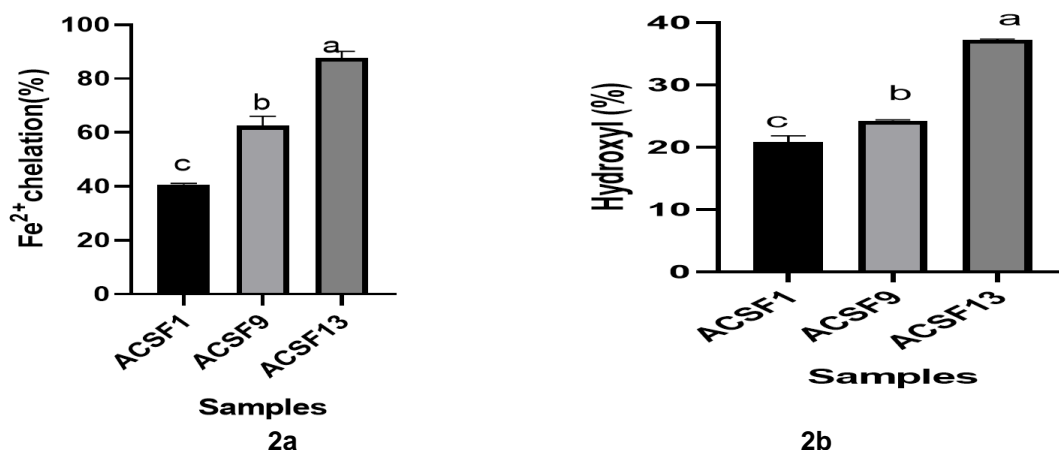
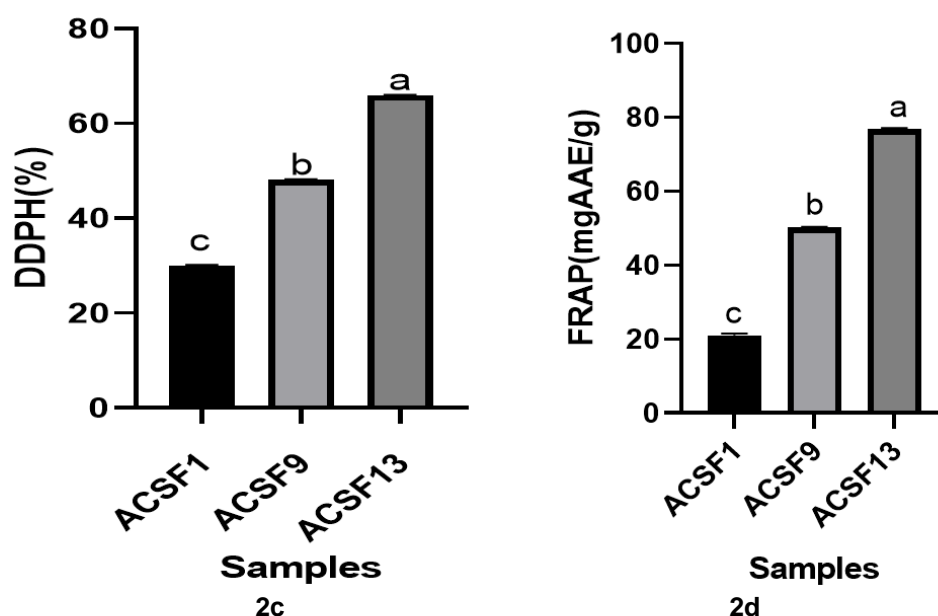


Fig. 1. The Total phenolic content and total flavonoid of the flour blends from acha, chia and soy cake

Values are Means ( $\pm$ SD) and bars with same alphabetic superscript are not significantly ( $p < .05$ ) different





**Fig. 2a to 2d are the Hydroxyl and. fe2+ chelation FRAP and DDPH of the flour blends from acha, chia and Soycake**

*Values are Means ( $\pm$ SD) and bars with same alphabetic superscript are not significantly ( $p < .05$ ) different*

the flour blends ranged from 76.85 to 21.04 mgAAE/g (Fig. 2d). The sample, with ACSF13 having the highest FRAP content possess the highest antioxidant activity. The hydroxyl radical (OH $\cdot$ ) scavenging assay and Fe $^{2+}$  chelation ranged from 37.18% to 20.84% (Fig. 2c) and 87.75 to 40.72% (Fig. 2c). The sample formulated (ACSF1, ACSF9 and ACSF13) shows a very significant amount of scavenging activities. The above scavenging radical assay also follows same trends with the FRAP and DDPH with ACSF13 having the highest hydroxyl and metal chelation in both.

#### 4. CONCLUSION

The results obtained in this study showed that Acha, chia, soycake flour blend formulation is with enhanced energy and nutrient concentrations (protein, carbohydrate and fat), minerals and antioxidative potential (FRAP, DDPH, OH and Fe $^{2+}$  chelation). The phytochemicals and the molarity ratio of all the sample were within the recommended values. The flours also have good functional properties. The composite flour obtained in this study can be used for so many purposes including dough meal, pap and bakery products. It will also be effective in tackling food insecurity by diversification and provide alternatives to enhanced health benefits. However, sample ACSF13 has the highest antioxidant and

polyphenol and may be suitable as a functional food for the treatment and prevention of hyperglycemia, hyperlipidemia, high blood pressure and other cardiovascular disease.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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