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Optimization of Protein, Total Minerals and Vitamin A Content of Orange-fleshed Sweet Potato, Amaranth Seed and Soybean Flour Blends

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Abstract

Food and nutrition security remain a major challenge facing the world and especially the developing world. This situation could be mitigated by utilization and blending locally available food crops. In this study, the nutritional contents of orange-fleshed sweet potato (OFSP), amaranth seed and soybean composite flours were optimized by Extreme Vertices Mixture Design using Minitab Software. The software generated 11 experimental runs from the flour blends. Each blend was analyzed for proximate, minerals and vitamin A contents. The analysis of blends lead to some useful conclusions, most important of which yielded high protein content (15.83%) and fat (6.16%) for blend C1 (50:25:25 for OFSP, amaranth seed, and soybean flour respectively), fiber (5.18%) for blend C11 (60:15:25 for OFSP, amaranth seed, and soybean flour respectively), total minerals (4.83%) for blend C2 (54.5:24:21.5 for OFSP, amaranth seed, and soybean flour respectively), energy value (359.75 kcal/100g) for C3 (50:30:20 for OFSP, amaranth seed, and soybean flour respectively), while blend C6 (75:15:10 for OFSP, amaranth seed, and soybean flour respectively) was higher in carbohydrate (66.60%), energy-to-protein ratio (37.98 Kcal/g of Protein) and vitamin A content (890.03 RAE μg/100g) than others. Generally, blend C1 was the highest in iron content (2.64 mg/100g), Zinc (0.56 mg/100g), magnesium (81.25 mg/100g) and calcium (58.10 mg/100g). The blend C6 was higher in sodium content (41.63 mg/100g) and potassium (65.18 mg/100g) than others, while blend C11 was high in manganese content (0.59 mg/100g) and the highest copper content (0.95 mg/100g) was observed in blend C8 (54.5: 26.5:19 for OFSP, amaranth seed, and soybean flour respectively). The most significant observation of this study is that the optimum blend was 57%, 24% and 19% of OFSP, amaranth seed, and soybean flour respectively for the production of protein (14%), total minerals (4.7%) and vitamin A content (813.6 RAE μg/100g). These findings could be applicable in cases of processing of nutritious foods for people in need in an economical way and promote the utilization of orange fleshed sweet potatoes.

Keywords: *Optimization; Mixture Design; Orange-fleshed Sweet Potato; Soybean; Amaranth Seed; Nutritional Content.*

Introduction

World hunger continues to be the major challenges and COVID-19 pandemic has further accelerated it. The undernourishment prevalence increased by 1.5% in one year [1]. A healthy diet is an important aspect of a person's defense against various diseases including Covid-19 [2].

Currently, 2.37 billion of people worldwide are suffered from severe food insecurity where Asia accounts half with 1.2 billion, Africa which is the second accounts for one-third with 799 million and lastly 11% (267 million) for Latin America and Caribbean year [1]. Access and affordability of healthy, sustainably produced food become even more difficult in the situation of food and nutrition scarcity due to the pandemic [2].

In addition, in 2020 many children under the age of five were suffered from stunting (22.0%) and wasting (6.7%) globally while child malnutrition continues to be the major challenge in Africa and Asia. In 2020, Africa was the first continent suffered from hunger with 21% of the population, followed by Latin America and Caribbean (9.1%) and Asia with 9.0% [1]. On the other hand, in 2018 vitamin A deficiency (VAD) prevalence for under five years children has reduced globally but no progress has been realized in South Asia and sub-Saharan Africa where

44% and 48% of under five years children are still affected respectively [3]. Furthermore, the number of people who are not able to afford healthy diets was 3 billion in 2019 globally where Africa and Latin America and Caribbean were affected from 2017 to 2019 [1].

Therefore, there is a need to formulate nutritious food that could be easily affordable by many people from locally available food crops. Processing of highly nutritious foods could be one way of reducing this severe situation. Sweet potato (*Ipomoea batatas* (L.) Lam.), is a high-fiber, beta-carotene, and vitamin C rich root crop, particularly in orange-fleshed cultivars [4]. Because of their high beta-carotene content and overall acceptability as a food product, OFSP types are some of the foods with the best potential for reducing VAD [5]. Sweet potato roots and leaves, depending on the type, are high in a range of food nutrients necessary to the human body. Sweet potato has got a lot of industrial applications and garnered considerable attention because of its high nutritional quality. Several researches have been also conducted on extraction of its different compounds, its structures, physicochemical and its functional properties. Currently sweet potato is processed into different staple products such as flour, flakes, granules, paste, purees, chips, canned products, beverages, steamed breads, baked breads, noodles, and pancakes, and various snack foods, among others [6]. Nevertheless, a significant number of by-products are developed in such manufacturing processes, including sweet potato juice, residues, peel, and cirrus. Protein, dietary fiber, pectin, anthocyanin, chlorogenic acid, and a variety of other functional components can be found in these by-products, all of which play significant roles in controlling the biological functions within the body [7].

According to FAOSTAT [2021] [8], the global production of sweet potato was 91.82 MT in 2019, Asia (59.10 MT), Africa (27.87 MT), and America (3.87 MT). Among the top ten producers of sweet potato, China is the first worldwide with 51.79 MT and accounts for 56.41%, followed by Malawi (6.44%, Nigeria (4.51%), Tanzania (4.27%), Uganda (2.12%), Indonesia (1.97%), Ethiopia (1.91%), Angola (1.83%), US (1.58%), and lastly Vietnam (1.53%) [9].

OFSP has been used in baby foods as an ingredient to improve the vitamin A content. OFSP is nevertheless low in protein and fat and must be blended with high protein and lipid-containing ingredients such as soy, fish powder and soybean oil to produce suitable nutritional products [10]. The nutritional properties of a particular food is extremely necessary for quality requirements for consumers.

In this study, OFSP was blended with soybean (*Glycine max* (L.) Merrill) and amaranth seed (*Amaranthus hybridus* L) flour for the production of a composite flour which is highly nutritious. Amaranth has both a higher protein content (12–18%) than other cereals, as well as a marginally greater lysine content and acceptable levels of tryptophan and methionine, which are found in cereal and legume grains at small quantities [11]. The amaranth is a good source of lipids, dietary fiber, and minerals (magnesium, phosphorus, copper, manganese, and so on). Amaranth also contains a lot of polyphenols (flavonoids) that have a lot of antioxidant activity. Caffeic acid, p-hydroxybenzoic acid, and ferulic acid are the main phenolics present in amaranth seeds [12]. The amaranth seed was preferred for fortification because it takes a huge amount of lysine, a nutrient that is lacking in other cereal grains [13] and its high biological value creates the potential to be used as a blending product to boost the nutritional value of processed foods [14]. Beta-carotene, L-ascorbic acid, polyphenol, lutein, and anthocyanins are among the many biological activities contained in it [15]. The storage proteins of amaranth seeds have been shown to have an outstanding amino acid balance, as well as antioxidant, antihypertensive, antiproliferative, antithrombotic, cholesterol reducing, and immune regulatory activities [Suarez & Añón, 2018] [16]. In contrast to traditional starchy staples, grain amaranth contains a higher concentration of vitamins A, E, and folic acid [17].

Soybeans is the most largely produced crop worldwide. According to FAOSTAT [2021] [8], the global production of soybean was 333.67 MT in 2019. Bresil is the leding country with 34.25%, followed by US (29.01%), Argentine (16.56 %) and China with 4.71% [9]. Soybeans are rich in protein and a good source of essential amino acids for body development, protection, and reproduction; they do have a lot of polyunsaturated fat and are low in cholesterol and lactose. Soybeans are a good source of nutrients and vitamins as well [18]. Soybeans are commonly used in the food industry to produce nutritious foods with a range of health benefits considering the nature of such bioactive components [19]. The main objective of this study was to optimize crude protein, ash (total minerals) and vitamin A contents in the composite flour in order to contribute to food and nutrition security.

Material and Methods

Raw Materials

Orange-fleshed sweet potato, variety, Kenspot 5 and soybean of variety DPSB 19 were bought from KALRO (Kenya Agricultural and Livestock Research Organization), Njoro, Nakuru, Kenya and amaranth seed of variety Katumani Amaranth (KAM) 001 was procured from KALRO (Kenya Agricultural and Livestock Research Organization), Katumani, Machakos, Kenya.

Production of Orange-fleshed Sweet Potato Flour

Orange-fleshed sweet potato tubers were washed with portable water to remove dirt and adhering soil and particle then peeled using a stainless-steel kitchen knife. The tubers were chopped to 2mm size using a universal kitchen machine (C-1RB, No.3601150, URDORF.ZURICH,Suissse). After blanching in hot water at 80°C for 3 min for enzyme inactivation, drying was carried out to achieve a moisture content of 10% at 55°C for 24 h. The dried potato was milled into flour using a

Perten milling machine (S-14105 huddinge, Perten Instruments AB, Finland) then packaged and stored [19].

Production of Amaranth Seed Flour

The grains were cleaned, carefully sorted, washed with portable water dried in an oven (Memmert GmbH+Co.KG, D-91126 Schwabach FRG, Germany) to moisture content of about 10.20±0.10, then milled using Perten milling machine (S-14105 huddinge, Perten Instruments AB, Finland), and then the flour was stored in paper bags inside of a polyethylene packaging at 15^{o}C of temperature before analysis [21].

Production of Soybean Flour

To begin the flour processing, the soybeans were sorted to remove any debris or damaged seeds. The soybeans were cleaned and soaked overnight at room temperature (25^{o}C) in tap water (soybean: water ratio 1:3, w/v) then manually drained, rinsing, and partially de-hulling of hydrated beans. Drying was done in oven at 55^{o}C for 18 h to achieve the moisture content of 10%, then milled. Soybean flour was packed in sealed polyethylene container and kept at 15^{o}C before analysis and food formulation [22].

Experimental Design

Minitab software version 19.2020.1 (Minitab, 2020) [23] was used to formulate mixture components. On a proportional basis, the extreme vertices method of mixture design was used with constraints that included orange-fleshed sweet potato flour at 50-75%, amaranth seed flour at 15-30%, and soybean flour at 10-25%, with the total mixture being 100%. This method has been useful in situations where all the components can vary from 0 to 1.0 or 0-100%. Furthermore, extreme vertices designs have been shown to be useful in analyzing the response of a mixture system with upper and lower constraints [24,25]. The model generated 11 runs (Table 1) which are composite flour from orange-fleshed sweet potato, amaranth seed and soybean flour at different proportions. The proximate composition, minerals and vitamin A content of each composite flour were determined in triplicate determination. In addition, the optimization of ingredients were carried out using response optimizer based on protein, total minerals and vitamin A content of the composite flour.

Table 1: Extreme vertices method of mixture design.

Run Order	Standard Order	Design Points	Ingredients Ratios				Bounds of Mixture Components	
Composite Sample	StdOrder	PtType	OFSP	Amaranth Seed	Soybean	Total	Lower	Upper
C1	2	1	50	25	25	100	50	75
C2	8	-1	54.5	24	21.5		15	30
C3	1	1	50	30	20		10	25
C4	10	-1	67	19	14			
C5	5	1	60	30	10			
C6	4	1	75	15	10			
C7	9	-1	59.5	19	21.5			
C8	7	-1	54.5	26.5	19			
C9	11	-1	59.5	26.5	14			
C10	6	0	59	23	18			
C11	3	1	60	15	25			

C1-C11: Composite flour number 1 to composite flour number 11 with their corresponding ingredients ratios from OFSP, amaranth seed and soybean flour

Proximate analysis of raw materials and composite flour

Proximate analysis was done according to the procedure described by AACC International (2010) methods as described below

Determination of dry matter content

The dry matter content was determined by air oven (AACC International, 2010) [26] Method 44-15.02 with modification. Briefly, 2 g ground portion were prepared by rapidly mixing with a spoon or spatula and weighed in moisture dishes. Dishes were sealed and measured at the same time. Pre-weighed weights were deducted, and the sample weight was registered. After the oven had recovered its temperature, the samples were heated at 103°C for precisely 60 min. The dishes were removed from the oven, insulated (using rubber finger insulators), and cooled in desiccators. Weights of dried samples were measured once they had reached room temperature about 25°C. Weight loss was calculated as dry matter.

$$\text{Dry matter content (\%)} = \frac{Dry\ weight\ of\ sample}{Total\ weight\ of\ sample} \times 100$$

Determination of crude fat

The crude fat content was determined by the Soxhlet extraction (AACC International, 2010, method 30-25.01. Sample (2 g) that had been previously dried in vacuum oven at 100°C, under pressure not exceeding 100 mm Hg (for about 5 h ± 30 min) were weighed. Sample was quantitatively transferred to extractor and was extracted with petroleum ether for 4 h at condensation rate of 5–6 drops/sec to 6 h at 2–3 drops/sec rate. Then ether was removed from collection flask or beaker at low temperature volatilization before oven drying. Fat that was remaining in previously dried and tared fat beaker was dried or flask in oven at 100°C for 30 min until constant weight. It was desiccated, cooled and weighed.

$$\text{Crude fat (\%)} = \frac{(\text{Weight of extract} - \text{Blank}) \times 100}{\text{Weight of sample}}$$

Determination of crude Protein

Crude protein was determined by the Kjeldahl method and boric acid with modification (AACC International, 2010) [26] method 46-12.01. Finely ground sample (1 g) was placed in digestion flask. Polyethylene packet of catalyst (9.9 g potassium sulfate, 0.41 g mercuric oxide, 0.08 g copper sulfate, and approximately 0.10 g pumice stone) and 25 ml concentrated H_2SO_4 was added to the flask. Digestion was carried out until solution was clear for 30 min; then the solution was removed and cooled. Boric acid-methyl red-methylene blue indicator solution (50 ml) was placed in flask under condenser tube with tip of condenser tube immersed under surface of solution. It was added to flask and cooling with 300 ml tap water. Concentrated NaOH (50 ml) was added, then the flask connected to condenser with tight-fitting rubber stopper, and swirled. It was boiled until all of the ammonia had distilled and then the receiving bottle was set down to drain the condenser tube totally. The distillate was titrated to neutrality with standard 0.1N HCl, using burette graduated in 0.1 ml. the volume of acid used was read, directly from burette. The blank determination was run using all ingredients except sample. The burette reading was corrected for nitrogen in reagents as shown below using blank reading.

$$\%\ \text{Protein} = \frac{(\text{Volume of standard acid } \times \text{N of HCl}) \times 1.4007 \times \text{f}}{\text{Sample Weight (g)}}$$

Where N = Normality of HCl; f = 6.25

Determination of crude ash

Ash content was determined by muffle furnace at 550 °C for 12 h [AACC International, 2010] method (08-01.01) where 3 grams of well-mixed sample were placed into a crucible that had already been burned, cooled in desiccators, and weighed shortly after reaching room temperature. Sample was placed in muffle furnace at 550°C and incinerated until light gray ash was obtained or constant weight was achieved. The sample was cooled in the desiccator and measured shortly after it reached room temperature.

$$\text{Ash (g/100 g)} = \frac{(\text{Weight of residue}) \times 100}{\text{Sample weight}}$$

Determination of crude fiber

Crude fiber was determined gravimetrically after chemical digestion according to AACC International (2010) [26] method 32-10.01. Ground sample (2 g) was extracted with petroleum ether. Sample was transferred to a 600 ml beaker while avoiding fiber contamination from paper. Prepared ceramic fiber (1.5-2.0 g), 200 ml boiling 1.25% H_2SO_4, and containing 1 drop diluted antifoam were added. The beaker was positioned with pre-adjusted hot plate on the digestive apparatus and boiled precisely 30 min, with periodic rotation to keep the solids from sidetracking. The beaker was removed. Using filter screen, suction was turned on and screen was inserted into beaker, face of screen was kept just under surface of liquid until all liquid was removed. Without breaking suction or raising filter, 50-75 ml boiling water were added. After wash was removed, repeated with three 50 ml washings. The filter was removed from beaker and drained all water from line by raising above trap level. The mat and residue were returned to beaker by breaking suction and blowing back. Two hundred milliliters (200 ml) boiling 1.25% NaOH were added and boiled exactly 30 min. The beaker and filter were removed as above. It was washed with 25 ml boiling 1.25% H_2SO_4 and three 50 ml portions boiling water without breaking suction. Free excess of water was drained by raising filter. Filter was lowered into beaker and washed with 25 ml alcohol. The line was drained, suction was broken, and mat was removed by blowing back through filter screen into ashing dish. The residue was treated. The mat and residue were dried at 130 ± 2°C for 2 h and then cooled in a desiccator and measured. It was ignited at 600 ± 15°C for 30 min and cooled and reweighed in a desiccator.

$$\%\ \text{Crude Fibre} = \frac{(\text{Loss in weight on ignition} - \text{Loss in weight on ceramic fiber blank}) \times 100}{\text{Weight of sample}}$$

Determination of carbohydrate content

The total carbohydrate content was determined by difference method where the sum moisture content, crude ash, crude fiber, crude protein and crude fat was deducted from the total of 100% [27].

Determination of Energy value and Energy-to-Protein Ratio

The calorific value was computed by summing up the values obtained by multiplying the values with Atwater constants for carbohydrates, crude fat, crude fiber and crude protein with the factors 4, 9, 2 and 4 respectively [28]. Energy-to-protein ratio was calculated by dividing energy value of sample by its corresponding crude protein content.

Determination of Vitamin A

The beta-carotene was determined by UV/Visible spectrophotometer according to Rodriguez-Amaya & Kimura [2004] [29] method with modification. Ground samples were saponified with potassium hydroxide and extracted with acetone. Carotene was eluted with 4% acetone (in hexane) and read by a spectrophotometer (UV-1700, Shimadzu, Japan). Two grams of sample were weighed, homogenized and color was extracted using a mortar and pestle with small portions of acetone until residual was colorless. All extracts were combined into 50 ml volumetric flask. Twenty-five milliliter (25 ml) of the extract was taken into round buttoned flask and it was then evaporated to dryness in rotary evaporator at about 60°C. One milliliter (1 ml) of petroleum ether was added to the evaporated sample so as to dissolve the beta-carotene. The elute was received into a 25 ml volumetric flask and the absorbance was read at 450 nm. Vitamin A in retinol activity equivalent was converted following the method of Trumbo *et al*. [2003] [30].

The beta-carotene was calculated from the beta-carotene standard curve

$$Conc = \frac{0.4}{0.12} \times \frac{Absorbance \times Final\ volume}{Weight\ of\ sample} \times Dilution\ factor$$

$$Vitamin\ A\ (\text{RAE}\ \mu\text{g}/100\text{g}) = \frac{\beta - Carotene}{12}$$

Determination of Minerals

Determination of minerals (Ca, Fe, Mg, Mn, Zn, and Cu) were carried out by Atomic Absorption Spectrophotometry (Model AA-6300, Serial No A30524300916 SA, Shimadzu corporation, Japan) (AACC International, 2010) [26] method 40-70.01. Sample (2 g) was accurately weighed into ashing crucible. The blank (empty crucible) was placed alongside the flour samples. The crucibles were placed in muffle furnace set at 500°C and allowed to ash for minimum of 6 h. The crucibles were removed from furnace and cooled to room temperature. HCl (10 ml) was added to each crucible and covered with a watch glass. The solution was boiled and evaporated nearly to dryness on hot plate. The residue was not allowed to cake up on the crucible. The residue was redissolved in 20 ml 2N HCl, and boiling gently. The crucibles were removed from hot plate and cooled to room temperature. The watch glasses were rinsed into crucibles with water. Solutions were quantitatively transferred into separate 100-ml volumetric flasks using a funnel. It was diluted to 100 ml and mixed then absorption of solution was directly measured. For calcium, there is sufficient supply. In this case, 5 ml of Lanthanum (La) stock solution was added in order to achieve a final dilution of 1%. For example, 20 ml of solution in a 25-ml flask, and so on.). The instrument was set up and at least 4 standard solutions within analytical range before and after each group of 6–12 samples was read. After each sample, the burner was rinsed with water to restore the zero absorption level.The calibration curve was prepared from average of each standard before and after sample group. The concentration of samples was read from plot of absorption against µg/ml. The following calculation was used:

$$Element\ (ppm) = \frac{\mu g/ml}{Sample\ weight\ (g)} \times 100$$

Determination of sodium and potassium

Determination of minerals (K and Na) were carried out by Atomic Absorption Spectrophotometry (Model AA-6300, Serial No A30524300916 SA, Shimadzu corporation, Japan) [AACC International, 2010] [26] Method 40-71.01. Sample (2 g) was accurately weighed into a ashing crucible. The control blank crucible was included. Sample was carefully charred mass on hot plate, or over Bunsen burner. Sample was not allowed to ignite. The dish with charred sample was placed in cold muffle furnace and slowly raise temperature to 525°C and ashing overnight. The crucibles were removed from furnace and cooled to room temperature. Hydrochrolic acid (10 ml) were added to each crucible and covered with a watch glass. The solution was gently heated on hot plate to dissolve. The watch glasses were rinsed into crucibles with water. Solutions were quantitatively transferred into separate 100-ml volumetric flasks. The crucibles were rinsed several times with water and rinsings were added to flask, cooled. It was diluted to volume and mixed and undissolved ash was allowed to settle. The dilutions were made to obtain solutions within ranges of instrument. For each sample and blank, sufficient Concentration of sample (Cs) stock solution was added to achieve a final dilution of 0.1% (i.e., 5 ml Cs to 25-ml flask, 10 ml Cs to 50-ml flask, etc.).The instrument was set up. After each sample, the burner was rined with water to restore the zero absorption level.The calibration curve was prepared from average of each standard before and after sample group. The concentration of samples was read from plot of absorption against µg/ml. The following calculation was used:

$$mg/100g = \frac{(C_s - C_b)}{S \times 10} \times \mathrm{V} \times \mathrm{D}$$

Where Cs = Concentration of sample (µg/ml), C_b = Concentration of blank (µg/ml), V = Original volume (ml), D = Dilution volume (ml)/aliquot for dilution (ml) if original solution is diluted, S = Sample weight (g).

Data Analysis

The data for proximate composition, minerals and vitamin A content were statistically analyzed using SAS version 9.4 TS Level 1M6 [SAS Institute Inc., 2016] [31] and Minitab software (version ® 19.2020.1) was also used for the analysis of data from mixture design. The software generated experimental runs with randomization. The mean values were analyzed statistically by analysis of variance (ANOVA). The coefficient of determination (R^2) value of the model was conducted to explain and predict the variability in the response data in order to fit the model. The regression models equations and graphical representations were observed. Each determination was performed in triplicate and the results were expressed as means ± standard deviation. Statistical differences between means ($p < 0.05$) were tested by Tukey's honest significant difference (HSD).

Results and Discussion

Proximate composition of raw materials

Grain and cereals are one of the major staple food in different area of the world. They are considered to be good sources of different many bioactive components, carbohydrate and fibre but are very low in protein content [32,33]. In addition, grain amaranth is exceptional because of its high protein content than most cereals, and also bioactive compounds [14]. The amaranth health advantages have also been established in homoeopathic medicine as well as ayurvedic medicines. Amaranth seeds and leaves are both utilized as herbal treatments and have nutraceutical qualities [34]. The moisture contents of all flour produced in this experiment are below 14%. The moisture content of flour above 14% favour mould growth and infestation by insects during storage [35].

The results (Table 2) indicated significant ($p<0.05$) differences in all tested parameters. The highest protein content was 31.53% from soybean flour followed by amaranth seed flour 19.80% then lastly OFSP had very low protein content (2.95%) but higher in carbohydrate content (75.73%) and observed the highest energy-to-protein ratio (118.37 Kcal/g of Protein) (Table 2). Soybean flour was also high in total minerals (5.98%), crude fat (13.13%) and crude fiber (6.29%), energy value (389.19 kcal/100 g) but very low in carbohydrate content (33.09%) and energy-to-protein ratio (12.35 Kcal/g of Protein) compared to others.

The dry matter content found in amaranth seed flour (89.80 %) was in the agreement with 10.50% reported by Miranda-Ramos *et al*. [2019] [36] for *A. hypochondriacus*. The high protein content of amaranth seed in this study was 19.80% which falls within the trends (13.8-21.5% for *A. cruethus*, 13.1-21% for *A. caudatus*, 15-16.6% for *A. hypochondriacus*, and 16-16.5% for *A. hypondriacus* x *A. hybridus*) reported by Mlakar *et al*. [2009] [33]. The carbohydrate content was found to be 54.94% and energy value to be 363.94 kcal/100g which is close to the value 60% and 391 kcal/100g respectively obtained by Narwade & Pinto [2018] [30]. The crude fat obtained in this experiment (6.23%) confirm well with the trends (5.6 - 10.9%) reported by Mlakar *et al*. [2009] [37] and also very close to the value 5.94% reported by Miranda-Ramos [2019] [32] for *A. hypochondriacus*. The fibre content was 4.47% which is within the range (3.1 - 5.0%) reported by Mlakar *et al*. 2009 [37]. Another study conducted by Antoniewska *et al*. [2018] [38] reported 51.70% of carbohydrate content which is closely to the value found in this study. The ash content of amaranth seed flour was

4.36% which is similar to the finding range (2.5 - 4.4% for *A caudatus*) reported by Mlakar *et al*. [2009] [37]. The Energy-to-Protein Ratio was 18.39 kcal/g of protein which is slightly below to the value 24.44 kcal/g of protein from the study carried out by Narwade & Pinto [2018] [34].

Apart from amaranth seed, the fiber content of OFSP was 3.66% and current similar results (3.78%) was obtained by Omoba *et al*. [2021] [39]. The fat, ash, and carbohydrate content got in this study are slightly close to the current findings (3.37%, 3.37%, and 78.65% respectively) reported by Adetola *et al*. [2020] [40]. The energy value in OFSP was 349.53 which is below to the value (359.71 kcal/100g) reported by Chikpah *et al*. [2020] [41]. This may be caused to different variety of OFSP. The protein content was 2.93% and this finding is in line with the value (2.90%) reported by Pereira *et al*. [2019] [42]. The Energy-to-Protein Ratio was 118.37 kcal/g of protein. This value is high and it could be attributed to very low protein content of OFSP observed in this study.

On the other hand, soybean flour had the highest energy value (389.19 kcal/100g) than amaranth seed and OFSP flour. The ash (5.98%) and fiber content (6.29%) of soybean flour obtained are very close to the findings (5.76% and 7.26% respectively) reported by Adetola *et al*. [2020] [40]. The crude protein and carbohydrate findings in this study, confirm well with the value (34.5% and 35.2 % respectively) reported by Serna-Saldivar *et al*. [2019] [43]. The fat content obtained was slightly above the value (10.95%) by Ndife *et al*. [2014] [44] but below to the high value (25.53%) obtained Adetola *et al*. [2020] [35]. This high fat content may be due to different varieties of soybean. The fiber content was similar to the value (6.74%) as reported by Ndife *et al*. [2014] [44]. The Energy-to-Protein ratio was 12.35 kcal/g of protein which is slightly above to the value 10.24 kcal/g got in the previous study conducted by Adetola *et al*. [2020] [40].

Table 2: Nutritional composition of OFSP, Soybean and Amaranth Seed flour.

Sample	Dry Matter (%)	Crude Protein (%)	Crude Ash (%)	Crude Fat (%)	Crude Fiber (%)	Carbohydrate (%)	Energy Value (Kcal/100g)	EPR (Kcal/g of Protein)
OFSP	90.00 ± 0.00^{b}	2.95 ± 0.04^{a}	4.60 ± 0.19^{a}	3.05 ± 0.28^{a}	3.66 ± 0.13^{a}	75.73 ± 0.53^{c}	349.53 ± 0.21^{a}	118.37 ± 1.69^{c}
Amaranth Seed	89.80 ± 0.10^{a}	19.80 ± 0.26^{b}	4.36 ± 0.12^{a}	6.23 ± 0.21^{b}	4.47 ± 0.29^{a}	54.94 ± 0.51^{b}	363.94 ± 0.42^{b}	18.39 ± 0.26^{b}
Soybean	90.00 ± 0.00^{b}	31.53 ± 0.46^{c}	5.98 ± 0.25^{b}	13.13 ± 0.24^{c}	6.29 ± 0.60^{b}	33.09 ± 0.50^{a}	389.19 ± 1.14^{c}	12.35 ± 0.18^{a}

Values are means ± Standard deviation replicated three times. Significantly different means are indicated by various superscripts along columns at p 0.05. EPR: Energy-to-Protein Ratio.

Nutritional composition of composite flour from OFSP, amaranth seed and soybean flour

The results of this analysis are summarized in Table 3. All tested parameters in composite flour differ significantly ($p < 0.05$). By carefully analyzing the proximate results, the highest protein content and fat content was observed in blend 50:25:25 for OFSP, amaranth seed and Soybean flour respectively while the lowest value was observed at ratio 75:15:10 for OFSP, amaranth seed and soybean flour respectively but the highest carbohydrate content was obtained in this blend. This is due to the fact that increasing amaranth seed and soybean in the mixture increased the protein content and fat content. However, increase of OFSP flour in the mixture resulted in reduction of the protein content and increased the carbohydrate content and energy-to-protein ratio.

The ash content was observed high in blend 54.5:24:21.5 and the lowest was seen in blend 75:15:10 while the highest fibre content was obtained in 60:15:25 for OFSP, amaranth seed and soybean flour respectively and the lowest was seen in blend 75:15:10. Generally, blend 75:15:10 was the lowest in crude protein, fat, ash, fiber content and energy value but the highest in carbohydrate content and energy-to-protein ratio and vitamin A content among others.

Table 3. Nutritional composition of composite flour from OFSP, Soybean and Amaranth Seed flour.

Sample	Dry Matter (%)	Crude Protein (%)	Crude Ash (%)	Crude Fat (%)	Crude Fiber (%)	Carbohydrate (%)	Energy Value (Kcal/100 g)	EPR (Kcal/g of Protein)	Vitamin A (RAE µg/100g)
C1	88.67 ± 0.58^{a}	15.83 ± 0.31^{e}	4.75 ± 0.14^{bc}	6.16 ± 0.19^{e}	4.94 ± 0.81^{ab}	56.99 ± 0.47^{a}	356.56 ± 0.89^{ef}	22.53 ± 0.49^{a}	780.17 ± 0.38^{a}
C2	88.90 ± 0.17^{abc}	14.50 ± 0.50^{de}	4.83 ± 0.06^{d}	5.07 ± 0.12^{abc}	5.00 ± 0.10^{ab}	59.50 ± 0.43^{bc}	351.61 ± 0.70^{ab}	24.27 ± 0.89^{abc}	800.00 ± 0.17^{c}
C3	89.66 ± 0.30^{abc}	15.77 ± 0.25^{e}	4.64 ± 0.06^{abc}	5.77 ± 0.25^{cde}	4.70 ± 0.18^{ab}	58.78 ± 0.48^{ab}	359.49 ± 0.34^{g}	22.80 ± 0.38^{ab}	785.03 ± 0.15^{b}
C4	89.55 ± 0.43^{abc}	11.50 ± 0.50^{b}	4.61 ± 0.03^{abc}	4.86 ± 0.31^{ab}	4.71 ± 0.17^{ab}	63.87 ± 0.46^{e}	354.62 ± 0.23^{d}	30.88 ± 1.34^{e}	860.02 ± 0.32^{i}
C5	89.82 ±0.31^{bc}	13.00 ± 0.01^{c}	4.52 ± 0.08^{a}	4.82 ± 0.29^{ab}	4.30 ± 0.26^{ab}	63.19 ± 0.83^{e}	356.68 ± 0.64^{ef}	27.45 ± 0.06^{cde}	837.03 ± 0.15^{h}
C6	88.87 ± 0.06^{abc}	9.26 ± 0.65^{a}	4.50 ± 0.10^{a}	4.32 ± 0.28^{a}	4.18 ± 0.16^{a}	66.60 ± 0.95^{f}	350.68 ± 0.73^{a}	37.98 ± 2.58^{f}	890.03 ± 0.15^{j}
C7	88.90 ± 0.17^{abc}	13.62 ± 0.40^{cd}	4.79 ± 0.11^{c}	5.17 ± 0.29^{bcd}	4.94 ± 0.05^{ab}	60.38 ± 0.32^{bc}	352.37 ± 0.33^{bc}	25.90 ± 0.79^{abcd}	825.03 ± 0.15^{g}
C8	89.67 ± 0.58^{abc}	14.68 ± 0.35^{de}	4.65 ± 0.05^{abc}	5.38 ± 0.42^{bcde}	4.79 ± 0.01^{ab}	60.16 ± 1.04^{bc}	357.36 ± 0.64^{f}	24.35 ± 0.63^{abc}	801.03 ± 0.15^{d}
C9	89.26 ± 0.22^{abc}	12.93 ± 0.40^{c}	4.56 ± 0.04^{ab}	4.99 ± 0.01^{abc}	4.33 ± 0.29^{ab}	62.44 ± 0.45^{de}	355.07 ± 0.50^{de}	27.48 ± 0.83^{cde}	825.03 ± 0.15^{g}
C10	88.72 ± 0.65^{ab}	13.54 ± 0.50^{cd}	4.70 ± 0.10^{abc}	5.50 ± 0.50^{bcde}	4.87 ± 0.32^{ab}	60.12 ± 0.72^{bc}	353.86 ± 0.41^{cd}	26.16 ± 0.97^{bcd}	820.00 ± 0.50^{f}
C11	89.87 ± 0.15^{c}	12.82 ± 0.83^{bc}	4.80 ± 0.02^{c}	5.97 ± 0.07^{e}	5.18 ± 0.15^{b}	61.11 ± 0.94^{cd}	359.75 ± 0.57^{g}	28.14 ± 1.77^{de}	810.07 ± 0.31^{e}

Values are means ± Standard deviation replicated three times. Statistical tests were conducted to see whether the means followed by different superscripts along columns were significantly different at $p < 0.05$. Where C1 (50:25:25), C2 (54.5:24:21.5), C3 (50:30:20), C4 (67:19:14), C5 (60:30:10), C6 (75:15:10), C7 (59.5:19:21.5) C8 (54.5: 26.5:19), C9 (59.5:26.5:14), C10 (59:23:18), C11 (60:15:25) are flour blends from OFSP: Amaranth Seed: Soybean respectively. EPR: Energy-to-Protein ratio.

OFSP has been used to reduce Vitamin A deficiency due to its excellent beta-carotene content but very low in protein and fat [10]. Therefore, enhancing OFSP and amaranth seed with soybean which is very high in protein resulted in high nutritional quality composite flour. The results indicated that, the highest protein content and fat content observed in the composite could be due to high soybean and amaranth seed flour inclusion in the mixture. Therefore, increasing OFSP flour in the blend reduced the protein and fat contents. However, it increases the carbohydrate (66.60%), protein energy ratio (37.98 kcal/100g of protein) and vitamin A content (890.03 RAE µg/100g). Soybean flour inclusion in the blends contributed to high amount of total minerals, fat, fibre and energy values observed in the composite flour. The tested parameters were significantly ($p<0.05$) different. Using these results, it was possible to investigate the impact of various blend proportions on protein, ash, vitamin A etc. The highest moisture content in the composite flour was 11.33% which is below the maximum (15.5%) moisture content designated for wheat flour. High moisture levels in flours can cause caking, a condition epitomized by particle agglomeration into aggregates [45]. In addition, microbial growth can take place and chemical deterioration of flour. This reduce the quality of the produced flour and loose its intend uses. In the other hand, low moisture content is one of the major factors which contribute to achieve long shelf-life or storage stability of a particular food.

The protein content ranged from 9.26-15.83%, ash content ranged from 4.5-4.83%, crude fat content ranged from 4.32 - 6.16%, fibre content ranged from 4.18-5.18%, carbohydrate ranged from 56.99-66.60%, energy value ranged from 350.68 - 359.75 kcal/100g. Apart from the ash content, these results have led to high levels of agreement of trends in the most current study conducted by Feyera *et al*. [2021] [46] for composite flour from finger millet, soybean, sweet potato and ground nut where protein (4.37 - 17.16%), fat (0.02 - 15.58%), fiber (2.85 - 13.43%), carbohydrate (61.99 - 71.57%), and energy value (297.67 - 428.80 kcal/100g) were reported. Energy is necessary to maintain the body's different processes, such as breathing, circulation, physical work, and protein synthesis [47]. The highest fibre content of the composite flour was 5.18%. High fibre consumption lowers the risk of digestive disorders and colon cancer. Another advantage is its capacity to regulate blood glucose levels [48].

The highest protein content (15.83%) was observed in blend 50:25:25 for OFSP, amaranth seed and soybean flour respectively. Proteins are biomolecules that are extremely complex. They are the most varied group of physiologically significant compounds and are frequently recognized as a significant compound required for living. They are important in cellular metabolism, defense, communication, transport, storage, and recognition; all of these functions are necessary for the construction, function, and control of the cells of the body. Proteins collaborate with other biomolecules to carry out biological activities [49]. The highest ash content of the composite was 4.83% which is very close to the value (4.88%) reported by Adetola *et al*. [2020] [40] from composite flour from OFSP, soybean and carrot. This difference could be attributed to high level (34.76%) of soybean flour inclusion in that mixture. High ash content indicates the high presence of minerals in the flour. The energy-to-protein ratio ranged from 22.80 - 37.98 kcal/g of protein which can be used as daily ration nutrient. The vitamin A content obtained in this study confirm well with trends (215.0 - 811.6 RAE) reported by Mitra [2012] [50], for different orange-fleshed sweet potato genotypes. These findings could also be applicable in cases of insufficient protein, mineral, energy intake and vitamin A situations to provide the human body with these nutrients.

Optimization of protein content, total minerals and vitamin A content

These results were obtained using the methods described in this work and optimized using Minitab Software. Optimization has been used in various areas including food science and technology. It has also been used extensively and successfully in food processing engineering like process optimization and also in ingredients optimization. The optimum blend proportions of the composite flour and the predicted responses values are illustrated in Figure 1. The values showed that the optimum value for model verification was 57, 24 and 19% of Orange-fleshed sweet potato, amaranth seed and soybean flour respectively for all three response variables. The results demonstrated the high adequacy of regression model to predict the responses where the coefficient of determination (R^2) of the model was 0.9973, indicating that the model explained 99.73% of the variability in the response data fitted the model. The target was to optimize the responses to the target value of 14%, 4.7% and 813.6 RAE µg/100g, crude protein, total minerals and Vitamin A content, respectively. This value could be used as daily recommended intake of 13 g of protein for children aged 1- 4 years and 800 RAE µg/100g as daily recommended intake for pregnant women [51]. The coefficient of determination for each component were 0.9995, 0.9927 and 0.9997 for crude protein, ash (total minerals) and Vitamin A respectively.

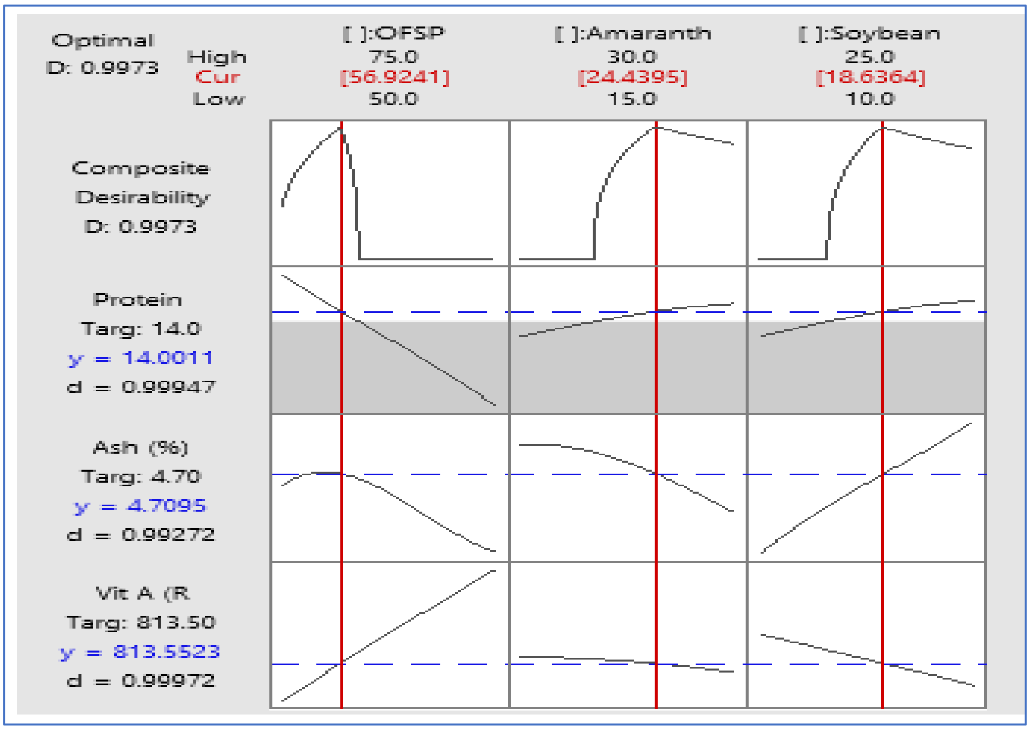
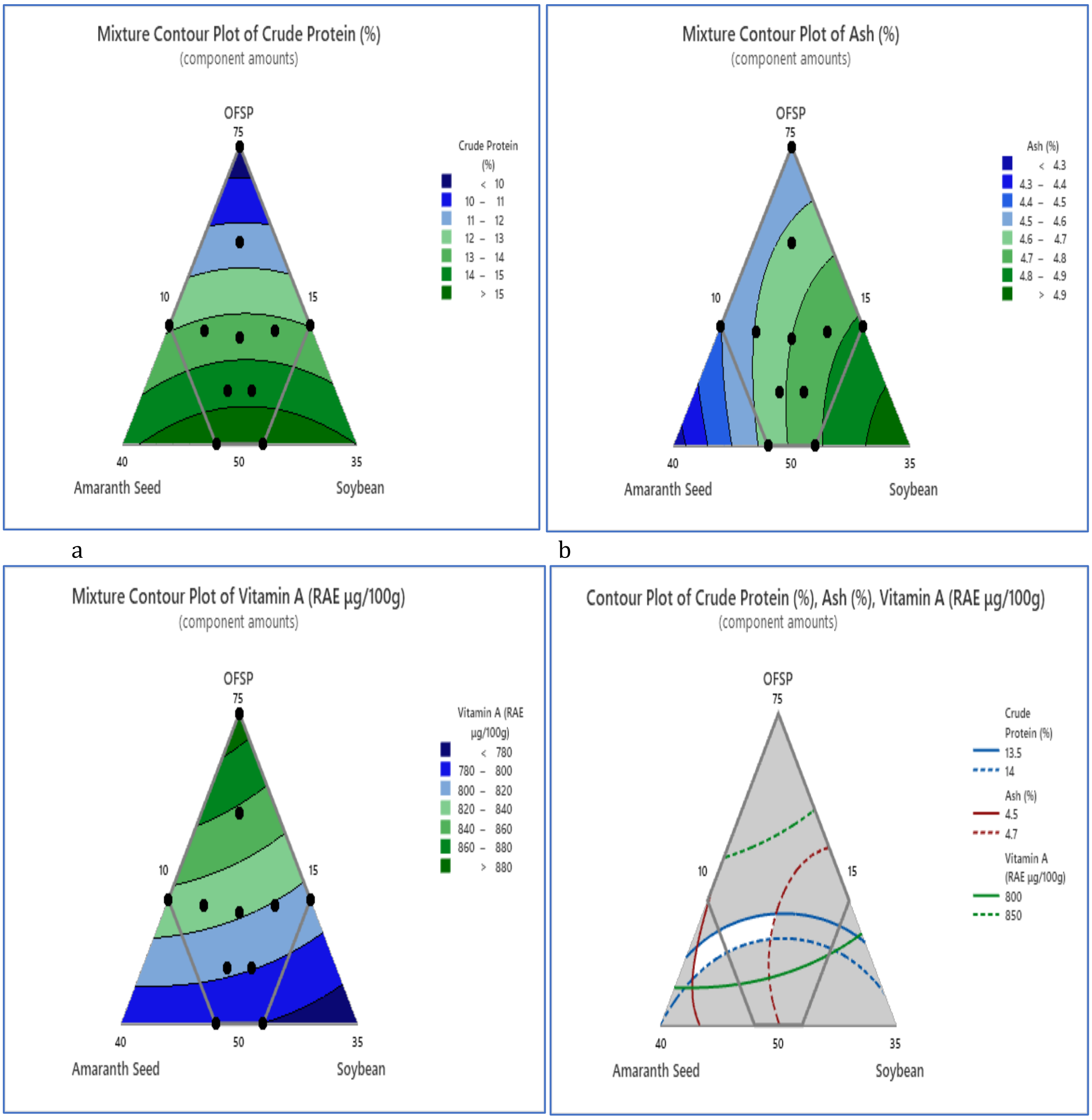


Figure 1. Variable optimization results. Key: D: Overall coefficient of determination of experiment (composite desirability), y= Optimum response value for each response, and d= Coefficient of determination for each response.

Based on available data in Figure 2.a, it can be concluded that highest protein content lies between amaranth seed and soybean flour inclusion in the mixture while its reduction observed in OFSP flour region. Figure 2.b shows proportion of OFSP, amaranth seed and soybean flour in relation to ash content where increasing soybean in the mixture resulted in high ash content. Figure 2.c shows the contribution of OFSP, amaranth seed and soybean flour in relation to vitamin A content where increasing OFSP flour in the mixture resulted in high vitamin A content followed by amaranth seed flour. Finally, Overlaid counter plot (d) shows optimum feasible (white) region for all ingredients.



a b

c d

Figure 2. Proportion of each component in relation to protein content (a), Proportion of each component in relation to ash content (b), Proportion of each component in relation to vitamin A content (c) and Overlaid counter plot for ingredients mixture composition for optimum blend (d).

Mixture Analysis of composite flour

After conducting regression for mixture data, it was observed that all response parameters tested were highly and significantly predicting data for production of composite flour from Orange-fleshed sweet potato, amaranth seed and soybean flour blends except for dry matter and energy value. The highest coefficient of determination was observed in protein with R^2=99.21% while the least was in crude fat with R^2=83.42%. The desirability (R^2) and regression model of each tested parameter is shown in Table 4.

Table 4. Regression model equations for response parameters.

Response	Model	R²
Crude protein	$Y=2.32X_1+12.7X_2+12.4X_3+20X_1X_2+16.7X_1X_3+60.4X_2X_3$	99.21%
Crude ash	$Y=3.66X_1-1.41X_2+5.19X_3+10.35X_1X_2+2.35X_1X_3+6.74X_2X_3$	90.41%
Crude fibre	$Y=2.10X_1-3.0X_2-3.2X_3+13.0X_1X_2+22.7X_1X_3+15.9X_2X_3$	92.24%
Crude fat	$Y=4.65X_1+25.6X_2+24.9X_3-31.4X_1X_2-15.2X_1X_3-50.8X_2X_3$	83.42%
Carbohydrate	$Y=78.16X_1+146.2X_2+14.9X_3-151.5X_1X_2+53.7X_1X_3-160.2X_2X_3$	98.03%
EPR	$Y=71.15X_1+109.4X_2+75.2X_3-223.5X_1X_2-150.4X_1X_3-198.1X_2X_3$	99.17%
Vitamin A	$Y=979.7X_1+284X_2+551X_3+564X_1X_2-164X_1X_3+494X_2X_3$	99.07%

EPR**=** Energy-to-Protein ratio, Y=Crude protein, Crude ash, Crude fibre, Crude fat, Carbohydrate, Energy-to-Protein ratio and Vitamin A content. X_1, X_2 and X_3 are OFSP, Amaranth seed and Soybean respectively.

Micro and macro minerals of composite flour

The minerals results shown in Figure 3 are from composite flour from OFSP, amaranth seed and soybean flour. The results showed significant differences (α<0.05, by Tukey) in macro and micro minerals composition of composite flour. Minerals play a crucial role in the human body because of their biochemical function, assisting cells in absorbing and storing energy from substances in foods [52]. Minerals are inorganic compounds that are found in all body tissues and fluids and are required for the regulation of certain physicochemical processes that are vital for life. Minerals are chemical elements that the body uses in a number of different ways [53]. Minerals are classed as macro if they occur in large quantities in the system and as micro or trace if their concentration is less than the critical mass threshold. Micro minerals include Fe, Zn, Cu, Mn, Co, Ni, Mo and I while macro minerals are Na, Ca, K, Mg and Cl. Mineral deficiencies can be serious, life-threatening conditions that affect the body in the same way that a vitamin or essential amino acid deficit does [52].

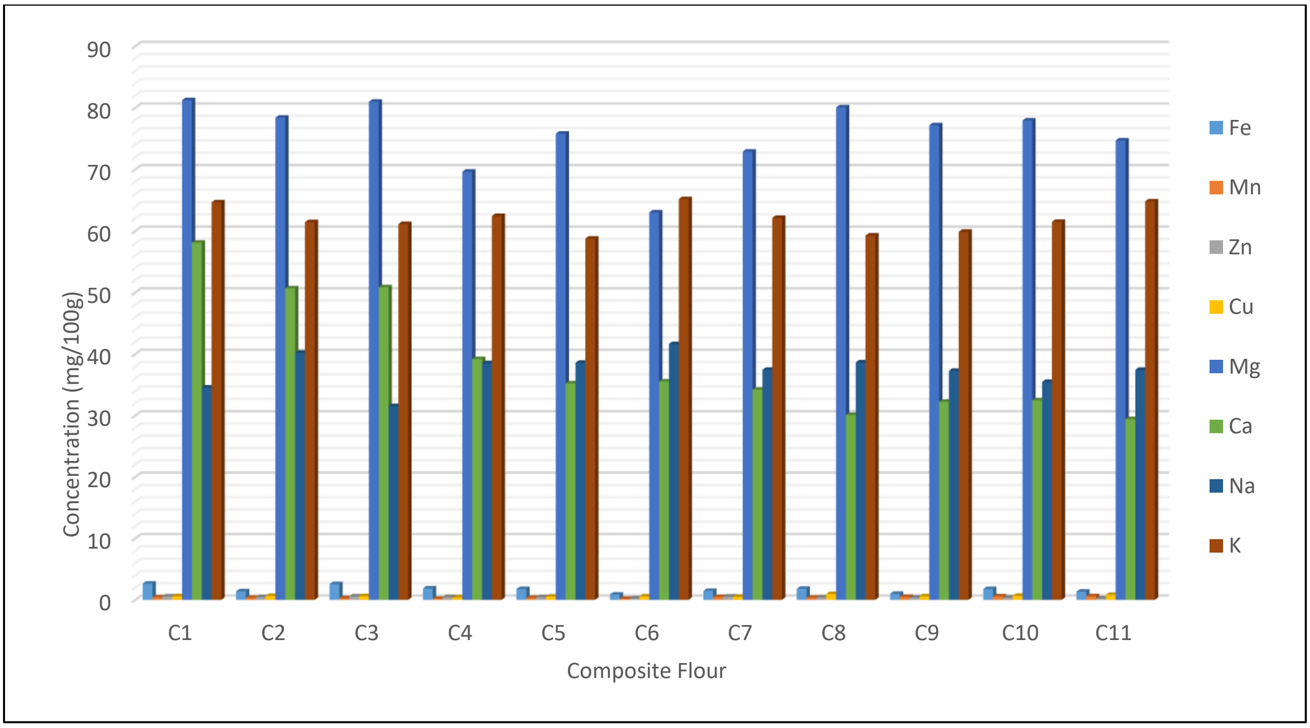


Figure 3. Mean of Micro and macro minerals of composite flour where C1 (50:25:25), C2 (54.5:24:21.5), C3 (50:30:20), C4 (67:19:14), C5 (60:30:10), C6 (75:15:10), C7 (59.5:19:21.5) C8 (54.5: 26.5:19), C9 (59.5:26.5:14), C10 (59:23:18), C11 (60:15:25) for OFSP: Amaranth Seed: Soybean respectively.

Calcium serves as a component of bones and teeth, as well as a regulator of nerve and muscle function [48]. Activates a range of processes, including fatty acid oxidation, mitochondrial ATP carrier (with magnesium), and glucose-stimulated insulin release [54]. The calcium content ranged from 29.41- 58.10 mg/100g, these values are above to the results (0.28 – 1.06 mg/100g) reported by Ikegwu *et al*. [2021] [55] for millet and soybean flour blends but below to the value (110.51 - 114.05 mg/100g) reported by Adetola *et al*. [2020] [140 for complementary foods from OFSP, soybean and carrot flour blends. The results were slightly below to the value (70 mg/100g) reported by Oguntoyinbo *et al*. [2021] [56] for wheat-banana peel flour but in agreement with the value (30 mg/100g) reported by Stathers *et al*. [2013] [51] for sweet potato. The calcium content was high in C1 and the least was observed in C11.

Iron acts as haemoglobin in the transfer of oxygen [53]. Iron assists in the transport of electrons in the respiratory chain and so plays a vital role in ATP production. It is required for the production and function of red blood cells [54]. The iron content of the composite flour ranged from 0.86-2.64 mg/100g. These findings are above to the values (0.30-1.10 mg/100g) reported by Adeoye *et al*. [2020] [57] for composite samples from rice flour, cassava flour, and soybean flour but below to the trends (8.45 - 9.95 mg/100g) reported by Adetola *et al*. [2020] [40] for complementary foods from OFSP, soybean and carrot flour composite. The highest value (2.64 mg/100g) of iron was observed in this study and confirm well to the value (2.55 mg/100g) of iron reported by Govender *et al*. [2019] [58] for cooked OFSP and close to the findings (1.94 mg/100g) found by Cayres *et al*. [2021] [51] for biscuit. Oguntoyinbo *et al*. (2021) reported similar trends (1.36 - 4.76 mg/100g) for wheat-banana peel flour. The iron content was high in C1 and the least was observed in C6.

Manganese is a cofactor in the different enzymes and is a constituent of mitochondrial superoxide dismutase and is essential in glycoprotein and proteoglycan production [53]. The manganese content of composite flour ranged from 0.17-0.59 mg/100g, these findings are in the agreement with the results (0.23 mg/100g) reported by Oguntoyinbo *et al*. [2021] [56] for wheat-banana peel flour. The manganese content was high in C11 and the least was observed in C6.

Zinc acts as a cofactor and is found in numerous enzymes [53]. The Zinc content ranged from 0.23-0.56 mg/100g, this value obtained confirm well with the value (0.45 mg/100g) reported by Govender *et al*. [2019] [58] for cooked OFSP. In addition, the obtained results are in agreement with the value (0.40 mg/100g) reported by Oguntoyinbo *et al*. [2021] [56] for wheat-banana peel flour. The zinc content was high in C1 and the least was observed in C11.

Copper is a component of many different enzymes [53]. The copper content ranged from 0.41 - 0.95 mg/100g. Similar results (0.31 mg/100g) were reported by Cayres *et al*. [2021] [59] for pre-gelatinized composite flours. The copper content was high in C8 and the least was observed in C4. Magnesium is an active component of various enzyme systems that contain thymine pyrophosphate as a cofactor. Mg is also required for the activation of the phosphate-transferring enzymes [53]. The magnesium content ranged from 63.02 - 81.25 mg/100g, these findings are above to the trends (184.67 - 221.33 ppm) reported by Adeoye *et al*. [2020] [57] for composite samples from Rice Flour, Cassava Flour, and Soybean flour and above to the value (0.09 mg/100g) reported by Govender *et al*. [2019] [58] for cooked OFSP. This could be due to different raw materials and proportions used in this experiment but similar findings (60.08 mg/100g) were reported by Cayres *et al*. [2021] [59] for pre-gelatinized composite flours. The magnesium content was high in C1 and the least was observed in C6.

Sodium is the most abundant cation in extracellular fluids. It helps to regulate plasma volume and acid-base balance, is actively engaged in the restoration of body fluid osmotic pressure, maintains normal irritability of muscles and cell permeability, stimulates nerve and muscle function, and is implicated in Na+/K+-ATPase, membrane potential general upkeep, nerve impulse transmission, and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts [53]. The sodium content ranged from 31.53 - 41.63 mg/100g, the results are high to the value (0.03 mg/100g) reported by Govender *et al*. [2019] [58] for cooked OFSP. This may be caused by soybean and amaranth flour incorporation into OFSP flour but confirm well to the trends (30.33-33.10 mg/100g) reported by Sanoussi *et al*. [2016] [60] for different OFSP. The sodium content was high in C6 and the least was observed in C3.

Potassium is the most abundant cation in intracellular fluid and is involved in acid-base balance, osmotic pressure regulation, nerve impulse conduction, muscle contraction, particularly cardiac muscle contraction, cell membrane function, and Na+/K+-ATPase. Potassium is also necessary in glycogenesis [53]. The potassium content was high in C6 and the least was observed in C5. The potassium content ranged from 58.76 - 65.18 mg/100g, these results are above to the value (1.70 mg/100g) reported by Govender *et al*. [2019] [58] for cooked OFSP but below to the range (98.32 mg/100g) reported Cayres *et al*. [2021] [59] for biscuit. These could be attributed by different raw materials used in this study. Furthermore, high temperature during ashing may reduce the minerals due to their volatility and interactions between minerals and crucibles.

Conclusion

The main conclusion that can be drawn from this work is that optimum blending ratio was 57, 24 and 19% for Orange-fleshed sweet potato, soybean, and amaranth seed flour respectively and nutrient content was 14%, 4.7% and 813.6 RAE µg/100g for crude protein, total minerals and vitamin A content respectively. Furthermore, overall this work offered a successful approach to follow for ingredients optimization. This can be easily used for the production of different food products which could meet the desired quality of end products. In addition, underutilized agricultural crops could be promoted by enhancing with potential crops for the production of various nutritious foods. That could increase its applicability and value addition, convenient, functional uses and achieve its marketability if standardized and commercialized.

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