Research Article

Advances in Nutrition & Food Science

Proximate Compositions, Anti-Nutritional Factors and Minerals Content of Composite Flours and Bread from Blends of Wheat-Anchote-Soyabean Flours

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Submitted: 24 Nov 2017; Accepted: 02 Dec 2017; Published: 03 Jan 2018

Abstract

The uses of wheat-Anchote-soyabean composite flours for bread making were not common at all. The aim of the study was to analyze nutritional content of Anchote-soyabean-wheat composite flours and its breads. The wheat flour was substituted by Anchote-sovabean flour at levels of 0, 10, 20, 30, 40 and 50%. The developed breads were subjected to proximate, minerals and anti- nutritional factor. The data were analyzed using SAS software version 9. Analysis of composite bread proximate composition revealed that the moisture, ash, crude fibre and crude protein contents increased significantly (p < 0.05) from 100% wheat bread (30.90)%, (1.8)%, (1.36)% and (10.4) to 50% Anchote-soyabean substituted bread which had (36.0)%, (3.02)%, (3.53)%, and (12.05)%, respectively. On the other hand, the crude fat, utilizable carbohydrate and gross energy contents varied significantly ($P \le 0.05$) from 100% wheat bread (1.77)%, (53.72)% and (272.62) % to 50% Anchote-soyabean substituted bread which had (1.50)%, (43.89)% and (237.34)%, respectively. Wheat bread had the lowest phytate and condensed tannin content (129.56) and (84.17) mg/100g, respectively. Whereas, 50% Anchote-soyabean substituted bread had the highest phytate and condensed tannin content (245.37) and (194.30) mg/100g, respectively. The mineral content of the bread also indicated that Ca and Zn contents increased with increasing Anchote-soyabean substituted bread from (29.27) to (35.93) mg/100g and (0.83) to (1.14) mg/100g, respectively. While the Mg content was decreased with increasing Anchote-soyabean substituted bread from 9.42 to 8.25 mg/100g. The nutritional content of wheat-Anchote-soyabean composite flours bread was nutrient dense product, while its anti-nutritional factors level increase with increasing Anchote-soyabean substitutes.

Keywords: Composite Flour, Bread, Proximate Composition, Anti-nutritional Factors, Anchote

Introduction

Food insecurity and malnutrition are among the urgent challenges that developing countries face these days. One of the indigenous Western regions of Ethiopian highlands roots and tuber crops which can have a potential to alleviate malnutrition is Anchote. Anchote (Coccinia abyssinica) is the Afan Oromo name for Coccinia abyssinica and there are about 10 species of Coccinia in Ethiopia found both cultivated and wild; however, only Coccinia abyssinica is cultivated for human consumption [1,2]. The juice prepared from tubers of Anchote has saponin as an active substance and is used to treat Gonorrhoea, Tuberculosis, and Tumor cancer [3]. Thus, Anchote is used as both traditional medicine and food source such as healing of broken/fracture bones and displaced joints, and believed that it makes lactating mothers healthier and stronger as it contains high calcium, and proteins than other commonly and wide spread root

and tuber crops [4]. Anchote is categorized as "poor man's food" has tremendous potential to contribute to a food based approach to promote food security, to alleviate poverty and to supplement as an alternative staple food for the resource poor farmers, because of its diverse range of positive attributes like high yield (42-76 t ha⁻¹) with limited inputs, short duration, high nutritional value and tolerance to various production stresses [1]. Traditionally, boiled after peeling or boiled before peeling and/ or further cooking are applied prior to consumption. Boiling makes most foods palatable, increases digestibility and bioavailability of some nutrients, inactivates some anti-nutritionals and enzyme inhibitors, and increases consumer preferences [5]. Comparing tuber crops the tuber yield of released potato varieties ranged from 22-47 t ha-1 while that of Anchote is 42-76 t ha ⁻¹ [6,2]. Anchote is a root crop, rich in starch and used as staple food in South-West of Ethiopia. The use of composite flours, in which a portion of wheat flour is replaced by locally grown crops, to be used in bread, thereby decreasing the cost associated with imported wheat.

Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking [7]. Among various antinutrients, and plant toxins, phytate, oxalate, tannin, cyanide and trypsin inhibitors are found in root and tuber crops, which may have adverse effects on health through inhibition of digestion, absorption, and growth. Anti-nutritional factors are known to reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals [8,9]. Habtamu reported that raw Anchote tubers were found to contain low antinutritional factors. except phytate [5]. Moreover, there were further reductions of the antinutritional factors during traditional processing. This implies, except phytate which might hinder iron bioavailability, traditional processing enables that the antinutritional factors in the Anchote could not hamper its nutritional value. Therefore, the boiling methods of traditional preparations of Anchote were effective to significantly reduce the levels of antinutritional factors, thereby improving the bioavailability of minerals such as zinc and calcium known to be affected by these antinutrients. Anchote, like many other root, and tuber crops, is rarely eaten raw. Presumed purpose of such processing is also make Anchote more palatable, digestible; inactivate enzyme inhibitors, and other anti-nutritional factors to qualify it for human consumption. Food processing technologies can contribute the alleviation of micronutrient deficiencies. Mechanical, thermal or biological processes have the potential to improve the nutrient availability in foods [10].

Composite flours are a mixture of flours from roots and tubers rich in starch (e.g. cassava, yam, sweet potato) and/or protein-rich flours (e.g. maize, rice), with or without wheat flour. For the developing countries the use of composite flours had the following advantages: A saving of hard currency; promoting of high-yielding, native plant species; a better supply of protein for human nutrition; eradicate glut, better overall use of domestic agriculture production [11]. Another advantage of root and tuber crops inclusion in composite flour is the effects on glycemic index. A recent study indicated that root and tuber flour had a lower glycemic index of 59.34 compared to wheat flour with a glycemic index of 70.10 among 10 diabetic patients [12]. The production of root/tuber-wheat composite bread provides opportunities for the fortification of the resultant bread for the purpose of improved nutrition. For instance, it has been recently demonstrated that the blending of root/tuber with wheat in composite flour can be used as a medium for vitamin A fortification and fibre enhancement [13,14]. When composite root and tuber-wheat flour is fortified with legumes, this can further increase the nutritional quality of the bread. Besides, the increase in the protein content of composite flour via legumes fortification has been reported to improve the baking quality of the dough [15]. The previous study showed that 2 to 10% non-wheat flour can be used in breads without undesirable changes in bread characteristics and sensory attributes of breads [16]. Moreover, cocoyam, cassava, plantain and other roots and tubers crops have been reported to be alternative sources of major raw materials for bread making [17,18].

Wheat is the most popular cereal grain that is consumed worldwide and is used for the production of numerous baked products such as bread, biscuits, cookies, doughnuts and cakes, of which bread is the most common. Bread is a staple foodstuff and is eaten in most countries around the world. It may be described as a fermented confectionary product produced mainly from wheat flour, water, yeast and salt by a series of process involving mixing, kneading, proofing, shaping and baking [19]. Cereals are the only source of nutrition for one-third of the world's population especially in developing and underdeveloped nations of Sub-Saharan Africa [20]. Ethiopia's commercial imports of wheat have risen in the last couple of years. USDAFAS estimates show that wheat deficit in the 2010/11 and 2011/12 seasons [21]. Ethiopian Grain Trade Enterprise imports wheat mainly from Russia and Argentina through food aid mainly by the United States. The Ethiopia Revenue and Custom Authority reported higher import levels, however; for example, USDAFAS estimated 900,000 MT of imports in 2011/12, compared to an estimate of 1,500,000 MT from the Authority [21]. The wheat bread is finally cleaning, drying and preparing it to domestic flour mills. The flour milling industry is the main consumer of wheat because these grains (wheat bread) are the key cereals used for bread production. Maize, oat, barley and rice are used in flour production in significantly lesser quantities where wheat flour is the most important product of wheat flour milling is used in the baking industries and for home cooking [22]. Before receiving wheat, samples are tested to ensure wheat quality and safety and to determine end use qualities and selecting adequate wheat flour, protein quality and gluten power are more important in protein quality in baking of bread. The detrimental effect of substituted flour on bread making potential increase with increasing levels of substitution. Some of the detrimental effects of wheat substitution on bread making include reduction in loaf volume, crumb structure, product shelf life, and impairment of sensory qualities such as appearance, texture, flavor, and mouth feeling [23]. Application of 2-3% of glyceryl monostearate produced cassava-wheat bread of acceptable quality reported by [24]. Bread is conventionally produced from wheat flour. Recently it is also made, though to a lesser extent, from the flour of other non-wheat cereals like; root/tuber and legumes crops with addition of wheat flour in a proportions to make economic use of local cultivated crops to produce high quality bread. It has now become one of the most widely consumed foods in the world. Bread is an important staple food both in the developed and developing world [25]. The rapid urbanization, increasing population and changing food habits have resulted in the preference for convenient foods such as bread, biscuits, and other baked products.

The production of Anchote flour is not as such practiced in the community level, but currently many researchers has been undertaking researches to evaluate the potential of Anchote for preparing varieties of food stuffs. It could increase opportunities to enhance the production and utilization of Anchote and could help improve the economy of various Anchote-producing areas. Therefore, the use of Anchote flour with addition of wheat flour is very beneficial. The production of root / tuber-wheat composite bread provides opportunities for the fortification of the resultant bread for the purpose of improved nutrition. For instance, it has been recently demonstrated that the blending of root/tuber with wheat in composite flour can be used as a medium for protein fortification and fibre enhancement [15,14]. Besides roots/tuber, legumes have a high potential for supplementing the cereal-and starch-based protein and energy intake of the populace especially the groups most vulnerable to malnutrition. Soyabean is an excellent source of protein (35-40%), rich in calcium, iron, phosphorus and vitamins that used in the production of bread as composite flour has been reported [26].

Due to the high cost and demand of wheat flour, efforts have been made to promote the use of composite flours in which flour from locally grown crops and replace a portion of wheat flour for use in bread, thereby decreasing the demand for imported wheat and producing protein enriched bread. According to reports the composite bread can be made by substituting 10, 20 and 30% root flour for wheat flour [27]. Anchote is under-utilized endemic crop. The development of bread products from Anchote flour is unknown, but might have good potential for a number of reasons. It could increase opportunities to expand the utilization of Anchote and could help improve the economy of various Anchote-producing areas. Most developing countries including Ethiopia are largest importer of American red winter wheat. Domestic wheat production has been insufficient to meet rising demand. Due to increased costs associated with production of bread from 100% wheat flour especially in developing countries, other cereal and tuber based flours are now being blended with wheat flour to produce bread. Therefore, the use of Anchote flour for production of baked foods would help to lower the dependency of developing nations on imported wheat. Thus the objective of this study aims to develop bread from composite flours and determine nutritional compositions, anti-nutritional factors and selected minerals content of the product.

Materials And Methods

Description of the Experimental Site

The laboratory work on this research such as; sample preparation, bread making, proximate composition was carried out in Hawassa University laboratory of, food science and technology. Whereas anti-nutritional factors and minerals determination were conducted at Jimma University College of Agriculture and Veterinary Medicine, Laboratory of Post-Harvest Management. All the chemicals used during this research were of analytical grade.

Source of Experimental Materials and Sample Preparation

Wheat flour was obtained from Hawassa Flour Share Company and baking ingredients (baking powder, baking yeast and common salt) were obtained from local super markets. Anchote roots (Coccinia abyssinica) were obtained from local markets of Nekemte town while Soyabean (Glycine max) variety of Afgat was collected from Awassa Agricultural Research Center.

Processing of Anchote Root into Flour

The Anchote flour was prepared by the method described for Anchote flour preparation with little modifications (sun drying instead of oven drying) [28]. The fresh matured and undamaged Anchote root was selected. The Anchote was washed in clean water in large bucket with frequently changing the water and dried in raised rack and peeled. The peeled Anchote was sliced with uniform size of 0.5 cm thickness, sun dried, milled and sieved at 1mm (Axel Kistner, London, England) to produce Anchote flour.

Processing of Soyabeans into Flour

The soybean flour was prepared using the method described by [29]. The seeds were cleaned by removing dirt and other foreign materials before being soaked in water for about 8 hours. The soaked soya beans was dehulled and dried in an oven at 80° C for 8 hr. The dried soybeans were allowed to cool, milled and sieved with a 250 μ m mesh packed into polyethylene bag and kept in dry and safe place until needed for further processing/analysis.

Preliminary Works and Bread Preparation

Preliminary works was carried out for optimizing the blend ratio which was comparable to the wheat flour (control) bread. The blend rations were selected based on the works of [30]. Bread was prepared by straight dough method bread production process (mixing and kneading, bulk fermentation, molding, rounding, intermediate

Blending	Wheat	Anchote	Soyabean
WAS ₀	100	0	0
WAS ₁	90	7	3
WAS ₂	80	14	6
WAS ₃	70	21	9
WAS_4	60	28	12
WAS ₅	50	35	15

WAS-Wheat: Anchote: Soyabean; WAS₀-100:0:0 (Wheat flour), WAS₁-90:7:3, WAS₂-80:14:6, WAS₃-70:21:9, WAS₄-60:28:12, WAS₅-50:35:15.

The breads were cooled to room temperature and packed in zip lock polythene bag with label. Till it was need for further nutritional composition and anti-nutritional factors.

Proximate Composition of Composite Flour and Bread

Samples of composite flour and developed breads were chemically analyzed to determine their moisture content, crude protein, crude fat, crude fiber, total ash, and utilizable carbohydrate and gross energy levels according to standard procedure [31].

Determination of Anti-nutritional Factors Phytate Content

The method described was used for phytate determination [32]. 5 g of dried sample was weighed and extracted with 10ml of 0.2N HCl for 1 hr at an ambient temperature and centrifuge (3000rpm/30minut). The clear supernatant was used for the phytate estimation. Then 2 ml of wade reagent was added to 3ml of the supernatant sample solution then homogenize and centrifuged the solution (3000 rpm/10 minute). The absorbance at 500nm was measured using UV-Vis spectrophotometer (CE1021, England). The phytate concentration was calculated from the difference between the absorbance of the blank (3ml of 0.2N HCl +2ml of wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytic acid standard curve and result was expressed as phytic acid in $\mu g/g$ fresh weight.

Standard Solution Preparation

A series of standard solution was prepared containing 4-40 μ g/ml phytic acid in 0.2N HCl. 3ml of standard was pipette in to 15 ml centrifuge tubes with 3ml of water used as a zero level (blank). Then 2ml of the Wade reagent was added to each tube, and the solution was mixed on a vortex mixer for 5 seconds. The mixture then centrifuged for (3000rpm/10min) and the supernatant read at 500nm by using water to make zero the spectrophotometer. Using SPSS plot the calibration curve (absorbance vs concentration) and find out the slope and intercept.

Calculation

$$\frac{\text{phytic acid in } \mu g}{g} = \frac{\text{(Absorbance-Intercept)}}{\text{(Slope \times Density \times weight of sample)}}$$

Condensed Tannin

Tannin content was determined by Vanillin assay [33]. About 2 gram of sample was weighed in a screw cap test tube. The sample was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000 rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol).

D-catechin was used as standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution. A standard curve was made from absorbance versus concentration and the slope and intercept was used for calculation.

Calculation: Concentration of tannin was read in mg per 100g of sample

Tannin in $\frac{\text{mg}}{100\text{g}}$ = (absorbance-intercept)/(slope×density×weight of sample×10)

Mineral analyses

Calcium, Magnesium and Zinc

The mineral analyses were determined by atomic absorption spectrophotometer method [31]. 1.5 g of sample was put in the oven at 100°C for 30 min. when dry, heat on hot plate until smoke finished and then the dish was placed in 525°C furnace (carefully avoiding ignition) for minimum time necessary to obtain ash that is white and free from carbon normally 3-5h but \leq 8h. The dish was removed from furnace and cooled. The ash should be white and free from carbon. If ash contains carbon particles (i.e. it is gray) wet with water and add 0.5-3ml HNO₃. It was dried on hot plate or steam bath and returned the dish to 525°C furnace for 1-2hr. The ash was dissolved in 5ml 1M HNO₃ warming on steam bath or hot plate 2-3 min to aid in solution. The solution was added in to 50ml volumetric flask and repeat with 2 additional portion of 1M HNO₃ (Nitric acid).

The minerals was determined by adding LaCl₃ (Lanthanum chloride) solution to final dilution of standard and test solution to make 0.1% (w/v) La for determination of Ca and Mg only. Prepare calibration curve (concentration vs absorbance) for each mineral to be determine using wavelength for Ca 422.7nm, Mg 285.2nm and flame for Ca reducing air C₂H₂ and for Mg oxidizing air C₂H₂. The apparatus was set according to the instructions, and a calibration curve prepared by plotting the absorption values against the metal concentration in μ g/ml. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption values of the samples, and the blank.

The metal contents were calculated by using the following formula: (A-B) x Vmetal content = (mg/100g)/10*WWhere:

W = weight of the sample (g), V = volume of the extract (ml), A = concentration (g/ml) of sample solution, B = concentration (g/ml) of blank solution

Experimental design and data Analysis

A simple comparative experiment was designed for the study. The experiment was completely randomized design (CRD). Data were reported as mean values and standard deviation. Comparisons among means were performed by one-way analysis of variance (ANOVA). The Fischer's least significant difference was used to test for comparison of the means among different samples. Significance was accepted at probabilities of less than 0.05. All statistical analyses performed used SAS software version 9.0 for windows. Measurements were performed in triplicate.

Results and Discussion

Proximate Composition of Wheat-Anchote-Soyabean Flours

The results of the proximate compositions of the wheat flour, Anchote flour and soybean flour showed in Table 2 that the Anchote flour had the highest moisture content of $(10.75\pm0.02\%)$ followed by that of the soybean flour $(9.81\pm0.01\%)$ and the wheat flour had the least moisture content of $(9.77\pm0.03\%)$. The above moisture content indicated that the Anchote flour had more moisture in it than either of the other two flours. This is consistent with the moisture contents of cocoyam flour (10.47%) reported due to the fact that root and tubers flour retain more moisture than that of cereals [34]. Also reported that moisture content of cassava flour (11.90 ± 0.01) is higher than that of maize flour (8.05 ± 0.04) and soya bean flour (6.38 ± 0.04) [35].

It was also observed from Table 2 that the ash content of soybean flour was the highest ($6.75 \pm 0.01\%$) followed by that of the Anchote flour ($3.96\pm0.03\%$). Wheat flour had the least ash content of ($0.78\pm0.01\%$); this value is close to the work conducted by Bekele and Shimelis [36]. The low ash content of the wheat flour could be as a result of the fact that wheat flours have very little amount of minerals compared to soybean and Anchote. The ash content results showed that the soybean flour contained the highest minerals.

Also from Table 2, the crude fibre content results indicated that the soybean flour had the highest crude fibre content of $(6.55\pm0.21\%)$. This is nearly the same with the crude fibre content (6.75 ± 0.19) of soyabean flour reported by Mesfin and Shimelis [35].

Wheat flour had the least crude fibre content of $(0.62\pm0.02\%)$. The high crude fibre content of soybean flour could be as a result of the remains of the dried radicles and hulls. The fat content of the soybean flour $(10.20\pm0.00\%)$ from the table above indicated that it was far greater than those of the wheat flour $(2.32\pm0.01\%)$ and Anchote flour $(1.85\pm0.03\%)$ Similar crude fat content $(10.20\pm0.03\%)$ of soyabean flour was reported by Ndife et al. (2011) [37]. Soybean, being an oil seed has more oil in it compared to wheat and Anchote tubers. This led to the high fat content of the soybean flour compared to the other flours.

The crude protein contents of the soybean flour had the highest crude protein content $39.40\pm0.28\%$. Wheat flour had 10.55 ± 0.02 of crude protein while Anchote flour had the least crude protein content of (6.02 ± 0.03). The protein content of the current wheat flour study was close to the protein content (11.07 ± 0.15) of wheat bread HAR 2501 varieties grown under Arsi and Bale climate condition [36]. And also the protein content of soyabean flour is in agreement with protein content reported by Kure while the protein content of Anchote flour [26,28].

Proximate Composition of Bread Prepared from Whole Wheat and Composite Flours

Moisture Content

Table 2: Proximate Composition of Wheat, Anchote and SoyabeanFlours (%)

Ingredients (g/100g)					
Nutrients	Wheat	Anchote	Soyabean		
Moisture	9.77±0.03	10.75±0.02	9.81±0.01		
Crude protein	10.55±0.02	6.02±0.03	39.40±0.28		
Crude fat	2.32±0.01	1.85±0.03	10.20±0.00		
Ash	0.78±0.01	3.96±0.03	6.75±0.01		
Crude fibre	0.62±0.02	3.94±0.01	6.55±0.21		

The moisture content of the composite breads was significantly affected (p<0.05) by blending ratios. The values were found to be 30.90 ± 0.01 , $31.09 \pm 0.05\%$, $32.50 \pm 0.35\%$, $33.99 \pm 0.04\%$, $34.93\pm0.07\%$ and $36.00\pm0.04\%$ for the bread samples of WAS_a, WAS₁, WAS₂, WAS₃, WAS₄, and WAS₅ respectively (Table 3). The results of these values are within the range for bread produced from composite flours of wheat, plantain and soybeans blend (30.98-35.59 [38]. Moisture content of control wheat bread ($30.90\pm0.01\%$) was significantly (p < 0.05) lower when compared with those of the composite bread samples. The moisture content of samples was increased as level of supplementation of Anchote-soyabean flour increased. Increase in moisture content has been reported to correlate with increase in fibre content [22]. The moisture content of the breads obtained from these composite flour were lower than the value of moisture content (10-40%) of defatted soya flour supplementation of wheat breads $(34.12\pm0.2-40.31\pm0.2)$ which was reported by Okafor and Ebuehi [39]. Ezeama reported that low moisture levels positively affects long shelf life of composite breads as they discourage microbial proliferation that lead to spoilage. In comparison to the Nigerian regulatory standards for moisture content values of whole wheat breads: the moisture contents of the bread of the current study were within regulatory specifications of moisture 40% maximum [40,41].

Crude Fat Content

Although the crude fat content of wheat flour bread (100% wheat flour) differed significantly at (p < 0.05) from the rest of the samples but there was no significant difference at (p < 0.05) in the crude fat contents of breads WAS_0 , WAS_1 and WAS_2 with the following crude fat contents 1.77±0.06, 1.76±0.04 and (1.71±0.07) % respectively. Similarly, there was no significant difference in the crude fat contents between WAS, and WAS, breads. The crude fat contents of the bread samples were low and also decreased significantly (p < 0.05) as the substitution of Anchote-soyabean flour was increased. Wheat bread had the highest fat content (1.77 ± 0.06) % may be because it was made from 100% wheat flour whose fat content was higher than that of Anchote flour initially and the substituted soyabean flour percent is could not compensate the low fat content of Anchote flour. WAS₅ bread on the other hand, had the least fat content (1.50 ± 0.02) and this may be attributed to the low content of wheat and Anchote used in making this bread. Similar trend was reported by Owuamanam et al. Where increasing the amount of cassava flour in the blends of wheat and soyabean flour in breads can decrease the crude fat content of the final products [42].

Wheat Ash Content

There was a significant difference (p<0.05) among blending ratios for ash content of composite bread samples (Table3). The ash content of the control bread was $1.81\pm0.00\%$; the ash content of WAS₁, WAS₂, WAS₃, WAS₄, WAS₅ breads were $2.11\pm0.02\%$, $2.30\pm0.01\%$, $2.49\pm0.02\%$, $2.88\pm0.01\%$ and $3.02\pm0.02\%$ respectively. The ash content results indicated that as the substitution of Anchote-soyabean flour was increased, the ash contents of the bread samples equally increased. This may be due to the high ash content of the Anchotesoyabean flour which indicates the high mineral contents in it. Total ash content is directly proportional with the amount of inorganic elements content of Anchote and soyabean [35]. Hence the samples with high percentages ash contents were expected to have high concentrations of various mineral elements, which are advantage to speed up metabolic processes and improve growth and development [28].

Crude Fiber Content

There was a significant difference (p<0.05) in crude fiber content of composite breads due to various blending ratios (Table 3). The crude fiber content of the composite breads increased with increase in substitution of Anchote-soyabean flour from $1.36\pm0.01\%$ in the control (WAS₀) bread to $1.64\pm0.02\%$, $2.16\pm0.04\%$, 2.59 ± 0.03 , and $3.53\pm0.01\%$ for the bread samples of WAS₁, WAS₂, WAS₃, WAS₄, and WAS₅ breads, respectively. The increase in crude fibre might have been due to the fiber content in the Anchote and soyabean flour which increased with increase in its level in the composite flour. According to Prosky and Schweizer report, wheat bread may contain 0.6-1.9% insoluble fibers and 0.1-2.8% soluble fiber making the total content of fiber up to 0.7 and 4.7\% [43]. The crude fiber in the composite bread is greater than 1.5% which is a maximum allowable fiber content of bread flour as stated by Omole [44].

Fibres exhibit beneficial physiological effects to human body, as they stimulate and accelerate intestinal contraction and transit, and increase feces volume [45]. Therefore, the high levels of crude fiber observed in the Anchote-soyabean flour containing breads could be an advantage of as it might help in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders and indigestion and prevention of colon cancer [46,47].

Crude Protein Content

Blending ratio had a significant effect (p<0.05) on the crude protein content of composite breads. The protein contents of the composite flour breads were significantly higher than that of the control sample. Increase in the blending levels of Anchote-soyabean flour resulted in increase in the protein content progressively (Table 3). This is because of the high protein content of the soyabean flour ($39.40\pm0.21\%$) and also indicates the Anchote flour ($6.02\pm0.03\%$) is not as poor as cassava and other root crops flour in protein content [48, 28]. This result also correlates with the studies of Mashayekh et al. who also reported increase in protein content of the bread as a result of the addition of soy flour [49].

Utilizable Carbohydrate

Carbohydrate content was observed to have decreased with increasing substitution of Anchote-soyabean flour in all the bread samples. This trend supports the claim of Akpapunam [50]. The carbohydrate content values was observed to have the highest amount in WAS₀ (100% wheat bread) (53.72±0.05%) and least in WAS₅ bread (43.89±0.02%). The low carbohydrate value was attributable to the low fat content of the wheat-Anchote flour and low percent substitution of soya flour functional breads. Similar experimental trends of results were reported by Islam et al. in their studies on

the fortification of wheat flours with non-defatted soy flour [51]. In comparison to the Nigerian regulatory standards for carbohydrate content values of whole wheat breads: the carbohydrate contents of the bread of the current study were greater than the minimum carbohydrate requirement (37%) regulatory specifications [41].

supports the claim of Okafor and Ebuehi [39]. The energy value was observed to have the highest amount in WAS₀ (100% wheat bread) (272.62 \pm 0.26Kcal) and least in the WAS₅ bread (237.34 \pm 0.07Kcal). The low energy values were attributable to the low fat content of the wheat and Anchote breads. Similar experimental trends of results were reported by Olaoye in their studies on the fortification of wheat flours with plantain and soybeans flour [38].

Gross Energy

Energy content was observed to have decreased with increasing substitution of soya flour in all the bread samples. This trend

Table 3: Proximate composition	s of wheat bread and	d composite flours breads (%	6)
1			

Code of blend	Moisture	Crude protein	Crude fat	Crude fiber	Ash	UCHO	Energy (kcal)
WAS ₀	30.90ª±0.01	$10.45^{f}\pm 0.08$	1.77ª±0.06	1.36 ^f ±0.01	$1.81^{\rm f} \pm 0.00$	53.72ª± 0.05	272.62ª± 0.26
WAS ₁	31.09ª±0.05	10.75°±0.01	1.76 ^{ab} ±0.04	1.64°±0.02	2.11°±0.02	52.66 ^b ± 0.12	$269.50^{b} \pm 0.15$
WAS ₂	32.50 ^b ±0.35	11.05 ^d ±0.03	1.71 ^{ab} ±0.07	2.16 ^d ±0.04	2.30 ^d ±0.01	50.28°±0.30	260.71°± 1.99
WAS ₃	33.99°±0.04	11.59°±0.02	1.65 ^{bc} ±0.03	2.59°±0.03	$2.49^{\circ}\pm0.02$	$47.67^{d} \pm 0.07$	251.93 ^d ± 0.48
WAS_4	34.93 ^d ±0.07	12.05ª± 0.01	1.57 ^{cd} ±0.02	1.57 ^{cd} ±0.02	$2.88^{\mathrm{b}}\pm\!0.01$	45.83°± 0.05	244.83°± 0.33
WAS ₅	36.00°± 0.04	12.05ª±0.01	$1.50^{\rm d}{\pm}0.02$	3.53ª±0.01	$3.02^{a}\pm0.02$	43.89 ^f ±0.02	$237.34^{\rm f}\pm 0.07$
Mean	33.23	11.28	1.66	2.37	2.43	49.01	256.16
CV (%)	0.45	0.19	2.76	1.12	0.71	0.28	0.335

WAS-Wheat:Anchote:Soyabean, WAS₀-100:0:0 (Wheat flour), WAS₁-90:7:3%, WAS₂-80:14:6-WAS₃-70:21:9, WAS₄- 60:28:12, WAS₅-50:35:15, UCHO- Utilizable Carbohydrates, CV-Coefficient of variation. The mean values of duplicate determination and means with the superscripts across the column are significantly different (p<0.05).

Anti-nutritional Factor Contents of Whole Wheat and Composite Flours Bread

complexes are insoluble and this decreases the protein digestibility by inhibiting the activities of digestive enzymes [55]. The results showed that the concentrations of anti-nutrients in the composite flour were below toxic levels.

Phytate Content

Analysis of variance showed that the phytate content of current study was significantly affected by blending ratio of wheat, soya bean and Anchote. Accordingly the highest phytate content was recorded from WAS, bread. While lowest value was obtained from bread developed from 100% wheat flour (Table 4). It was observed that the phytate content were in the ranges of 129.56 ± 0.48 to 245.37 ± 0.62 mg/100g. The level of phytate content significantly increased with increase in Anchote-soyabean substitution for bread. However, the levels of phytate in the breads were low as to have a binding effect on some minerals such as Ca, Mg, Fe and Zinc and make them unavailable. High levels of phytates in human nutrition are toxic and limit the bioavailability of calcium, magnesium, iron and phosphorus by the formation of insoluble compounds with the minerals [52]. This is due to more phytate content in soybean and Anchote. The Phytate concentration range obtained in the present study is lower compared to the acceptable concentrations. In average, the daily intake of phytate was estimated to be 2000-2600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries and 150-1400 mg for mixed diets [53].

Condensed Tannin Contents of the Composite Breads

The condensed tannin contents of present experiment showed a highly significant difference (p<0.05) due to effect of blending ratio of wheat, Anchote and soya bean. WAS₅ bread shows the highest mean values of tannin content (194.30±0.57 mg/100g) while the least tannin content (84.17±0.23 mg/100g) was recorded from WAS₀ bread (Table 4). Bread samples from all blends could be considered safe with regard to tannin, since the tannin content is far below the total acceptable tannin daily intake for man, 560mg [54]. Tannin-protein

Table 4: Anti-nutritional factor contents of wheat bread and composite breads (mg/100g)

Code of blend	Phytate	Tannin
WAS_0	129.56f±0.48	84.17f±0.23
WAS ₁	145.59e±0.55	101.89e±0.43
WAS ₂	157.41d±0.28	116.61d±0.62
WAS ₃	175.33c±0.91	127.18c±0.71
WAS_4	194.64b±0.78	172.03b±0.34
WAS ₅	245.37a±0.62	194.30a±0.57
Mean	174.649	132.69
CV (%)	0.37	0.35

WAS-Wheat:Anchote:Soyabean, WAS₀-100:0:0 (Wheat flour), WAS₁-90:7:3%, WAS₂-80:14:6-WAS₃-70:21:9, WAS₄- 60:28:12, WAS₅-50:35:15, CV- coefficient of variation. The mean values of the superscripts across the column are significantly different (p<0.05).

Mineral contents

Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body [56]. Calcium is a mineral required by the body for a variety of physiological functions and the maintenance of bone tissues throughout life [57]. As an essential nutrient for human health, zinc also plays an important role in normal growth and development [58]. Effect of blending ratio of Wheat, Anchote and Soybean flours on mineral contents of bread was shown on Table 5. The mineral contents of bread samples analyzed in the present study ranged between 29.27 ± 0.07 to 35.93 ± 0.4 , 8.25 ± 0.31 to 9.96 ± 0.05 and 0.83 ± 0.10 to 1.14 ± 0.04 mg/100g for calcium, magnesium and zinc respectively. The variation observed could be due to the compositional difference in terms of mineral content between the crops used in the blends. For calcium and zinc an increasing trend was observed with increasing the Anchote and soyabean flour. While magnesium content of the bread decreased as the amount of Anchote-soyabean increased.

The calcium content of the bread samples investigated in the present study was significantly affected (p< 0.05) by the blending ratio. There was no significance differences observed among WAS₁, WAS₂, WAS₃, WAS₄, and WAS₅ bread at p < 0.05 for calcium contents. WAS₀ (100% wheat bread) was significantly different from other breads in terms of calcium contents at p<0.05. The trend showed that there was increment in the amount of calcium as the amount of Anchote and soybean increased in the blend.

The zinc content of the bread samples investigated in the present study was significantly affected (p < 0.05) by the blending ratio. There was no significance differences observed among WAS₁, WAS₂, WAS₃, WAS₄ and WAS₅ composite bread at p < 0.05 for zinc contents. Bread prepared from wheat flour was significantly different from other breads in terms of Zinc contents at p < 0.05. The results also revealed that an increased Zn contents were observed when there was high concentration of Anchote and soybean flour in the bread. Higher mineral content in the present study found in different breads may be attributed to higher concentration of calcium and zinc in the Anchote and soybean flour used for supplementation of composite flours [59,60]. Even though there was decrement in the amount of magnesium as the amount of Anchote and soybean increased, it did not show significance difference in all bread prepared from different blends.

Table 5: Mineral contents of wheat and Anchote-soybe	ean flours
blended bread (mg/100g)	

Code of blend	Ca	Mg	Zn
WAS ₀	29.27 ^d ±0.07	9.96ª ±0.05	0.83°±0.10
WAS ₁	33.93 ^{abc} ±0.11	9.59 ^{ba} ±0.02	0.95 ^{bcde} ±0.01
WAS ₂	34.00 ^{abc} ±0.13	9.42 ^{ab} ±0.07	1.01 ^{abcd} ±0.2
WAS ₃	34.00 ^{abc} ±0.6	9.42 ^{ab} ±0.04	1.09 ^{ab} ±0.07
WAS ₄	35.00 ^{ab} ±0.01	9.03 ^{abc} ±0.12	1.13 ^{ab} ±0.03
WAS ₅	35.93ª±0.04	8.25 ^{abc} ±0.31	1.14ª±0.04
Mean	33.688	9.278	1.025
CV (%)	0.78	2.85	25.81

WAS-Wheat: Anchote: Soyabean; WAS₀-100:0:0 (Wheat flour), WAS₁-90:7:3%, WAS₂-80:14:6-WAS₃-70:21:9, WAS₄-60:28:12, WAS₅-50:35:15, Ca-Calcium, Mg-Magnesium, Zn-Zinc, CV-Coefficient of variation. The mean values of the superscripts across the column are significantly different (p<0.05).

The result of the current research was agreed with a research finding by Ndife et al. who concluded that zinc content of wheat ratios increased with supplementation of soy flour [37]. Another research done by Jan et al. reported that oilseeds flour contained appreciable quantity of mineral which resulted in increase in mineral contents of composite flours [61]. A similar result has also been reported by Rawat et al. as soy fortified chapattis contained higher iron, zinc and calcium than whole wheat flour chapattis [62].

Conclusion

This study was carried out to assess the effect of substitution level of Anchote-soyabean flour on composite flour. Increased substitution level of Anchote-soyabean flour correspondingly increased the crude protein, crude fibre, and ash level of the breads while the fat content was decreasing with increased Anchote-soyabean flour substitution level. Due to the low attention given to the research and development of Anchote root, there is no variety of food products prepared from Anchote flour. Bread production from Anchotesoyabean-wheat composite flour should be given due emphasis and processors should be encouraged to utilize the potential of Anchote flour thereby diversify their products for better income and service for the consumer. A comprehensive study on optimization of ingredient and baking condition and shelf life stability of baked products of Anchote-soyabean blended with wheat should be conducted to come-up with complete and usable information in order to improve the composite bread qualities.

Acknowledgements

We would like to acknowledge the School of Nutrition, Food Science and Technology, Hawassa University and ENGINE project for their financial support. Our special appreciation goes to Hawassa Agricultural research center and Hawassa Flour Share Company for providing raw materials for this research.

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