

Bio-fortification of Cereals Based Bread Using OFSP for Alleviating of Vitamin-A Deficiency

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Abstract

Ethiopia is one of the developing countries with a high prevalence of micronutrient deficiencies and protein-energy malnutrition. Vitamin A is one of the most versatile vitamins with roles in various functions such as vision, immune defense, maintenance of body linings and skin. Fortifying cereals through processing and conventional breeding can tackle micronutrient deficiency. Orange Fleshed Sweet Potato (OFSP) is a source of food that contains useful β -carotene, starch, mineral, dietary fiber, and vitamins. The inclusion of OFSP enhances the beta-carotene content of bread. This research aims to develop Vitamin A rich cereals-based bread enhanced with orange-fleshed sweet potato. The straight dough baking method was used to develop bread. Beta-carotene content and physical properties of bread were done based on standard methods. Blending of OFSP flour in cereals significantly influenced the physical properties of bread. The total carotenoid content of bread products with 10% of OFSP Flour was 3.998mg/100g while that of beta-carotene is 23.217mg/100g. Inclusion of OFSP in composite flour increased the total carotenoid and beta-carotene content of bread. Generally, formulated flour has huge advantageous as a means of enhancing beta-carotene content and helps to alleviate vitamin A deficiency.

Key Words: Bread, Micro-nutrients, Vitamin-A, malnutrition, Orange Flashed Sweet Potato

1. Introduction

Malnutrition refers to deficiencies, excesses, or imbalances in a person's energy intake and/or nutrients. It usually refers to several diseases, each with a specific cause related to more nutrients, such as protein, iodine, vitamin A or iron (Saunders *et al.*, 2015). The most crucial malnutrition in most developing countries, including Ethiopia, is micronutrient deficiencies and protein-energy malnutrition (Urigacha, 2020).

Vitamins are a group of organic compounds that play important functions in body but cannot be made by the body. Some vitamins can be stored in the body so need to be eaten often but not every day (fat soluble vitamins A, D, E and K), while others cannot be stored and should be eaten daily (water soluble B vitamins, vitamin C). Vitamin A is one of the most versatile vitamins with roles in various functions such as vision, immune defense, maintenance of body linings and skin, bone and body growth, normal cell development, and reproduction (Aslam *et al.*, 2017). Vitamin A also helps to form and maintain healthy teeth, skeleton and soft tissue, mucous membranes, and skin. Therefore, vitamin A and related nutrients are collectively important in protecting against conditions related to oxidative stress, such as aging, cancer, cardiovascular disease, cataracts, diabetes mellitus and infection (Laquatra, 2003). According to the World Health Organization (WHO), vitamin A deficiency affects approximately 190 million children of pre-school ages and 19 million pregnant women, mainly in Africa and South-East Asia (WHO, 2019). In Africa, 2% of preschool-age children are affected by night blindness which is four times higher than the proportion in South East Asia (0.5%). In Ethiopia, the prevalence of Vitamin A deficiency among preschool children was found 13.9 % at a national level while that of school age children was found 10.9%. Among the regions the prevalence of vitamin A deficiency of school age

children who live in Harari is the highest as compared to other region at a prevalence of 25.0%. Numerous studies have shown that vitamin A deficiency is a moderate public health problem. According to WHO classification (Mild: ≥ 2 to ≤ 10 , Moderate: ≥ 10 to < 20 and Severe: > 20 $\mu\text{mol/l}$), Vitamin A deficiency is a moderate public health problem in Ethiopia (EPHI, 2016). Ethiopia supports and has adopted policies supporting regular vitamin A supplementation of children (27). This policy calls for children aged 6-12 months and over to receive 2 doses of vitamin A, once every four to six months. In addition to supplementation there is an act of food fortification to overcome vitamin A deficiency.

Cereals are climate-resilient, widely grown, accessible, and affordable crops to vulnerable populations. Foods prepared out of whole cereals contain increased concentration of phytochemicals along with other vitamins and minerals (Aune et al, 2011). In addition to this, a smaller percentage of population used cereals like sorghum and other millets and their food products have received the attention in new food formulations as they are proved to be the good and comparable sources of proteins and other functional components (Kumari & Sangeetha, 2017). After few mechanical processes like steel cutting, rolling or flour making, most of the cereals can be eaten as whole, refined or as breakfast foods which may add required nutritional profile like high fiber, low fat and retention of some micronutrients. Fortifying cereals such as wheat and sorghum through processing and formulations can tackle global micronutrient deficiency (CGIAR, 2021). Incorporation of roots and tubers has found to be increased in the proximate composition, minerals and vitamin content of supplementary food mix which meets nutritional requirements of malnourished and underweight children (Eke-Ejiofor, & Friday, 2019).

Fortification is the practice of deliberately increasing the content of one or more micronutrients (i.e., vitamins and minerals) in a food or condiment to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health (WHO, 2022). Staple foods such as wheat flour, maize flour, rice, oil, pulses and salt, are consumed by most of the global population consistently throughout the year. They can be fortified with micronutrients, including iron, folic acid, vitamin A, iodine and others, without affecting taste, texture, or color and with a negligible cost to the consumer (Nutrition International, 2022). Bio-fortification or biological fortification refers to nutritionally enhanced food crops with increased bioavailability to the human population that are developed and grown using modern biotechnology techniques, conventional plant breeding, and agronomic practices (McGuire, 2015). Orange-fleshed sweet potato (OFSP) is one of the biofortified crops and it is an excellent source of pro-vitamin A in the diets of most people living in developing countries. It is an essential industrial crop and a source of food that contains useful β -carotene, starch, minerals, dietary fiber, and vitamins (Neela and Fanta, 2019). Orange Flashed Sweet potatoes can add natural sweetness, color, and flavor to bakery products (Edun et al, 2019). OFSP is taken into account as an upscale source of non-digestible dietary fiber, specific minerals, vitamins, and antioxidants (Endrias et al., 2016). Phenolic compounds and carotenoids are accountable for distinguishing flesh and skin colors (deep yellow, red to orange, purple, and pale) of Sweet Potato together with antioxidant properties (Steed & Truong, 2008). Recent scientific reports concluded the ant-oxidative and radical scavenging activity of phenolic acid components in Orange Flashed Sweet Potato with beneficial health-promoting activities (Rumbaoa et al., 2009). Incorporation of OFSP flour into other foods appears to be the most effective way for increasing the vitamin A content of OFSP enriched food products (Kidane *et al.*, 2013). Bread is a bakery product whose main ingredients are comprised of water, flour, salt, yeast, sugar, and fat, which are mixed and fermented to form viscoelastic dough before being baked (Goesaert et al., 2009). It is the important vehicle to fortify micronutrients such as vitamins. Therefore, the objective of this research is to address problems associated with vitamin A deficiency by developing nutritious foods and enhancing palatability based on wheat-sorghum and orange flashed sweet potato composite flour bread.

2. Materials and methods

2.1. Study area

The sample preparation and experimental analysis were conducted at Melkassa Agricultural Research Center (MARC). It is found in the Great Rift Valley, 117 km away from Addis Ababa in the southeast direction.

2.2. Sample collection, preparation and flour extraction

Sorghum (Melkam, white sorghum variety), five kilograms was brought from sorghum and millet improvement, Melkassa Agricultural Research Center (MARC). It was cleaned, sorted, washed with tap water, drained, de-hulled (manually), sun-dried, milled, packed in a plastic bag, and stored in a dry and cool place until further use. The sorghum sample was milled by a hammer miller (XFYC 810, China) at Food Science and Nutrition Research, a food product development laboratory with a sieve size of 0.50mm. The flour was packed in a polyethylene bag and stored at room temperature until further formulation with wheat and Orange flashed sweet potato flour.

Five kg of Orange fleshed sweet potato (Vita variety) were brought from Jimma Agricultural Research Center. Then it was sorted, weighed, washed with clean tap water, peeled and cut into thin slices by stainless steel knife, spread in a tray and oven (TR-TC-YHG-300-BS-11), dried at 60°C for 10 hr. and milled into flour by hammer miller (XFYC 810, China) at MARC, Food Science and Nutrition Research, food product development laboratory with a sieve size of 0.50mm. The flour was packed in a polyethylene bag and stored at room temperature for the further formulation, product development, and composite flour nutritional and anti-nutritional analysis.

Wheat grain (Daka) variety was collected from Kulumsa Agricultural Research Center, National wheat breeding program. Then it was cleaned manually, picking foreign materials (stone, straw, and defective seeds), winnowed, sorted, and stored in a cool and dry place until milling. After a week, the cleaned wheat grain was conditioned to a 16.5% of moisture level with distilled water in a plastic container and left for 18 hours to facilitate a tempering situation. Then flour was extracted using a Chopin laboratory mill (Moulin CD1 mill, Chopin technology, France) and sieved through a fine sieve of size 0.50 mm at Kulumsa Agricultural Research Center, Food Science, and Nutrition Research laboratory. The flour was packed in a polyethylene bag and stored at room temperature for further formulation, product development, and beta-carotene analysis.

2.3. Flour formulation

Mixture design (D-Optimal) was used for flour formulations. Maximum and minimum levels of blending ratio were based on previous studies recommendation. The formulation of composite flour was done based on the study previously conduct by Wu et al., (2018). The author recommended that the best optimized value for good physical properties and beta-carotene content of formulated bread was for sample of wheat (60), sorghum (30%) and OFSP (10%). Accordingly, the study designed has been summarized in Table 1.

Table 1 Formulation of wheat-sorghum and OFSP composite flour for bread making

Runs	Crops		
	Wheat (%)	Sorghum (%)	OFSP (%)
R0 (Control)	100	0	0
R1	90	0	10
R2	70	30	0

2.4. Product development

Bread baking was performed based on the method of Malik *et al.*, (2015) bread baking procedure (straight dough method). All ingredients flour (100 g blended), salt (2.5 g), water (65 ml), and yeast (2.5g) were added at the mixing stage and kneaded to obtain uniform dough. The dough samples were placed in baking pans smeared with vegetable oil and was covered for the dough to ferment resulting in gas production. The dough was then baked in an oven (Electric ovens SIMPLY 2T, China) at an average temperature of 230°C for 30 minutes. The baked loaf was carefully removed from the pans and allowed to cool and then packaged in Polyethylene bags for further analysis.



Figure 1 Bread baking and nutritional analysis

2.5. Bread physical properties

The evaluated bread physical characteristics were loaf weight, loaf volume and specific loaf volume. Loaf weight was measured 30 minutes after the loaves were removed from the oven using a weighing balance. It was determined by weighing the bread loaves after cooling by using a laboratory scale electronic balance (CE- 410I, Camry Emperors, China) and the readings recorded in grams. The loaf volume was measured using the rapeseed displacement method (AOCC, 2000, Standard 10-05). Rapeseed grain was poured into a container of known volume until the bottom was covered. The loaf was placed inside the container which was then filled to the top with more seeds. Then the extra rapeseed grain which had equal volume with the loaf was measured in a graduated cylinder and recorded as loaf volume. Specific volume was obtained by dividing the loaf volume of bread by its corresponding loaf weight.

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{Loaf volume (cm}^3\text{)} \dots\dots}{\text{Loaf weight (g)}} \quad \text{Equation 1}$$

2.6. Analysis of Total Carotenoid and Beta-carotene

2.6.1. Beta-carotene

Total carotenoid and beta carotene were extracted in the dim-light room. For the extraction of β-carotene, the procedure outlined in AOAC Official Method 941.15- (Pritwani & Mathur, 2017) was followed. Five grams of flour sample were measured and blended with 40 ml acetone, 60 ml petroleum ether, and 0.1 g magnesium/sodium carbonate, extracted for 5 min using mortar and pestle. Then filtration was done through the aid of a suction pump (Halstead Essex, England) and the sample was decanted into the separator funnel. The residue was washed with 25 ml portions of acetone, then with 25 ml petroleum

ether, and then extracts were combined. The combined extract was evaporated to dryness and the residue re-dissolved in acetone. The volume made up to 5 ml using acetone depending upon the matrix. Before reading with HPLC (model) the sample was filtered through a syringe of 0.45µm pp then 1 ml samples were put into a vial and then the sample was analyzed by high-performance liquid chromatography (Shimadzu HPLC, Japan, Kyoto). The peak responses of β-carotene were measured at wavelengths of 450 nm (Sungpuag et al., 1999).

Standard Preparation of beta-carotene

Standard of beta carotene (1g enclosed in the vial) (Sisco Research Laboratories Pvt. Ltd. (India) was purchased. The stock solution of beta-carotene was prepared by taking 10mg in 100ml acetone with a concentration equal to 100 ppm. Then series of standard solutions were prepared from known concentrations of stock solution (15, 30, 45, and 60 ppm dilutions with 5 ml of each acetone solution. The formula to calculate the beta-carotene contents of a sample in the extract was calculated as follows

2.6.2. Total Carotenoids ($\frac{mg}{100g}$)

Harvest plus crops methods were employed for total carotenoid analysis. Total carotenoids were performed spectrophotometrically (Shimadzu -UV-1800/Japan) using the method described by (Rodriguez-Amaya and Kimura, 2004). Five grams (5 g) flour samples were ground with 40 ml of cold acetone using mortar and pestle until the residue became colorless and then vacuum-filtered using a Buchner funnel. The extract was partitioned with 60 ml of petroleum ether, and then each fraction was washed with distilled water for complete acetone removal. The extracts were made up to a volume of 50 mL with petroleum ether. All of the procedures were performed in dim light. The extracted carotenoids were collected and measured at 452 nm using a UV spectrophotometer (Shimadzu -UV-1800, Japan). Total carotenoids were calculated with the following equation.

$$\text{Total Carotenoid } (\mu\text{g/g}) = [A \times \text{volume (mL)} \times 104] / [A1\%1\text{cm} \times \text{sample weight (g)}]. \text{Equation.. 3}$$

Where A = absorbance; volume = total volume of extract = 50 mL; A1%1cm=absorption coefficient of β-carotene in petroleum ether (2592).

2.7. Statistical Analysis

The results were subjected to Analysis of Variance (ANOVA) technique by using Completely Randomized Design (CRD) method and all pair wise comparison tests were used for mean comparisons whereas Duncan’s Multiple Range test (SPSS version 21.0 for Windows, SPSS Inc, Illinois, and USA) were carried out to determine level of significance within means (at $p \leq 0.05$). Both numerical optimization and graphical optimization technique were employed using the Design Expert™ version 7.0 software (State Ease Inc.).

3. Results and Discussion

The bread physical properties, total carotenoid and beta-carotene, and vitamin-A content of OFSP enhanced bread were discussed in detail below.

3.1. Bread physical properties

The bread-making quality parameters measured for the pup loaf straight-dough procedures baking were color, loaf volume, loaf weight, and specific loaf volume. For the improvements in physical and sensory properties and shelf life of bread product, quality is very important. The quality of bread is normally defined based on its volume, color, texture, and flavor of bread (Quilez *et al.*, 2006).

Table 2 Bread loaf volume, weight and specific volume

Sample code	Loaf volume (cm ³ /100g)	Loaf weight (g)	Specific volume (cm ³ /100g)
T0	290.97 ^a	84.73 ^a	3.43 ^a
T1	232.45 ^a	75.10 ^b	3.16 ^a
T2	224.79 ^a	74.94 ^b	3.01 ^{ab}
T3	152.14 ^a	73.79 ^b	2.06 ^b
Grand Mean	226.88	76.69	2.97
CV	17.78	3.97	18.97

Means in the same column with different letters are significantly different based on Duncan's multiple range test ($p < 0.05$). Where T0 (1.000:0.000:0.000), T1 (0.900:0.000:0.100), T2 (0.700:0.300:0.000), and T3 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

Loaf volume

Loaf volume is used as criteria to measure the quality of fresh bread in research quality control in industry and by consumers. The bread loaf volume of our experiment ranged from 152.14 cm³/100g to 290.97cm³/100g (Table 2). The samples were not significantly different from each other except sample 60% wheat: 30% sorghum: 10% OFSP. Bread developed from 100% wheat has high bread volume while sample 60% wheat: 30% sorghum: 10% OFSP has low bread volume. As we have observed from our result, as the ratio of wheat decreased, the bread volume decreased. Even though there was a decrease in bread volume as the ratio of sorghum and OFSP increased, acceptable bread was developed. Our finding is in agreement with the Hugo *et al.*, (2003) who concluded that the substitution of wheat flour with up to 40% sorghum flour decreased the bread volume and Mahendran & Hariharan, (2018) who reported that the addition of sorghum to wheat flour negatively influence the loaf volume.

Loaf weight

Bread loaf weight was ranged from 67.854 g to 84.728 g (Table 2). There were no significance difference between samples except sample of (100%) wheat and sample of (70% wheat: 22.5% sorghum: 7.5% OFSP). Sample of 100% wheat has high loaf weight while sample of (70% wheat: 22.5% sorghum: 7.5% OFSP) has low loaf weight when compared to other samples.

Specific Loaf volume

Specific volume is an indication of the gluten content of the bread but, other constituents such as starch and fiber also contribute to the specific volume of bread. It ranged from 2.06cm³/g to 3.434 cm³/g in our research finding. The highest and lowest level of specific volume was observed in sample of (100% wheat) and sample of (60% wheat: 30% sorghum: 10% OFSP) respectively. Aprodu, (2020) also reported in their finding that, the composite flour of 70% wheat and 30% sorghum had the specific loaf volume of 2.87 cm³/g which is in range with our finding. This was due to the highest and lowest ratio of loaf volume and loaf weight of samples respectively. Edun *et al.*, (2018) also reported that a decrease in bread specific volume when wheat flour was replaced with 10-30% OFSP flour.

3.2. Total carotenoid and beta-carotene content

Total and beta carotene content of composite flour were listed and discussed below in Table 3.

Table 3 Total carotenoid and beta-carotene content of OFSP enhanced bread.

Sample Code	Total Carotenoids (mg/100g)	Beta-carotene (mg/100g)
T0	BDL	BDL
T1	32.89 ^{ab}	21.30 ^d
T2	6.22 ^d	0.140 ^d

T3	35.90 ^a	23.22 ^a
Grand mean	18.05	10.01
C.V	21.65	14.27

Means in the same column with different letters are significantly different based on Duncan’s multiple range test ($p < 0.05$). Where T0 (1.000:0.000:0.000), T1 (0.900:0.000:0.100), T2 (0.700:0.300:0.000), and T3 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively. BDL: Below detection level.

The highest value of total carotenoid and beta carotene content of bread samples were 35.90 mg/100g and 23.22 mg/100g respectively. As the ratio of OFSP in composite flour increased, the total carotenoid and beta-carotene content of bread increased. Nzamwita *et al.*, (2017) found in their previous study that, the composite flour of OFSP (10%) and wheat (90%) has the average value of 29.17 mg/100g beta carotene which was in range with our finding while that of 100% wheat has no beta-carotene which our experiments also confirmed.

3.3. Vitamin-A value of OFSP enhanced bread.

The vitamin-A content of OFSP enhanced bread value was presented in figure 1. The vitamin A equivalency ratio for β -carotene to vitamin A is currently estimated as 12:1, by weight (12 μg β -carotene is equal to 1 μg retinol), for plant sources of β -carotene in a mixed diet (FDA, 2016). Bread sample of treatment 3 (60% wheat, 30% sorghum and 10% OFSP) had high value (1940 μg retinol) of vitamin A while bread sample of 100% wheat (control) has no vitamin A. The result revealed that as the ratio of OFSP increased, the vitamin A content was increased.

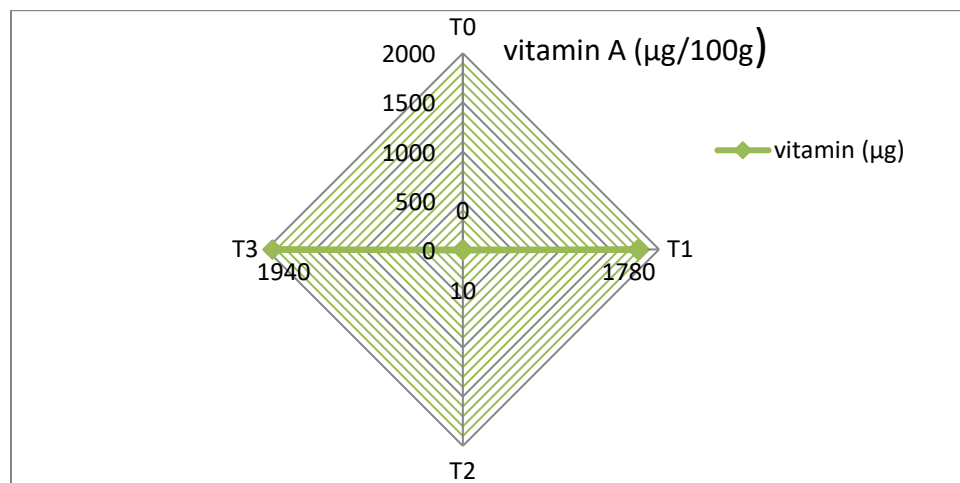


Figure 2 Vitamin-A content of OFSP enhanced sorghum-wheat based bread.

The Recommended Dietary Allowance for adults 19 years and older is 900 mcg RAE for men and 700 mcg RAE for women. The Tolerable Upper Intake Level (UL) is the maximum daily intake unlikely to cause harmful effects on health. The UL for vitamin A from retinol is 3,000 micrograms of preformed vitamin A (NIHODS, 2018). This study showed that inclusion of OFSP in bread can boost the chance of meeting recommended dietary allowance of men and women.

4. Conclusions and Recommendation

Vitamin-A is one of essential vitamins that help for vision, immune defense, maintenance of body linings and skin, bone and body growth, normal cell development, and reproduction. Most of leafy vegetables

and roots and tuber are rich sources of pro-vitamin-A. OFSP is an excellent source of pro-vitamin A in the diets of most people living in developing countries. Generally, this study revealed that bread samples enriched with OFSP potato had high amount of total carotenoids and beta-carotene, and sensory acceptance. The author recommends that inclusion of OFSP in bread products has huge advantages as a mean to alleviate vitamin-A deficiency and cheap technology to enhance health benefits for the whole community.

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