

Carotenoid Retention and Nutritional Value of Biscuits from blend of Orange-Fleshed Sweet Potato, Wheat flour and Palm Oil

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Abstract

Vitamin A deficiency, largely affects children under five years of age and women within the reproductive ages in Sub-Saharan countries in Africa. The aim of this study was to develop biscuits with high carotenoid contents, from Orange-Fleshed Sweet Potato, wheat-flour and crude palm oil and also to determine carotenoid retention in the flours and in the biscuits. The Orange-Fleshed Sweet Potato (OFSP) flour was prepared from fresh OFSP root after sun and oven drying before the determination of carotenoid content and retention. We analysed carotenoid content in crude palm oil (CPO) before its incorporation in biscuit formulations. The flour from oven dried showed high carotenoid content, therefore was preferred in biscuit formulations. One control biscuit (A0) was prepared using 100g of wheat flour and 30 g of margarine. Then formulation A was: 100g of wheat flour +10g of palm oil+20g of margarine, the formulation B, was 70g of wheat flour +30g of OFSP flour +10g palm oil and 20g of margarine and the formulation C, was 50g of wheat flour +50g of OFSP flour+10 palm oil +20g of margarine. The carotenoid content was determined using the method described by Rodriguez Amaya and Kimura (2004) and the proximate composition by the Association of Official Analytical Chemistries (AOAC) methods. The analysis of variance was used to determine the significant difference in the sample according to the formulation with a significant level of 0.05. After all the data treatments, the following results were obtained:

The carotenoid retention was significantly higher in the oven dried than the sun-dried flour (90.76% vs 66.09%); in the biscuits, retention levels were 77.43% in formulation A, 78.62% in the treatment B and 74.48% in treatment C. The carotenoid contents in the biscuits were 55.77 µg/g, 115.68 µg/g and 146.89 µg/g, in treatments A, B and C respectively. The loss of carotenoid in biscuits increases proportionally with concentration of carotenoids in the blend before baking.

The protein content ranged from; 4.83 to 6.35% and energy from; 468.46 to 487.35 Kcal/100g of biscuits.

Composite wheat-OFSP flour appears to be useful to produce biscuits rich in provitamin A carotenoids, which could be useful in a fortification program to manage vitamin A deficiency in high-risk people.

Key words: Orange-fleshed sweet potato, biscuits, palm oil, carotenoids retention

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Introduction

Vitamin A deficiency largely affects children under five years of age and women within the reproductive ages in Sub-Saharan African countries. Micronutrients deficiency or hidden hunger does not only contribute to 45% of children deaths in Africa, it also exacerbates poor health and poverty, reducing productivity and country's economic growth [1]. Chronic malnutrition affected more than 165 million children worldwide, a quarter of children under 5 years and about 190 million children under five (33.3% of the preschool age population) are vitamin A deficient (VAD), with about 5.2 million affected by night blindness [2].

Selected VAD surveys present, 9.23% among Chinese children [3], 5.3% among Nigerian's children [4] and alarming 61,1% for children aged from 6-36 months in the Democratic Republic of Congo (DRC) [5]. Moreover, Mukund et al [6], reported 20.3% of VAD among malnourished and healthy children in Kisangani in DRC.

Biscuits are widely consumed and its quality can be modified to suit specific nutritional needs of any target population [7]. However, Burrier et al [8] stated that, the quality of biscuit depends on the type of ingredients and particular improvement needed. Wheat flour the main ingredient for biscuit was reported to have less than 1 µg/100g of beta-carotene and after baking, the biscuit showed 14 µg/100 g of beta-carotene [9].

Orange-Fleshed Sweet Potato (OFSP) is a potential source of dietary provitamin A. The predominant carotenoid is beta-carotene [10], which may reach up to 20000 µg/100 g wet basis depending on OFSP variety [11]. On the other hand, the carotenoid content in crude palm oil ranged from 13mg.Kg⁻¹ to more than 1000 mg. Kg⁻¹ depending on the variety (Santos et al. 2015). Being rich in carotenoids, these two ingredients were used in bakery products to improve carotenoid content [13]. Addition of Orange-Fleshed Sweet Potato flour in wheat flour during

biscuit formulation increased beta-carotene significantly in biscuit [14]. The same effect was reported when shortening was substituted by red palm oil in biscuit formulation [15]. However, carotenoid is more sensitive for its degradation during processing. The retention or loss of carotenoids during processing and storage of food has been reported in numerous studies [16-17]. The approach in this study was to produce OFSP flour from OFSP root, then to replace wheat flour in biscuit by OFSP flour and margarine by crude palm oil in order to increase carotenoid contents. The objective was to develop biscuit with high carotenoid contents meanwhile to evaluate the carotenoid retention levels in OFSP flour and in biscuits, with regard to the high carotenoid degradation during processing.

Material and Methods

The experimental studies were carried out in the laboratory of food science at the Faculty of Engineering, Eduardo Mondlane University (UEM), Maputo, Mozambique.

Procurement of raw materials

The Orange Fleshed Sweet Potato (unknown variety) and others ingredients like wheat flour, margarine, sugar, salt, baking powder were purchased in local markets (Maputo city) and brought immediately to the laboratory. Palm oil was brought from Kisangani, Democratic Republic of Congo, and kept in a well-sealed plastic box.

Orange-Fleshed Sweet Potato (OFSP) Flour preparation

OFSP flour was processed according to the method reported by Silva et al. [18]. The Orange-Fleshed Sweet Potato (OFSP) (*Ipomea batata*) tubers were cleaned and washed with tap water to remove any adhering soil and dust. The tubers were then mechanically peeled using a stainless kitchen knife and immersed in chlorinated solution (200 ml of sodium hypochlorite in 10 litres of water) for 10 minutes, then washed with distilled water. After that, the peeled OFSP was cut in 2-3mm thickness shape to facilitate drying and milling. Two methods of drying were used: sun drying for three days (depending on the weather condition) and oven drying at 60°C for 18 hours. After the drying process, the samples were milled into flour by a laboratory grinder (Retsch GmbH, 36 42781, made in Germany) and passed through 250µm mesh sieve to obtain flour of uniform size. The flour was then packed in black plastic bags and stored at ambient temperature till further used.

Preparation of wheat-OFSP flour

The wheat-OFSP flour composites were prepared at different ratio (A = 100% wheat flour, B= 70% wheat and 30% OFSP flour and C= 50% wheat and 50% OFSP flour). including other ingredients which were formulated and weighed accurately as shown in table 1.

Table 1. Ingredients for biscuit formulations

Ingredients	Formulations (g)			
	A0	A	B	C
Wheat/OSP flour	100	100	100	100
Crude palm oil	-	10	10	10
Margarine	30	20	20	20
Baking powder	1	1	1	1
Sugar	15	15	15	15
Salt	0.5	0.5	0.5	0.5
Water ml	Variable	Variable	Variable	Variable

A0: Control= 100% Wheat Flour biscuit with 30g margarine without crude palm oil; A: biscuit A =100% Wheat Flour+10g of crude palm oil and 20g of margarine; B: biscuit B =70% Wheat Flour + 30% OSP Flour+10g of crude palm oil and 20g of margarine; C= biscuit C: 50% Wheat Flour + 50% OFSP Flour+10g of crude palm oil and 20g of margarine.

The carotenoid contents in raw materials are shown in the table 2

Table 2. Carotenoid content in raw materials

Ingredient	Carotenoid (µg/100g)
Red palm oil	72023,4µg
OFSP flour	25012,3 µg
Wheat flour	No determine
Margarine	No determine

Biscuit preparation

Biscuits were prepared using the method described by Gernah et al [19] with small modifications. Fat, sugar and salt were mixed together for 3 minutes, using a mixer (Kenwood chef Serial: 0662544). After that, the flours thoroughly mixed with baking powder was added and mixed for further 7 minutes to form dough. The dough was then rolled to a uniform thickness (4-5mm) on a rolling board and cut into a uniform diameter using a biscuit cutter. The batter was shaped and baked in an oven (MACADAMS CONVECTA, 8 MR11-01314-11/2008, made in South Africa) at 150⁰C for 20 minutes. The samples were then removed from the oven, allowed to cool on a rack, packaged in black polyethylene bags and stored at room temperature [19].

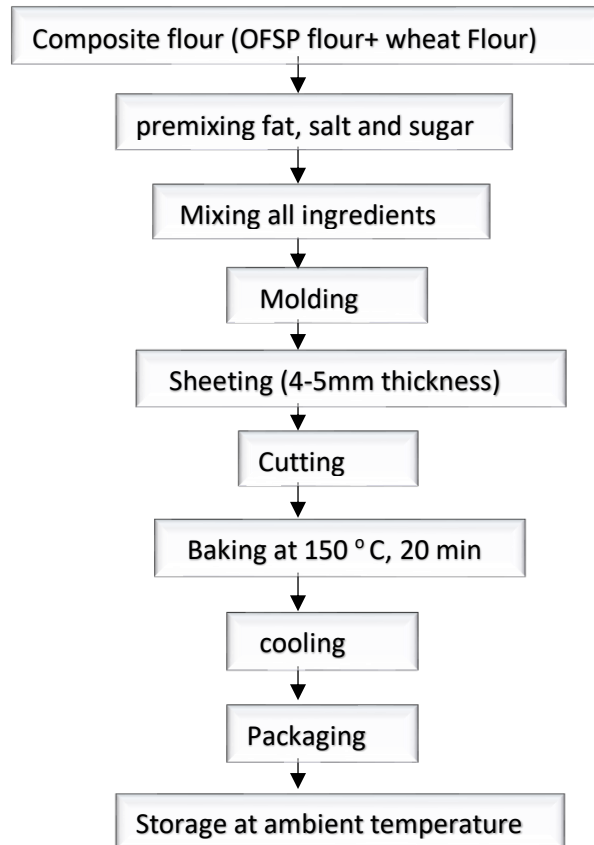


Figure 1: flow chart for the preparation of Biscuits

Proximate composition determination

Determination of Moisture content

Moisture was determined according to the Association of Official Analytical Chemistries (AOAC, 2000). About 5 g of samples were weighed and transferred to the pre-weighed crucibles. The crucible and their contents were placed in the drying oven for 3 hs at 105°C, then the crucibles were removed from oven and cooled in desiccators to room temperature and reweighed. All the results were in triplicate.

The results were expressed according to the formula 1.

$$\% \text{ Moisture} = \left(\frac{\text{weight wet sample} - \text{weight of dry sample}}{\text{weight of wet sample}} \right) \times 100 \quad (1)$$

Determination of Protein

Protein analysis was carried out using Kjeldhal method (AOAC, 2000). About 0.1g of pre-dried Orange-Fleshed Sweet Potato samples were taken in a Kjeldahl flask (tubes) and 15ml of concentrated sulfuric acid was added, mixed, thoroughly with potassium sulfate as a boiling point raising agent and selenium as a catalyst. Digestion rack with Kjeldahl tubes were placed in the digestion block and covered. The temperature of the digester was adjusted to 420°C and

digested for 45 minutes. After digestion, the solution obtained was taken off and allowed to cool to ambient temperature. About 70ml of distilled water was added to dilute the solution, and shaken, to avoid precipitation of sulfate in the solution. A conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. then 15ml of digested and diluted solution was transferred into the sample compartment of the distiller. A 30ml of sodium hydroxide solution (40%) was added into the compartment containing the sample solution and the steam switched on. The distillation was continued until a total volume of 150ml is collected. The distilled solution was then titrated with 0.1N hydrochloric acid by colorimetric endpoint to green to blue colour. All the results were in triplicate and expressed according to the formula 2.

$$\% \text{ Protein} = \%N * 6.25 \quad (2)$$

Determination of Carbohydrate

Carbohydrate (CHO) was determined by difference and the results were expressed according to formula 3.

$$\% \text{ CHO} = 100 - (\text{Moisture} (\%) + \text{Ash} (\%) + \text{fat} (\%) + \text{protein} (\%) + \text{fiber} (\%)) \quad (3)$$

Determination of Energy

The value of the energy in the biscuits was estimated considering the conversion factors of Calory, according to the formula 4.

$$\text{Calorie (Kcal/100g)} = (4 \times \text{HCO}) + (4 \times \text{protein}) + (9 \times \text{fat}) \quad (4)$$

Determination of total Ash

The ash content was determined according to the AOAC (2000) method. About 5g of the samples was accurately weighed into clean, dry, weighed and tared silica crucible (W1). The initial ashing was carried out over a low flame to char the sample. The crucible was then transferred to a muffle furnace maintained at 500-550°C to ignite for about 18 hours to get ash. The crucible was then cooled until a constant weight (W2) was obtained and expressed as g/100 g of sample. The formula 5, was used to express the percentage of total Ash. All the results were in triplicate.

$$\% \text{ Ash content} = \frac{(W1 - W2) \times 100}{\text{Weight of sample}} \quad (5)$$

W1 = Weight of sample + crucible before ashing (g)

W2 = Weight of sample + crucible after ashing (g)

Determination of Fat

Fat was determined by Soxhlet method AOAC (1990). About 5 g of sample was added into the extraction thimbles, and then covered with layer of fat free cotton. The thimble with the sample was placed into soxhelt extraction chamber. The cooling water was switched on, and an adequate amount of petroleum ether (100ml) was added to the extraction flask and connected to extraction tube, then to condenser. The tap water was opened to flow through the condenser throughout the extraction process. The extraction was conducted for about 4 hours. After counting 7 refluxes, the flask was removed and placed in oven at 105⁰C for about 1h, for complete evaporation of the solvent and cooled to room temperature in the desiccators for about 30min and re-weighed. According to formula 6.

$$\% \text{Fat on dry weight basis} = \left(\frac{\text{weight of fat in sample}}{\text{weight of dried sample}} \right) \times 100 \quad (6)$$

Determination of Crude fiber

Crude fiber was determined according to AOAC (2000): About 1 g of sample was placed in beaker, 150ml of H₂SO₄ (0.128M) was added, and boiled/digested for 30 min. the digested sample was filtered through a filter paper using the vacuum pump and the residue was washed three times with hot water. A 150ml of KOH added to the resulting solution and boiled again gently for a further 30 min. the solution was than filtered and washed three times with hot water. The final residue with filter paper was transferred into a crucible, dried in the oven at 105⁰C for 2 h, and cooled for 30 min in desiccator and then weighed (W1). The crucible was transferred to a small muffle furnace and incinerated for 2h at 550⁰C. The crucible was cooled in the desiccators and weighed (recorded as W2). Formula 7 was used to determine the crude fiber.

$$\% \text{Crude fiber} = \left(\frac{W1 - W2 \text{ of ash}}{\text{Wt of sample}} \right) \times 100 \quad (7)$$

Carotenoid extraction and determination

Carotenoids extraction and determination in Sweet potato and Biscuits

Carotenoid extraction was carried out according to the method described by Rodriguez-Amaya and Kimura [20]. Fresh OFSP tuber was washed, peeled sliced and grinded. About 5g of grinding sample was carefully weighted and put in an extraction tube and homogenised with 50 ml of acetone (previously refrigerated for about 2 hours) for 30 minutes, centrifuged at 4000 rpm by a laboratory centrifuge (ThermoFisher, made in China, 2013) for one minute, then was filtrated.

Carotenoids in the biscuits and in OFSP flour were extracted in the same way after grinding three biscuit samples in a mortar using a pestle to get powder. About 3g was weighted and put in extraction tube followed by rehydration in 10 ml of distilled water for 30 minutes. Then about

20 ml of cold acetone was added and let stand for 15 min; the homogenized sample was centrifuged at 4000 rpm by laboratory centrifuge (ThermoFisher, made in China, 2013) for one minute and then filtrated.

The extraction from both fresh sample and the flour was repeated in the extraction tube using 50 ml of cold acetone, the process was repeated until there was no yellow colour in the residue. The total extract was transferred to a separating glass funnel (500 ml). The Partition between the aqueous phase and organic phase containing the carotenoids was achieved by the addition of 40 ml and 20 ml of petroleum ether (PE) for the fresh and the flour samples respectively. These were washed three times with 300 ml of distilled water then, the PE phase was collected in a volumetric flask which made up the total volume with including the petroleum ether (50 ml for the fresh sample and 25 ml for the flour). The analysis was carried out under low light conditions, using aluminium foil to cover the extract tubes to minimise carotenoids' oxidation.

Carotenoids determination

Total carotenoid was determined in both fresh and flour by UV-visible spectrometer using a spectrophotometer (Agilent Technologies No: G6860A, made in Malaysia) to measure the absorbance at 450 nm in a glass cuvette using PE as the blank. The total carotenoid content was calculated using the formula 8.

$$\text{Total carotenoid content}(\mu\text{g/g}) = \frac{A \times \text{volume}(\text{mL}) \times 10^4}{A_{1\text{cm}}^{1\%} \times \text{sample weight}(\text{g})} \quad (8)$$

Where A= absorbance; volume=total of extract (50 or 25 mL); $A_{1\text{cm}}^{1\%}$ = absorption coefficient of β -carotene in PE (2592); multiplied by 100 to give the carotenoid content in $\mu\text{g}/100\text{g}$.

Carotenoid determination in crude palm oil

The carotenoids content in crude palm oil (CPO) was determined according to the Palm Oil Research Institute Malaysia (PORIM) test method reported by Nokkaew et al.[21]. The method was developed by Swoboda [22]; About 0.1g of CPO was weighed and dissolved in 25 ml of n-hexane solvent. The solution sample was placed in a 1 cm wide cuvette and absorbance measured at 466 nm using a spectrophotometer (Agilent Technologies No: G6860A, made in Malaysia) and n-hexane as blank. The carotenoid contents were calculated using the formula n°10.

$$\text{Carotenoids (ppm)} = \frac{383 \times A_{446} \times v}{w \times 100} \quad (10)$$

Where: A_{446} = absorbance at 446nm; 383= diffusion coefficient; V= value of hexane (ml) and W= weight of CPO sample (g)

Carotenoid retention

Retention of carotene was calculated using simplified formula n° 11 and 12 of the true retention in OFSP flour and in biscuits respectively, assuming that the dry matter content was constant Chijioke et al [23] and De Moura et al [16].

$$R (\%) = \frac{\text{carotenoid content in dry matter of flour}}{\text{carotenoid content in dry matter of fresh}} \times 100 \quad (11)$$

$$R (\%) = \frac{\text{carotenoid content in dry matter biscuit}}{\text{carotenoid content in mixed ingredient}} \times 100 \quad (12)$$

Sensory evaluation

The acceptability of the biscuits was evaluated using a 9-point hedonic scale [24]. Fifty untrained judges comprising of staff and students of the Faculty of Engineering, University Eduardo Mondlane were used for the evaluation of the quality parameters of the biscuits (color, taste, flavour, and the overall acceptability). They were requested to indicate their preference using the panelist method for a nine-point hedonic scale with 1 and 9 representing disliked extremely and liked extremely, respectively.

Statistical Analysis

All analytical determinations were conducted in triplicate. Mean \pm standard deviation (SD) values were calculated and the data were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 20 and the Analytical software Statistics 10. If a significant *F*-test was noted, significance was accepted at $P < 0.05$. Results were expressed as the mean value \pm standard deviation of triplicate determinations.

Results and Discussion

Proximate composition in fresh Orange-Fleshed Sweet Potato and flour

The results showed a high moisture content in the fresh sample: $79.97 \pm 0.26\%$ (20.03% dry matter), $10.98 \pm 0.03\%$ in oven dried flour and $12.41 \pm 0.47\%$ in sun dried (table 3). The moisture ranged between 58.7 to 80.8% (18.8 to 34.6% of dry matter) were reported in sweet potatoes by Hagenimana et al [26]. Moreover, the dry matter in OFSP cultivar in Gaza province (Mozambique) ranged from 20.1% and 27.2% were reported by Mazuze [27]. These results are in agreement with the results found in this study. In addition, the moisture content in sun and oven-dried flours were within the values reported of OFSP flour by Olatunde et al [28].

It was stated that more factors could influence moisture content in sweet potato, including genetic composition, cultivation practices and the application of fertiliser Takayata [30]. Fat content was $0.52\pm 0.03\%$, $1.51\pm 0.05\%$ and $1.96\pm 0.04\%$, respectively in fresh, sun-dried and oven-dried (table 3). This result was similar to other study findings [31-32]. Sweet potato is poor in fat as other roots and tubers.

Crude protein was $1.27\pm 0.07\%$, $4.57\pm 0.17\%$ and $5.24\pm 0.63\%$, respectively for fresh, sun-dried and oven-dried flour. Similar protein content in sweet potato ranged between 1.91 to 5.83% were reported by Alam et al [31] while Silva et al.[18], observed the highest protein content (10.45%) in sweet potato flour. The protein content in sweet potato is more variable depending on the variety, and fertilizer practice [33]. Protein content in diets of the low-income population in developing countries is derived mostly from plant origin.

The crude fiber was $1.26\pm 0.57\%$, $3.62\pm 0.85\%$ and $3.80\pm 0.66\%$, respectively, for fresh, sun-dried and oven-dried flours. This finding result was high than the crude fiber reported in fresh sweet potato by Alam et al [31], but was in accordance with crude fiber stated in sweet potato flour according to Olatunde et al [28]. Dietary fiber has recently received much importance as it is believed to reduce the incidences of colon cancer, diabetes, heart diseases and certain digestive diseases. Ash content was $0.53\pm 0.02\%$, $2.62\pm 0.04\%$ and $2.86\pm 0.01\%$, respectively, for fresh, sun-dried and oven-dried flour. This result was in the range of other reported ash content in OFSP tuber and flour [18, 28, 32]. The ash content represents the minerals in the foodstuff. It was stated that the ash content in OFSP can be influenced by some factors, like soil, climatic conditions and sweet potato variety [31].

Carbohydrate obtained in this study was 16.45%, 75.07% and 75.16%, respectively for fresh, sun-dried and oven-dried flour. Carbohydrate content in fresh sample was lower than that reported in other study [33]; the reason could be the high moisture content in OFSP analysed which decrease the dry matter content and thus, less carbohydrate. However, after drying, the flour showed high carbohydrate, which was in the reported range (74.55 and 90.54%) in sweet potato flour [28]. Sweet potato is the main staple food crops of the people and contribute up to 90% of the people diet in some countries. It can sustain the growing population for many generations [34]. The energetic value of fresh sweet potato was low (75.56 Kcal/100g) due to the high moisture content, and low-fat content which decrease considerably the energy value. This property may be explored by a hypo-energetic diet to fight against obesity. On the other hand, the energetic value increased significantly in the flour, 339.24 and 332.15 Kcal/100g,

respectively, for the oven and sun-dried methods. Processing OFSP crops to flour is a good way to minimize post-harvest losses making them more stable intermediate product to increase the utilization. The flour can be added to bakery products including breads and biscuits [35].

Table 3. Proximate composition of the fresh sweet potato samples

Proximate composition	Treatment of sample		
	Fresh	Oven drying	Sun drying
Moisture (%)	79.97 ± 0.26	10.98±0.03	12.41±0.47
Fat (%)	0.52±0.03	1.96±0.04	1.51±0.05
Crude fiber (%)	1.26±0.57	3.80±0.66	3.62±0.85
Ash (%)	0.53±0.02	2.86±0.01	2.62±0.04
Crude Protein (%)	1.27±0.07	5.24±0.63	4.57±0.17
Carbohydrate (%)	16.45	75.16	75.07
Gross energy (Kcal/100g)	75.56	339.24	332.15

Values are mean ± standard deviations of three (n = 3)

Carotenoid content in Orange-Fleshed Sweet Potato and retention

In table 4, the total carotenoid contents in the fresh OFSP, the flours and its retention can be observed. The result showed the mean carotenoid content to be 5525.17±98µg/100g, 15970±56 µg/100g and 22287± 58 µg/100g (wet basis) respectively in the fresh, sun-dried and oven-dried sample. In dry matter basis it was 27584.61±21µg/100g, 18232.67±12µg/100g, and 25036.02±14 µg/100g, respectively, for the fresh OFSP, sun-dried and oven-dried flour and were significantly higher than that reported in wet basis (p<0.05). This difference could be due to drying effect which decreases the moisture content significantly and increases dry matter. These values are similar to those found by other researchers in fresh wet basis OFSP: 5310µg/100g Tumwegamire et al [36], ranged between 0.38 to 7.34 mg/100g Alam et al [31]; and between 0.01 and 26.6mg/100g Takahata and Noda [29]. Growing conditions, stages of maturity, harvesting and post-harvest handling, processing, air and soil temperature, radiation, soil moisture and fertilization, storage and variety are such, the main factors that may influence variation of β-carotene content in orange-fleshed sweet potato [20,33,37]. The more predominant carotenoid in OFSP is the beta-carotene [11,38]. Being excellent source of this provitamin A carotenoid, OFSP consumption could make a major contribution to controlling vitamin A deficiency in many developing countries especially in Sub-Saharan Africa [39].

The carotenoid retention was significantly high in oven-dried than sun-dried; 90.76% and 66.09% respectively ($p < 0.05$). Cooking and processing have a degrading effect on β -carotene content. Sun drying was observed to retain 63-73%, oven drying 89-96%, boiling 84-90% and frying 72-86% of β -carotene in OFSP varieties studied [17]. The influence of different procedures on carotene have been reported in sweet potato [38], in cassava [40], and in carrots [41]. Retention is defined as the proportion of carotenoids remaining in the processed sweet potato root in relation to the amount of carotenoids originally present in the sweet potato [38]. Although the cheapest and accessible mean of food preservation in poor regions, sun-drying, causes considerable carotenoid destruction. The alteration or loss of carotenoids during processing and storage of foods occurs through removal (e.g., peeling), geometric isomerization, and enzymatic or non-enzymatic oxidation [20].

Table 4. Carotenoid content in orange sweet potato and retention

Parameter	Moisture	Carotenoid	Carotenoid	Carotenoid	Carotenoid
Sample	(g/100g)	$\mu\text{g}/100\text{g}$ (w.b)	$\mu\text{g}/100\text{g}$ (d.b)	loss (μg)	Retention %
Fresh	79.97 ± 0.26	5525.17 ± 98^c	27584.61 ± 21^a	-	100
Oven drying	10.98 ± 0.03	22287 ± 58^a	25036.02 ± 14^b	2548.59^a	90.76
Sun drying	12.41 ± 0.47	15970 ± 56^b	18232.67 ± 12^c	9351.94^b	66.09

Means \pm standard deviation with different superscript letters in the same column are significantly different from each other ($p < 0.05$) w.b= wet basis; d.b= dry basis

Carotenoid content in crude palm oil

Carotenoids in palm oil was $72023,4 \pm 4 \mu\text{g}/100\text{g}$ (720.23ppm) (table 2). The similar carotenoid content was reported ranged from 500 to 800 ppm and 1000-1600 ppm in palm oil, respectively, from Zaire (DRC) and Cote d'Ivoire and Benin [42]. However, variable carotenoid content, from 13mg.Kg^{-1} to more than 1000mg.Kg^{-1} were reported by Santos et al [12]. Palm oil possesses several characteristics that are important in determining its incorporation into food products: it has high solid-glyceride content, giving the required consistency without hydrogenation. It is very resistant to oxidation and therefore has a long shelf life. Palm oil has good oxidative stability due to the presence of natural antioxidants (tocopherol and tocotrienols) and the absence of linolenic acid. Comparatively to other oils, it is cheap to use and produces fried food products with good flavor and long shelf life [43].

Result of biscuits

Figure 2 shows biscuits produced from the experiment alongside their respective formulations

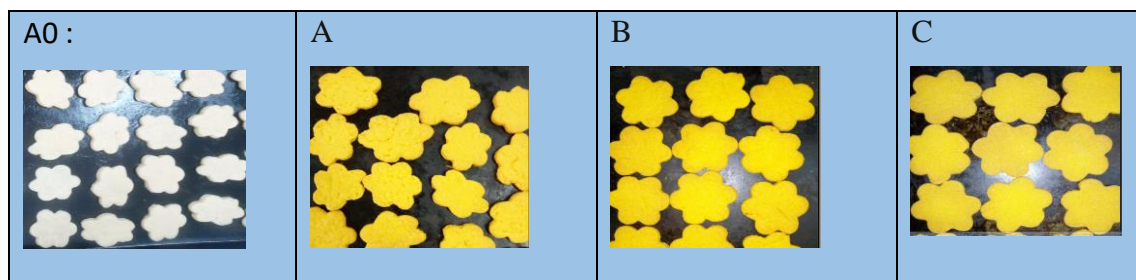


Figure 2. biscuits samples for analysis. A0= Control (100% wheat+30g margarine) ; A= (100% wheat +10g CPO+20g margarine); B= (70% wheat +30% OFSP+10 CPO+20g margarine); C= (50% wheat+50% OFSP+10g CPO+20g margarine).

Proximate composition

In the table 6, related to the proximate composition of biscuits, a significant change in the values of moisture, crude protein, crude fiber, ash and fat was observed ($p < 0.05$). However, no significant difference was found between the control (A0) and the formulation A where one-third of margarine was substitute by palm oil. The moisture content was between 3.21 ± 0.25 to $4.37 \pm 6\%$ and decreased significantly with OFSP flour incorporation in the biscuit formulation. This effect was stated by many researchers [35, 44-45]. This effect could due to the increasing fiber content in the formulation from OFSP flour which decreased the water retaining capacity of biscuits after baking [19], and the difference in the flour structure and water holding capacities [45-46]. However, lower moisture content in biscuits (1.329 % to 1.369%) and the higher (4.3 to 5.9%) were reported respectively by Srivastava et al [47] and Songre-Ouattara et al [48]. The biscuit with low moisture content has a longer shelf life since many chemical reaction and microbial activity depend on the water content.

The protein content ranged between 4.83 ± 0.03 to $6.35 \pm 0.05\%$. The substitution with OFSP decreased significantly the protein content in biscuits ($p < 0.05$). This result was in accordance with the reported protein (5.6 to 6.3%) in biscuit from OFSP and sorghum flour [48], and in biscuit from Cassava starch, OFSP puree and cowpea flour (5.14 to 7.21%) [49]. However, the lowest protein content in biscuit (4.50 to 4.70%) and the highest (8.84-14.5g) were reported by Silva et al [50], and Jumirah [13], respectively. The difference in protein content observed in this study could be due to the low protein content of OFSP which decreased significantly the protein in the treatment B and C compared to the control. Proteins are involved in numerous

physiological function. Protein intake is particularly important in childhood, during the period of when rapid growth which requires amino acids to build new tissues, its deficiency lead to protein-energy malnutrition in children. Hundred grams of these biscuits provide about 33.3% (biscuit C) to 43.8% (biscuit A) of dietary allowances of protein for children between 1-3 years old. The recommended protein intake per day being 14.5g according to WHO reported by [51].

Crude fiber ranged from 0.59 ± 0.07 to $1.55 \pm 0.04\%$ and ash from 1.25 ± 0.04 to $2.15 \pm 0.02\%$. The similar crude fiber (0.33 to 0.91%) and ash (0.92 to 1.50%) in biscuit from purple sweet potato flour and wheat flour were reported [35]. Moreover, Kolawole et al [52], found crude fiber between 1.01 to 3.68% and ash 1.27 to 3.70% in other biscuits. The difference of crude fiber and ash contents in biscuits could be due to OFSP substitution in the blend. In fact, the different levels of substitution of sweet potato flour were found to increase significantly for both crude fiber and ash content in biscuits made from sweet potato flour and wheat flour [47], and in biscuit from OFSP and sorghum flour [48]. Ash content is an indication of the availability of minerals, which are very essential in the normal functioning of the body, since they are involved in many biochemical reactions [19].

The fat content found in experiment biscuits was between 18.66 ± 0.56 to $22.48 \pm 0.27\%$, and increased significantly with OFSP flour substitution ($p < 0.05$). Data collected from numerous researches on biscuit have shown that, the fat content in biscuits ranged from 9.72-26.69% [35, 48, 49, 52]. The difference in fat content of biscuits may be attributed to the fat ability retention of OFSP flour when comparing with that of wheat flour during baking process. the higher fat retention may improve the mouth feel and retains the flavor of biscuits [35].

The results showed the mean of the carbohydrate content ranged from 65.09 to 68.81% and was in the range of reported carbohydrate in numerous studies [18, 48, 49, 52]. The control (A0) biscuit and the formulation A, exhibited high carbohydrate contents (68.78 and 68.81%) than the formulation B and C (67.88 And 65.89%). This difference could be due to the effect of substitution of OFSP flour which increased ash, crude fiber and fat contents, as a result decreasing of carbohydrate. A similar finding was also stated when biscuit was produced from blends of wheat and sesame flour [19].

The energy content ranged significantly between 468.46 to 489.33 Kcal/100g (table 5). The similar energy content (469.0 to 474.6 Kcal) was reported in biscuit from OFSP and sorghum flour [48]. Energy is required for tissue maintenance, growth and physical activity. For young children, weight gain is a sensitive indicator of the adequacy of energy intake [51].

Biscuits are ready to eat food which is widely consumed nearly by all parts of the world due to its good nutritional quality, affordable cost and availability in different taste and longer shelf life [53]. Hundred grams of biscuits could cover more than one-third of total daily energy required (1230 Kcal) for children aged 1-3 years. The fortified micronutrient biscuits (BP100) had been used in DRC as food supplement for alleviation and prevention of protein-energy malnutrition by the national program of nutrition.

Carotenoid content in biscuits

The content of carotenoid for all biscuits varied significantly between 0.96 $\mu\text{g/g}$ for the control biscuit and 146.89 $\mu\text{g/g}$ for the biscuit C according to the treatments (figure 3). The similar lowest β -carotene (0.54 $\mu\text{g/g}$) was reported in the control biscuit made from wheat flour and shortening [45]. It was stated that β -carotene is only a trace constituent in wheat and other grains [9]. A significant relationship was observed ($r = 0.98$, $P < 0.007$) between different treatments. Crude palm oil incorporation showed an increase of carotenoid from almost 1 μg in the control up to 55.77 μg in treatment A; with 30% of OFSP flour substitution, the carotenoids increased significantly up to 115.68 μg and till 146.89 μg with 50% of substitution. Similar increasing of beta-carotene content (14.93 to 17.28 mg/100g) was observed in sweet potato biscuits and tempeh substituted by red palm oil [13]. In addition, substitution of shortening by red palm oil in biscuit, increased the carotenoid from 25 $\mu\text{g}/10\text{g}$ to 853.52 $\mu\text{g}/10\text{g}$ [54].

Substitution of wheat flour by OFSP flour or butter by palm oil is among the strategies used to produce enriched carotenoid food products but, in most cases, they are used separately. It was stated that, incorporation of 15 to 45 % sweet potato yielded approximately similar results compared wheat flour biscuits with improved nutritional value, texture and overall acceptability, but at 60% of incorporation, the sensory attributes score decreased [55-56]. On the other hand, biscuit made by replacing white shortening by 40% of red palm oil produced significantly superior biscuit based on overall acceptance [15,57]. In this experiment, a composite wheat-OFSP flour (30%) with substitution of margarine by about 30% of crude palm oil appears to be more efficient to produce biscuits with high carotenoid content and good acceptability than these two ingredients used separately.

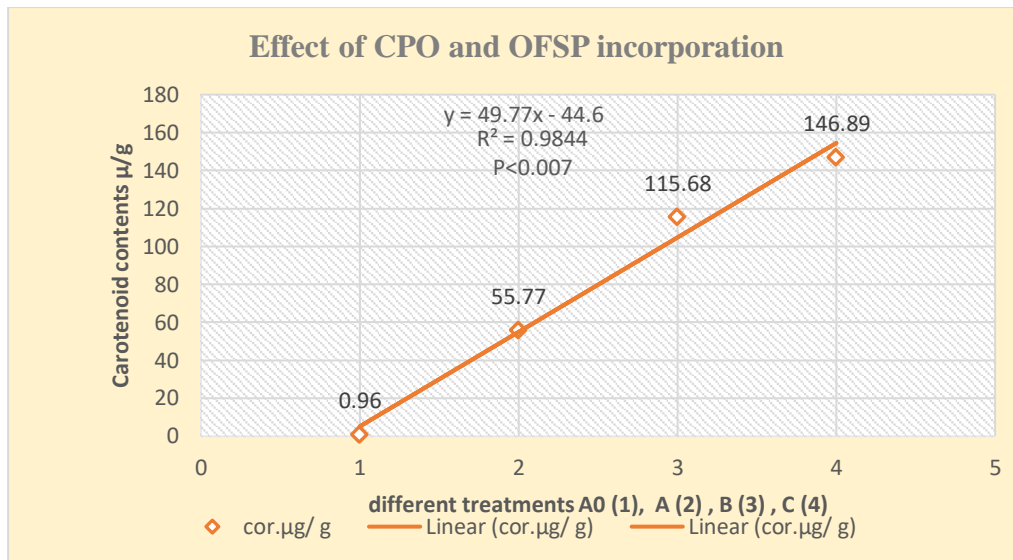


Figure 3. Carotenoid contents in biscuits

Carotenoid retention in biscuits

The carotenoid retention in biscuits was 77.43% in formulation A, 78.62% in formulation B and 74.48% in formulation C. This result was in accordance with carotenoid retention reported in different food items subjected to different types of heat treatment; total retention of carotene ranged from 69 to 86% and β -carotene from 70 to 88% [58]. The formulation C which was more concentrated in carotenoid, exhibited significantly less retention (about 25.52% loss) compared to the formulation A and B (table 5). Similar observation was stated by Ahmad and Idris [59], in cake baked at 180°C. The blends which contained at least 30% red palm oil retained highest carotene (78.9%) compared to (71%) for the blends with 50, 70 and 100% of red palm oil. Since palm oil is thoroughly blended with other ingredients, thereby avoiding direct exposure to heat, which increase carotene retention after cooking [58]. It was reported that destruction of carotene was lower when the initial concentrations were low [60].

Table 5. Carotenoid retention in biscuits

Formulations	Carotenoids in mixed ingredients ($\mu\text{g}/100\text{g}$)	Carotenoid after baking ($\mu\text{g}/100\text{g}$)	Carotenoid retention (%)	P-value
A	7202.34	5576.8 ± 65	77.43	0.0413*
B	14713.15	11568 ± 111	78.62	
C	19720.37	14689 ± 18	74.48	

*Fisher- test ($P < 0.05$)

A: biscuit A =100% Wheat Flour+10g of red palm oil and 20g of margarine; B: biscuit B =70% Wheat Flour + 30% OSP Flour+10g of red palm oil and 20g of margarine; C= biscuits C: 50% Wheat Flour + 50% OFSP Flour+10g of red palm oil and 20g of margarine;

Sensory evaluation

In this study, the mean sensory score was presented in table 6. The control (A0) and the formulation (A) recorded higher scores for colour, texture, flavour and acceptability with no significant difference between them. The attractive yellow colour observed in biscuit A and B could be due to the combined effect of red palm oil and OFSP flour which gave an orange colour and was probably improved by the Maillard reactions due to the baking temperature. The taste acceptability scores ranged between 6.29 ± 1.64 and 7.74 ± 0.81 with a significant difference between formulation C and other formulations ($p < 0.05$), which could be due to high OFSP flour incorporated into the formulation to give a slight sweet potato taste. The overall acceptability score of the biscuits was in the acceptable range (6.36 ± 1.44 to $7.63 \pm 0.94\%$). The result revealed that the flavor and the overall acceptability score decreased in the formulation C where 50% OFSP flour was the substitution. This could be due to the high amount of OFSP flour which is not familiar with most people as compared to other biscuits. The substitution up to 30% OFSP flour could be more beneficial since it increases the carotene content in the biscuit and was more acceptable to the consumers.

Table 6. Proximate composition and sensory evaluation of biscuits

Proximate compositions	Formulations			
	A0	A	B	C
Moisture (%)	4.37±0.6 ^a	4.32±0.26 ^a	3.33±0.04 ^b	3.21±0.25 ^c
Crude Protein (%)	6.35±0.05 ^a	6.33±0.11 ^a	5.67±10 ^b	4.83±0,03 ^c
Crude fiber (%)	0.59±0.07 ^c	0.58±0.05 ^c	0.91±0.04 ^b	1.55±0.04 ^a
Ash (%)	1.25±0.04 ^c	1.26±0.34 ^c	1.56±0.04 ^b	2.06±0.02 ^a
Fat (%)	18.66±0.56 ^c	18.70±0.27 ^c	20.65±0.28 ^b	22.46±0.25 ^a
Carbohydrate (%)	68.78	68.81	67.88	65.89
Energy (Kcal/100g)	468.46	468.86	480.05	487.35
Sensory parameters				
Color	7.68±0.89 ^a	7.12±1.65 ^{ab}	6.88±1.52 ^{bc}	6.44±1.35 ^d
Taste	7.74±0.81 ^a	6.94±1.23 ^{bc}	7.12±1.30 ^b	6.29±1.64 ^d
Flavor	7.48±1.14 ^a	7.03±1.24 ^{abc}	7.09±1.26 ^{abc}	6.46±1.69 ^c
Overall acceptability	7.63±0.94 ^a	7.23±1.03 ^{ab}	7.24±1.13 ^{ab}	6.36±1.44 ^d

*Means ± standard deviation with different superscript letters in the same raw are significantly different from each other (p<0.05) A0: Biscuit control= 100% Wheat Flour biscuits with 30g margarine without red palm oil; A: biscuit A =100% Wheat Flour+10g of red palm oil and 20g of margarine; B: biscuit B =70% Wheat Flour + 30% OSP Flour+10g of red palm oil and 20g of margarine; C= biscuits C: 50% Wheat Flour + 50% OFSP Flour+10g of red palm oil and 20g of margarine;

Conclusion

It was found in this study that, the inclusion of orange fleshed sweet potato significantly affected the carotenoid content in the end product. Carotenoid retention was significantly higher in the oven-dried flour than that of the sun-dried. Suggesting that, the sun drying method of OFSP, though a more accessible method of flour, results in a high loss of carotenoid. The carotenoid retention in biscuits was correlated to the blend concentration in ingredients before baking.

Using composite wheat-orange sweet potato flour and crude palm oil in biscuit formulation appears to be more efficient than OFSP flour or palm oil alone. Due to the high carotenoid content and high acceptability especially at 30% for both OFSP flour and palm oil substitution. The treatment A and B showed no significant difference with the control, but that of treatment C recorded a lower score in all parameters analysed including the overall acceptability.

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