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Production of Beta-Carotene Rich Biscuits from Composite Wheat-Orange-Fleshed Sweet Potato Flour, Palm Oil and Eggs

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ABSTRACT

One of the main micronutrient deficiencies in children under five years is vitamin A deficiency (VAD). This study aimed to produce biscuit from composite wheat-orange-fleshed sweet potato flour (OFSP), palm oil and eggs and to determine its contribution to the diet of children on the age group of 1-3 years. For the biscuit formulations codified ''AE, BE and CE'' were prepared by partial replacement of wheat with OFSP flour in proportion at 0%, 30% and 50% respectively, and margarine by crude palm oil 1/3 (33%), this combination was supplemented by 20g of egg. While the control biscuit codified A0, was prepared using 100g of wheat flour and 30g of margarine. The carotenoid contents of the prepared biscuit were determined by using the method describe by Rodriguez Amaya and Kimura (2004). The analysis of variance was used to determine the significant difference in the samples with a significant level of 0.05.

Results of the study revealed that the carotenoid content in the biscuits ranged from 96.03 µg/100g to 15251 µg/100g (86.82µg/100g to 12200.8µg/100g of beta-carotene) and the Retinol Activity Equivalent (RAE) was reported from 6.4 to 1016.7µg RAE/100g in the control and the formulation CE respectively. The protein content ranged from 5.48 to 7.91%, and the energy level was reported from the 468.46 to 484.6 Kcal/100g in the biscuits. All biscuits were in the acceptable range (6.36 to 7.63) related to the sensory score.

From the results of study, biscuits formulation appears to be efficient, since it produced biscuits of high nutritional values within the RAE range which could prevent the VAD in young children since 2 to 5 portions of biscuits could cover the retinol equivalent for children aged 1-3 years- old.

Keywords
Biscuits, Orange-Fleshed Sweet potato, palm oil, beta-carotene, Children 1-3 years.

Introduction
Hidden hunger, a challenging public health concern in Sub-Saharan regions, is prevalent in children under five years and contribute to poor growth, intellectual impairments, and increased risk of morbidity and mortality [1]. Vitamin A deficiency (VAD), one of the main micronutrient deficiencies in children under five years, affects about 190 million children under five (33.3% of the preschool age population) with about 5.2 million affected by night blindness [2]. In the Democratic Republic of Congo (DRC), the latest national investigation reported that 61.1% of the 6 to 36 months aged children were suffering from VAD in 1998 [3].

In Sub-Saharan Africa countries where most people are unable to get required preformed vitamin A due to the unaffordability of meat, provitamin A remains the main source of vitamin A [4]. Whereas in developed countries provitamin A account for only about 35% of dietary vitamin A [5], in developing countries provitamin A covers more than 80% of vitamin A needs [6].

Orange-Fleshed Sweet Potato (OFSP) is a potential source of dietary provitamin A, which may reach up to 20000 µg/100 g (wet-basis) depending on the OFSP variety [7]. On the other hand, the carotenoids content in crude palm oil ranged from 13µg/g to more than 1000µg/g depending on the variety of the palm oil tree [8]. Being rich in carotenoids, these two ingredients (palm oil and OFSP flour) were used in bakery products to improve carotenoid content [9].

Biscuit can be described as a dry bakery product made mainly from wheat flour, oil/fat with or without addition of other food ingredients [10]. However, biscuit quality depends on the type of ingredients and particular improvement required. The nutritional value of biscuit can be increased by fortification with nutritious ingredients rich in protein, vitamin and minerals [11].

In Democratic Republic of Congo, the complementary foods for young children are made mainly from cereals, which are low in essential nutrients, therefore contributing less to the nutritional needs of young children. In contrast, biscuits play a vital role in a food vehicle to fight against malnutrition. Fortified biscuits with minerals and vitamins were used in DRC as a therapeutic food for malnourished children under five years [3]. Biscuits are particularly well accepted by young children; they are directly consumed or can be rapidly transformed into flour for preparation of porridge.

The present study examined the effect of substitution of wheat flour with the OFSP flour and margarine with crude palm oil for the carotenoid contents in biscuit. Also, the contribution of biscuit in the diet of 1-3 years old children relating it to the energy and daily retinol equivalent (RE) supplied was also evaluated.

Material and Methods

Current study was carried out in the laboratory of food sciences and technology of the Faculty of Engineering, Eduardo Mondlane University (UEM), Maputo, Mozambique.

Procurement of raw materials

Palm oil was brought from Kisangani, Democratic Republic of Congo, and kept in a well-sealed plastic box. Other ingredients such as margarine, sugar, eggs, baking powder and wheat flour were bought from the local markets in Maputo.

The orange-fleshed sweet potato flour was made from the OFSP (*Ipomea batata*) tubers and then, oven dried and milled to flour. The proximate composition was 10.98% for fat, 1.96% for crude, 3.80% for fiber, 2.86% for ash, 5.24% for crude protein and 75.16 for carbohydrate [12].

Preparation of wheat-sweet potato biscuit

The wheat-sweet potato flour composites were prepared at different ratios and codified as **A** = 100% wheat flour, **B**= 70% wheat and 30% OFSP flour and **C**= 50% wheat and 50% OFSP flour. Other ingredients which were formulated and weighed are shown in table 1.

Table 1: Biscuit formulations with different ingredients.

	Formulations (g)			
Ingredients	A0	AE	BE	CE
Wheat/OSP flour	100	100	100	100
Crude palm oil	-	10	10	10
Margarine	30	20	20	20
Eggs	-	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)	20	15	15	15

A0: Control= 100% Wheat flour biscuits with 30g margarine without crude palm oil;
AE: Biscuit AE=100% Wheat flour+10g of crude palm oil and 20g of margarine + 20g of eggs;
BE: biscuit BE =70% Wheat+ 30% OFSP flour+10g crude palm oil and 20g of margarine +20 g of eggs; CE: biscuit CE=50% Wheat + 50% OFSP flour+10g of crude palm oil and 20g of margarine+ 20 g eggs.

Biscuit preparation

Biscuits were prepared by the method of Gernah et al. [13] with some modifications in baking time and basic formulations. Fat (palm oil and margarine), sugar and salt were mixed together for 3 minutes, using a mixer (Kenwood chefserial N°0662544). After that, the flours thoroughly mixed with baking powder. The dough was then rolled to uniform thickness (4-5mm) on a rolling board and cut into a uniform diameter using a biscuit cutter (Star-Shaped Steel made in China). The batter was shaped and baked in an oven (MACADAMS CONVECTA 8 MR11-01314-11/2008 made in South Africa) at 150°C 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 [13].

Measurement of the physicochemical properties of biscuit

Determination of Water activity:

Water activity (a_w) of the biscuit was determined by the previously calibrated water activity meter (AQUA Lab Serial 08038551B, USA). The measurement was done in triplicate to get reliable results [14].

Determination of color

Color of the biscuit samples was determined by the previously calibrated colorimeter device (CR-10, Minolta CO LTD, made in Japan). The biscuit sample, previously ground, was placed into a sample cup and the color was measured in triplicate. The CIE (International Commission of Illumination) system was used for color measurement using as L* – lightness, a* – hue on a green (-) to red (+) axis, b* – hue on a blue (-) to yellow (+) axis [14].

Weight Measurement:

The weight (in grams) of biscuits was measured by a digital scale (Dalco chromtech ACADAM®No, 17250), with max 250 grams.

Determination of Diameter

The diameter was measured in millimetre (mm) by digital calliper (ABSOLUTE DIGIMATIC, ref.3472, China).

Proximate composition determination

The AOAC [15] method determined the content of moisture, ash, crude fiber, fat and crude protein. and the difference determined the carbohydrate [16]. The gross energy was estimated using the relationship from fat, carbohydrate and protein contents of the Atwater's Conversion Factors, (4 Kcal/g) for protein, (4 Kcal/g) for carbohydrates and (9Kcal/g) for fat and expressed in Calories [17].

Carotenoid extraction and determination

Carotenoid extraction

Carotenoid extraction was carried out according to the method described by Rodriguez-Amaya and Kimura[18]. Three biscuit samples were ground in a mortar using a pestle to get fine powder, and then 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 to stand for 15 minutes; the homogenized sample was centrifuged at 4000 rpm by a laboratory centrifuge (ThermoFisher, made in China,2013) for one minute, then was filtrated. The extraction was repeated in the extraction tube using 50 ml of cold acetone, 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 20 ml of petroleum ether (PE), these were washed three times with 300 ml of distilled water before collecting the PE phase in a volumetric flask which made up the total volume including the petroleum ether (25ml). The analysis was carried out under low light conditions, using aluminium foil to cover the extract tubes to minimise carotenoids' oxidation.

Carotenoid determination and beta-carotene content

Total carotenoid was determined by UV-visible spectrometer using a spectrophotomer (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 1 described by Rodriguez-Amaya and Kimura [18] as showed below:

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

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

Among the all-analysed carotenoids content of the biscuits, 80% of all carotenoids are beta-carotene considering the previous published results on beta-carotene content in Orange-Fleshed Sweet Potato [7, 19] and in palm oil [20, 21].

Number of biscuits needed to cover the µg RE/day for children aged 1-3 years

Considering that previous studies on carotenoid found 25% (ranging from 11% to 48%) of bioaccessible fraction in the OFSP [22], and that the bioaccessible fraction of carotenoid in palm oil ranged from 33% to 75% [23], in this study, we considered the bioaccessible fraction of the beta-carotene in biscuit to be 0.25 (1/4) of total beta-carotene content. Therefore, the bioaccessibility of beta-carotene in biscuit was calculated as: Total beta-carotene of biscuit X 0.25. This formula was used to calculate the amount of biscuits needed to meet the daily retinol equivalent (RE) required for infant with vitamin A deficiency according to the formula 2 as describe by Burri [7].

$$\text{Biscuit per day (g/day)} = \frac{\mu g\ RE/day\ (400\ required\ for\ children)}{\mu g\ bioaccessible\ \beta-carotene/gram\ of\ biscuit} \quad (2)$$

For children with good vitamin A status, we used the Retinol Activity Equivalent (RAE) conversion factor 12 µg of β-carotene = 1 µg of trans-retinol [24]. The amount of biscuit required was calculated according the formula 3 as described by Burri [7].

The amount of biscuit per day (g/day) =

$$\frac{\mu g\ RE/day\ (400\ required\ for\ children)}{\mu g\ of\ \beta-carotene/gram\ of\ biscuit} x\ 12 \quad (3)$$

Sensory evaluation

The acceptability of the biscuits was evaluated using a 9-point hedonic scale [25]. Fifty untrained judges comprising of staff and students in the Engineering Faculty of University Eduardo Mondlane participated in the evaluation of the quality parameters of the biscuits (color, taste, flavour, and the overall acceptability). Judges 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 ± 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. When a significant *F*-test was noted, significance was accepted at $P<0.05$. Results were expressed as the mean value ± standard deviation of triplicate determinations.

Results and Discussion

Physicochemical property of biscuits

The weight of the experimental biscuits was between 6.61 ± 0.2g for formulation CE to 6.98 ± 0.1g for formulation A0 and AE (table 2). The results differed significantly among samples ($p<0.05$). These findings were similar to those of Van Toan &Thu [26] which were 6.07 to 6.62g reported on biscuits prepared from wheat substitute by sweet potato flour. The weight of biscuits decreased with increasing level of replacement of wheat flour by the OFSP flour.

The water activity in the biscuits analysed ranged between 0.27 ± 0.00 and 0.38 ± 0.00. The results differed significantly among samples ($p<0.05$). The lower a_w (0.22 to 0.26) were observed in biscuits made from wheat flour and red palm oil [27]. The difference in a_w found in this study could be due to the higher loss of water during baking of biscuit with sweet potato flour and red palm oil substitution. Biscuit is a baked product with moisture content less than 5% and low water activity which increases its shelf life [28].

The diameter of the biscuits ranged from 45.51 ± 1to 47.02 ± 0.1mm, these results are in accordance with values reported by Hadnađev et al. [28]. The regularity of biscuit diameter will assist in better and economical packaging size while an irregular one will cause the reject of a lot of products [29].

The color values of biscuits in this study was significantly different (p<0.05) for each sample (table 2): L* 40.10 ± 0.2 to 49.9 ± 0.6; a* from 4.86 ± 0.4 to 13.66 ± 0.23 and b* from 20.26 ± 0.7 to 50.66 ± 1. Only the formulation AE was not different from the control for the L* parameter. The substitution of wheat flour by the OFSP flour decreased significantly the L* values which indicate the brightness thus increase the darkness. Meanwhile the a* values, showing the redness increased. The b* values which indicate the yellowness of all biscuits supplemented with red palm oil and the OFSP flour were higher than that of the control. Since the red palm oil and the OFSP flour are rich in beta-carotene, they have an orange color. Moreover, the browning of the biscuit surface was induced by non-enzymatic browning reaction (Maillard reactions) which involves the interaction of reducing sugars with proteins, and also due to starch dextrinization and sugar caramelisation [28]. The similar results were observed in biscuits from sweet potato flour and wheat flour [30].

Proximate composition of biscuit

Except from the control biscuit (AO) and the formulation AE, the proximate composition of biscuits showed significant change in the values of moisture, crude protein, crude fiber, ash and fat with p<0.05 (Table 2). The moisture content was between 3.25 ± 0.14 to 4.37 ± 0.6%. However, in their studies, Srivastava et al. [31] found a lower moisture content in biscuits (1.329 % to 1.369%) and Songre-Ouattara et al. [11] found a higher moisture (4.3 to 5.9%). Moisture content decreased significantly with the increasing of concentration the OFSP flour in the biscuit formulation. Similar effects were also observed by Van Toan & Thu [26] and Amir et al. [32], and it could be due to the increasing fiber content in the formulation from the OFSP flour which decreased the water retaining capacity of biscuits after baking [13].

The protein content ranged from 5.48 ± 0.03 to 7.91 ± 0.61% and it was significantly different between various samples (p<0.05). However, Lusamaki et al. [12] reported lower protein contents (4.83% to 6.59%) in biscuit made from wheat-OFSP flour, crude palm oil and margrine. Similarly, Songre-Ouattara et al. [11] reported the similar proteins value (5.6 to 6.3%) in the biscuit supplemented by the OFSP and sorghum flour. However, Agbemafle et al. [33] reported high protein content (11,95%) in the porridge from the OFSP flour and soyabeans which was useful for children under five. The difference in protein content observed in this study could be due to the addition of whole egg in biscuit formulation as well as wheat flour substitution.

Protein intake is particularly important in childhood, during the period of rapid growth which requires amino acids to build new tissues. Its deficiency leads to protein-energy malnutrition in children. Hundred grams of these biscuits provide about 37.8% (formulation CE) to 54.8% (formulation AE) 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 Kim et al. [34].

The range of crude fiber was reported from 0.59 ± 0.05 to 1.55 ± 0.5% and ash from 1.25 ± 0.04 to 2.15 ± 0.01% and these were significantly different among various treatments. The similar range of crude fiber (0.33 to 0.91%) and ash content (0.92 to 1.50%) was reported by Van Toan & Thu [26] in the biscuit made from purple sweet potato flour and wheat flour. The observed difference of crude fiber and ash in different formulations could be due to wheat-OFSP flour substitution in the blend. The similar observation was reported by Srivastava et al. [31]. 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 [13].

Further, the fat content found in experiment biscuits was between 18.66 ± 0.6 to 22.48 ± 0.3%, and was in the range of 9.72-26.69% reported by Oduro et al. [35] and Kolawole et al. [36]. The difference in fat content of biscuits may be attributed to the fat ability retention of the OFSP flour when comparing with the wheat flour during the baking process. The higher fat retention may improve the mouth feel and retains the flavor of biscuits [26].

The results showed that the mean of the carbohydrate content ranged from 65.09 to 68.78% and was in the range of reported carbohydrate content in numerous studies [11,37]. The control, exhibited high carbohydrate contents (68.78 %) than the other formulations, probably due to the effect of substitution of the OFSP flour and addition of egg which increased ash, crude fiber and fat contents, as a result decreasing the carbohydrate contents.

The energy content ranged significantly between 468.46 to 484.6 Kcal/100g (Table 2). A similar energy content (469.0 to 474.6 Kcal/100g) was reported in biscuit from the OFSP and sorghum flour [11]. Energy is required for tissue maintenance, growth and physical activity. Weight gain is a sensitive indicator of the adequacy of energy intake for young children [34]. The affordable cost and availability in different taste and its longer shelf life make biscuits preferably ready to eat food which is widely consumed nearly by all parts of the world [38]. 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) have been used in DRC as food supplement for alleviation and prevention of protein-energy malnutrition by the national program of nutrition.

Table 2: Physical properties, proximate composition and energetic value of biscuit.

	Formulations			
Physical properties	A0	AE	BE	CE
Weight (g)	6.98 ± 0.1ª	6.98 ± 0.1ª	6.66 ± 0.1ᵇ	6.61 ± 0.2ᵇᶜ
Water activity	0.38 ± 0.00ª	0.28 ± 0.00ᵇ	0.27 ± 0.00ᶜ	0.27 ± 0.00ᶜ
Diameter (mm)	47.02 ± 0.1ª	46.99 ± 0.7ª	46.80 ± 0.3ª	45.51 ± 1.1ᵇ
Color				
L*	47.96 ± 3ᵃᵇ	49.9 ± 0.6ª	40.10 ± 0.2ᵈ	41.53 ± 1ᶜᵈ
a*	4.86 ± 0.⁴d	10.86 ± 0.5ᶜ	13.66 ± 0.23ª	12.70 ± 7ᵇ
b*	20.26 ± 0.7ᵈ	50.66 ± 1ª	40.1 ± 0.10ᶜ	40.63 ± 0.5ᵇᶜ
Proximate composition				
Moisture (%)	4.37 ± 0.6ª	4.37 ± 0.33ª	3.37 ± 0.1ᵇ	3.25 ± 0.14ᶜ
Crude Protein (%)	6.59 ± 0.05ᵇ	7.91 ± 0.61ª	6.57 ± 0.13ᵇ	5.48 ± 0.03ᶜ
Crude fiber (%)	0.59 ± 0.0ᵈ	0.61 ± 0.06ᵈ	1.01 ± 0.08ᵇ	1.55 ± 0.5ª
Ash (%)	1.25 ± 0.04ᶜ	1.25 ± 0.07ᶜ	1.64 ± 0.03ᵇ	2.15 ± 0.01ª
Fat (%)	18.66 ± 0.6ᶜ	20.46 ± 0.3ᵇ	20.66 ± 0.1ᵇ	22.48 ± 0.3ª
Carbohydrate (%)	68.78	65.40	66.75	65.09
Energy (Kcal/100g)	468.46	477.38	479.22	484.6

*Means ± standard deviation with different superscript letters in the same row are significantly different from each other (*P* <0.05)

A0: Controle =100% Wheat Flour+30g of margarine; **AE**: biscuit 100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **BE**: biscuit BE =70% Wheat + 30% OFSP Flour+10g crude palm oil and 20g of margarine +20 g of eggs; **CE:** biscuit CE: 50% Wheat + 50% OFSP Flour+10g of crude palm oil and 20g of margarine+ 20 g eggs.

Carotenoid contents in biscuits

As discussed in material and method section of this report, we assumed the beta-carotene of total carotenoid content in biscuit to be 80% as found in previous studies.

Table 3 presents the carotenoid content, beta-carotene and the retinal activity equivalent of biscuits. Observed data were significantly different between various formulations (*P* <0.05). Among the various tested combinations, carotenoid content ranged between 96.03 ± 0.46 μg/100g (86.82 μg/100g of β-carotene) for the control and 15251 ± 46 μg/100g (12200.8μg/100gof β-carotene) for the formulation CE. The lowest β-carotene (14 μg/100 g) and (0.54 μg/g) were found in biscuits by Heinonen et al. [39] and Afework et al. [16], respectively. According to Heinonen et al. [39] this lowest beta-carotene value is because of trace constituent of beta-carotene in wheat and other grains.

Incorporation of crude palm oil showed an increase of carotenoid from almost 96 μ/100g in the control up to 5613.3 ± 18μ/100g in formulation AE; and up to 15251 ± 46 μ/100g with 50% of wheat flour substitution (formulation CE). This could be due to the high carotenoid content in crude palm oil (720.23ppm) used in biscuit formulations [12]. Substitution of shortening by red palm oil increased carotenoids in biscuit from 25 μg/10g to 853.52 μg/10g [40]. Similarly, Lusamaki et al. [12] reported values ranged from 5576.8, 11568 and 14689μ/100g in biscuits with some level of replacement of margarine and OFSP flour. This suggests that, addition of 20g of whole egg in formulation does not significantly increase carotenoid content of biscuit even though egg contains some carotenoid.

It was stated that, incorporation of 15 to 45 % sweet potato flour yielded approximately similar results compared to wheat flour biscuits with a good score of overall acceptability, but at 60% of incorporation, the sensory attributes score decreased [41]. On the other hand, biscuits made by replacing margarine by 40% of red palm oil produced significantly superior biscuit based on overall acceptance [27].

The retinol activity equivalent (RAE) was established by United State Institute of Medicine (USIOM) to replace the retinol equivalent (RE) as a measure of the vitamin A activity of dietary provitamin A carotenoid [24]. One (1) μg RAE is defined as 1 μg of all-trans-retinol that is equivalent to 12 μg of all-trans-β-carotene and 24 μg of other provitamin A carotenoids (usually limited to α-carotene and β-cryptoxanthin) [24]. These are similar to retinol equivalencies (RE) calculated for fruits and vegetables (6 μgβ-carotene: 1 μg retinol and 12 alpha carotenes: 1μg retinol) [42]. The daily RAE requires for children 1-3-years being 300-400μg, 100g of biscuits could cover from 100% for formulation AE to more than 200% for the formulation CE. In this experiment, a composite wheat-OFSP flour (30%) with substitution of margarine by 33% (1/3) of crude palm oil appears to be more efficient for producing biscuits with high carotenoid content and a better acceptability than these two ingredients used separately.

Table 3: Carotenoids, Beta-carotene and RAE of the biscuits.

Formulation	Carotenoid μg/100 g	Beta-carotene μg/100 g	μg RAE /100 g (12 μg of β-carotene = 1 μg of trans-retinol)
A0	96.03 ± 0.46ᵈ	76.82	6.402
AE	5613.3 ± 18ᶜ	4490.64	374.220
BE	12001 ± 36ᵇ	9600.8	800.067
CE	15251 ± 46ª	12200.8	1016.733

*Means ± standard deviation with different superscript letters in the same column are significantly different from each other (*P* <0.05).

A0: Controle =100% Wheat Flour+ 30g of margarine; **AE**: biscuit 100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of eggs; **BE**: biscuit BE =70% Wheat + 30% OSP Flour+10g crude palm oil and 20g of margarine +20 g of eggs; **CE:** biscuit CE: 50% Wheat + 50% OFSP Flour+10g of crude palm oil and 20g of margarine+ 20 g eggs.

Amount of biscuit need to cover retinol equivalent require for children 1-3 years

The conversion ratio used in this study was dependent on the vitamin A status of the children. Many authors stated that well-nourished individuals, with good vitamin A status, convert less beta-carotene to vitamin A than poorly nourished people with low vitamin A status [43, 44]. Current study estimated that children with good vitamin A status had a retinol equivalency ratio of 12-μg beta-carotene: 1- μg retinol [24]. Poorly nourished women and children are likely to have a smaller retinol equivalency ration of perhaps 3-μg beta-carotene:1-μg retinol, which depends mainly on carotenoid bioaccessibility [22].

The amount of biscuit required to cover 400μgRE/day for a child aged between 1–3-year-old with VAD ranged from13g for the biscuit (formulations CE) (2 biscuits) to 36g for the (formulation AE) (5 biscuits) and from 39g for biscuit (formulation CE) (6 biscuits) to 108g for biscuit (formulation AE) (16 biscuits) for Well-nourished child (Table 4).

This high retinol equivalent shown in this biscuit is mainly due to mixed ingredient rich in carotenoids (palm oil and OFSP flour). In fact, palm oil has been used for many bakery products to improve the carotenoid content [9]. On the other hand, the OFSP is rich in carotenoids and has been widely used in bakery products. In southern India, the vitamin A deficiency was observed in poor communities where children 1-6 years old reported to have intakes of about 100-200µg RE/day. These signs were relieved and risk of mortality was reduced when the equivalent of 350-400 µg RE/day was given to children weekly [46]. Moreover, in the United States, most preschool-age children maintain serum retinal levels of 0.70µmol/l or higher while consuming diets providing 300-400µg RE/day [46]. Since 2, 3 or 5 small biscuit, respectively, formulations CE, BE and AE produced in this study would cover the daily requirement of retinol equivalent for a child with vitamin A deficiency, these biscuits could serve as a useful strategy in African countries to reduce vitamin A deficiency for people at risk like children and pregnant women in developing countries like the Democratic Republic of Congo.

Table 4: Quantity of biscuits required to cover the dietary allowance of vitamin A.

Formulation	β-carotene µg/g	Child with VAD g/day of biscuit= 400µgRE / (0.25x µg β-carotene/g of biscuit) (*)	Well-nourished child g/day of biscuit = 400µgRAE / (µg β-carotene/ g of biscuit) x 12 (*)
AE	44.9064	36 (5)	108 (16)
BE	96.008	17 (3)	50 (7)
CE	122.008	13 (2)	39 (6)

(*) amount of biscuit needed = grams of biscuit require to cover the daily allowance / the weight of biscuit

AE: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **BE**: biscuit BE =70% Wheat + 30% OFSP Flour+10g crude palm oil and 20g of margarine +20 g of eggs; **CE:** biscuit CE: 50% Wheat + 50% OFSP Flour+10g of crude palm oil and 20g of margarine+ 20 g eggs.

Sensory evaluation

In this study, the mean sensory score is presented in table 5. The control and the formulation (AE) recorded higher scores for the color with no significant difference between them. But the sensory score was significantly different compare to others parameters (p<0.05). The attractive yellow color observed in formulations AE and BE could be due to the combined effect of red palm oil and the OFSP flour which gave an orange color and was probably improved by the maillard reactions due to the baking temperature (figure 1 shows the color of different biscuit formulations). The taste acceptability scores ranged between 6.48 ± 1.6 and 7.74 ± 0.81 with a significant difference between formulation CE and other formulations (p<0.05), which could be due to high OFSP incorporated into the formulation which gave a slight sweet potato taste. The overall acceptability score of the biscuits was in the acceptable range (6.72 ± 1.55 to 7.63 ± 0.94) (Table 5). The result revealed that the flavor and the overall acceptability score decreased in the formulation CE where 50% of the OFSP flour substituted wheat flour. This could be due to the high amount of the 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 5: Sensory evaluation of biscuits.

Sensory parameter	Formulations			
	A0	AE	BE	CE
Color	7.68 ± 0.89^{a}	7.21 ± 1.5^{ab}	6.64 ± 1.6^{cd}	6.80 ± 1.4^{bcd}
Taste	7.74 ± 0.81^{a}	6.59 ± 1.7^{bcd}	6.72 ± 1.6^{bcd}	6.48 ± 1.6^{cd}
Flavor	7.48 ± 1.14^{a}	6.74 ± 1.68^{bc}	6.71 ± 1.41^{bc}	6.51 ± 1.75^{bc}
Overall acceptability	7.63 ± 0.94^{a}	6.87 ± 1.4^{bc}	6.81 ± 1.4^{cd}	6.72 ± 1.55^{cd}

*Means ± standard deviation with different superscript letters in the same row are significantly different from each other (p<0.05).

The experiment produced biscuits according to different formulations are presented in figure 1.

Conclusion

Biscuits made from composite wheat and orange fleshed sweet potato flour, crude palm oil, margarine and eggs showed higher values of carotenoid content (beta-carotene), the energy and moderate amount of protein. This could be useful for fortification programs for children deficient in vitamin A. The consumption of 2, 3, or 5 portions of biscuit respectively for the formulations CE, BE, AE could cover the daily requirement of retinol for children aged 1-3 years with VAD. Incorporation of eggs into biscuit formulation increased the protein content which significantly enhance the nutritional value of the biscuits. All biscuits were in the acceptable range related to the overall acceptability. This kind of biscuit formulation appears to be more efficient than the use the OFSP flour or palm oil alone.

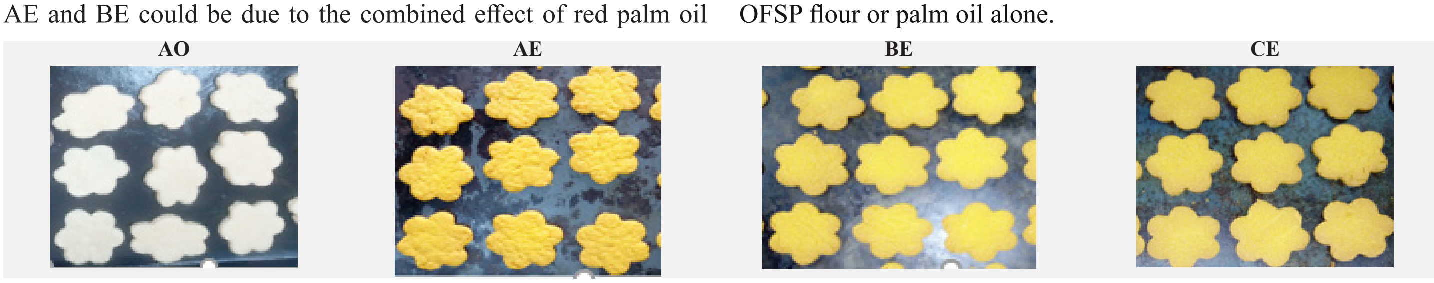

Figure 1. Sample of produced biscuit for analysis.

A0: biscuit A0 =100% Wheat Flour+30g of margarine; **AE**: biscuit AE=100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of eggs; **BE**: biscuit BE =70% Wheat + 30% OSP Flour+10g crude palm oil and 20g of margarine +20 g of eggs; **CE:** biscuit CE= 50% Wheat + 50% OFSP Flour+10g of crude palm oil and 20g of margarine+ 20 g eggs.

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