UNIVERSIDADE EDUARDO MONDLANE

FACULTY OF ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

Biscuits made from composite Wheat- Orange-Fleshed Sweet Potato (*Ipomoea batata*) flour, Palm oil and Eggs: Nutritional value and carotenoid retention

(contribution in the diet of children 1-3 years old)

M.Sc. Dissertation

by

Lusamaki Mukunda François

March, 2021

Maputo, Mozambique

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Supervisor: Doctor Irene Stuart Torrié De Carvalho, Eng[a]

A DISSERTATION REPORT SUBMITTED TO EDUARDO MONDLANE UNIVERSITY, FACULTY OF ENGINEERING, DEPARTMENT OF CHEMICAL ENGINEERING FOR PARTIAL FULFILMENT OF DEGREE OF MASTERS SCIENCE IN FOOD TECHNOLOGY

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DEDICATION

This work is dedicated to Mukunda Wayama Prosper and Almadanga Keogyaboni Delphin families for their constant love and spiritual support during our life that makes us succeed in this world. May our Almighty God bless you and your future generation.

STATEMENT OF ORIGINALITY

I, **Lusamaki Mukunda François,** with the student registration number 20196267 at UEM, hereby declare that this dissertation entitled '**Biscuits made from composite Wheat- Orange-Fleshed Sweet Potato (*Ipomoea batata L*) flour, Palm oil and Eggs: Nutritional value and carotenoid retention** (contribution in the diet of children 1-3 years old).' is the result of my own investigations accept where otherwise stated. This dissertation is presented in partial fulfilment of the requirement for the award of Master's Degree (MSc) in Food Technology from Faculty of Engineering, Eduardo Mondlane University, Mozambique.

Signature: 

Lusamaki Mukunda François

20196267

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MASTER IN FOOD TECHNOLOGY

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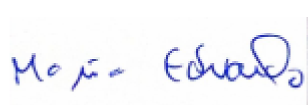
Examination board:

Supervisor	Co-Supervisor	President	Opponent
			

ABBREVIATIONS

AOAC: Association of Official Analytical Chemistries

A_w: water activity

CHO: Carbohydrate

CMBO1: β-carotene15,15'-monooxygenase

CMDO2: β-carotene 9',10'-dioxygenase

CPO: Crude Palm Oil

d.b: dry basis

DRC: Democratic Republic of Congo

DRI: Dietary Reference Intake

EPOA: European Palm Oil Alliance

EDS: Enquête Démographique et de Santé

FAO: Food and Agriculture Organization

GHI-PD: Global Hidden Hunger Index

IOM: Institute of Medicine

Kcal: Kilocalorie

MJ: Mega Joule

µg: Microgram

N: Nitrogen

OFSP: Orange-Fleshed Sweet Potato

PE: Petroleum Ether

Pronanut: Programme National de Nutrition

ppm: Part per million

RAE: Retinol Activity Equivalent

RBP: Retinol Binding Protein

RE: Retinol Equivalent

RDA: Recommended Dietary Allowance

rpm: Revolution Per Minute

SSA: Sub-Saharan Africa

UEM: Eduardo Mondlane University

USIOM: United States Institute of Medicine

UNIKIS: Kisangani University

UL: the tolerable Upper intake Level

VA: vitamin A

VAD: vitamin A deficiency.

w.b: wet basis

WHO: World Health Organization

Abstract

Hidden hunger is a challenging public health concern in Sub-Saharan regions, is prevalent for children under five-year-olds. Vitamin A deficiency (VAD), is one of the main micronutrient deficiencies in children under the age of five. The aim of this study was to produce biscuit from composite wheat-orange sweet potato flour, palm oil and eggs and to determine its nutritional value including retinol equivalent and energy content.

One control biscuit (formulation A0) was prepared using 100g of wheat flour and 30g of margarine. Then, the first formulation (A) was performed: 100g of wheat flour +10g of palm oil+20g of margarine, the second formulation (B), 70g of wheat flour +30g of OFSP flour +10g palm oil and 20g of margarine and the third formulation (C), 50g of wheat flour +50g of OFSP flour+10 palm oil +20g of margarine. The fourth, fifth and sixth formulations (AE, BE, CE) were same as the 1st, 2nd and the 3rd, but 20g of egg was added to each of them. We determined the total carotenoid content and the proximate composition in both, Orange Fleshed-Sweet Potato (OFSP) and biscuit. The analysis of variance was used to determine the significant difference in the samples according to the treatment with a significant level of 0.05. After data analysis, the following results were observed:

The carotenoid retention was significantly higher in oven dried than sun-dried flour (90.76% vs 66.09%); The carotenoid content in the biscuits ranged from 96.03 µg/100g to 15251 µg/100g (86.82 µg/100g to 12200.8 µg/100g of beta-carotene) and Retinol Activity Equivalent (RAE) from 6.4 to 1016.7µgRAE/100g in the control and the formulation CE, respectively. The protein content ranged from 4.83 to 7.91%, and energy from 468.46 to 485.38 Kcal/100g in the biscuits. All biscuits were in the acceptable range (6.36 to 7.63) related to the sensory score.

This biscuits formulation appears within the RAE range which could prevent the VAD for young children, since 2 to 5 biscuits could cover the retinol equivalent for children aged 1–3-year-old. Thus, it could be useful in a fortification program to minimize the Vitamin A Deficiency.

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

Resumo

Fome oculta é um desafio a saúde pública como preocupação em regiões da África Subsaariana, e é prevalente em crianças com idade inferior a cinco anos. A Deficiência de Vitamina A (DVA), é uma das principais deficiências de micronutrientes em crianças come idade inferior a cinco. O objectivo deste estudo foi o de produzir biscoitos a partir de farinhas compostas de trigo e de batata-doce de polpa alaranjada, óleo de palma e ovos, a fim de se determinar o valor nutricional e incluindo o Retinol Equivalente (RE) e o valor energético.

Um biscoito controlo (formulação A0) foi preparado usando 100g de farinha de trigo e 30g de margarina. Então, a primeira formulação (A) 100 g de farinha de trigo + 10 g de óleo de palma + 20 g de margarina, a segunda formulação (B), 70 g de farinha de trigo + 30g de BDPA farinha + 10 g de óleo de palma e 20 g de margarina e a terceira formulação (C), 50 g de farinha de trigo + 50 g de farinha de BDPA + 10 óleo de palma + 20 g de margarina. A quarta, quinta e sexta formulações (AE, BE, CE) foram preparadas da mesma forma que a A, B e C, mas 20 g de ovo foi adicionado a cada uma delas. Foi determinado o teor de carotenóide total e a composição centesimal em ambos, Batata-doce de Polpa Alaranjada (BDPA) e biscoito. A análise da variância foi utilizada para determinar a diferença significativa nas amostras de acordo com o tratamento com um nível de significância de 0.05. Após a análise dos dados, os seguintes resultados foram observados:

A retenção de carotenóides foi significativamente maior na farinha seca ao forno do que na farinha seca ao sol (90,76% vs 66,09%); o teor de carotenoide nos biscoitos variou de 96,03 µg/100g a 15251 µg /100 g (86,82 µg/100g a 12200,8 µg/100g de beta - caroteno) e Actividade Equivalente de Retinol (ERA), de 6,4 a 1016,7 µg RAE / 100 g para a formulação de controlo e formulação CE, respectivamente. O conteúdo de proteína variou de 4,83 a 7. 91 %, e o valor energético de 468,46 a 485, 3 8 kcal / 100g de biscoitos. Todos os biscoitos estavam na faixa de aceitação de 6,36 a 7,63 em relação a análise sensorial. As formulações destes biscoitos encontram-se dentro da faixa da Actividade de Retinol Equivalente, o que poderá prevenir a Deficiência de Vitamina, visto que o consumo de 2 a 5 biscoitos poderia cobrir o equivalente de retinol para crianças com idades compreendidas entre 1-3 anos de idade. Assim, poderá útil em um programa de fortificação para minimizar a Deficiência de Vitamina A.

Palavras-chave: Batata-doce de polpa alaranjada, biscoitos, óleo de palma, retenção de carotenóides

1. GENERAL INTRODUCTION

1.1 Background

It is estimated that two billion people worldwide suffer from hidden hunger, a condition of chronic micronutrient deficiency (Muthayya et al., 2013). In Sub-Saharan Africa (SSA) countries, the most widespread micronutrient deficiencies are iron, zinc, vitamin A, iodine and folate, and all of these are common contributors to poor growth, intellectual impairments, perinatal complications, and increased risk of morbidity and mortality (Bailey et al., 2015). However, deficiency of vitamin A affects largely children under five years old and women of reproductive age. About 190 million children under five (33.3% of the preschool age population) are vitamin A deficient, with about 5.2 million affected by night blindness (FAO/WHO, 2013). In the Democratic Republic of Congo (DRC), the national investigation realised in 1998 that included children aged from 6 to 36 months, found a Vitamin A Deficiency (VAD) rate at 61,1% (Pronanut, 2002). This finding propelled the semi-annual government supplementation strategy of vitamin A for children aged from six to fifty-nine months, for each six months.

Since humans cannot synthesise Vitamin A, VAD is due to an insufficient dietary intake of performed vitamin A from animal sources or provitamin A from plant sources (fruits, vegetables, oil, tubers and roots) (Rodriguez-Amaya and Kimura, 2004). In SSA countries where most people are unable to get performed vitamin A because of unaffordability of meat, provitamin A remains the most abundant form of vitamin A (Souganidis et al., 2013). Whereas in developed countries provitamin A account for only about 35% of dietary vitamin A (Weber and Grune, 2012), in developing countries it covers over 80% of vitamin A needs (Codjia, 2001). Vitamin A, molecules derived from provitamin A carotenoids, play an essential role in cell differentiation, morphogenesis and vision in vertebrates (Nagao, 2014).

Nowadays, bakery products such as biscuits, breads and cakes are becoming more important as part of the daily diet in SSA (Harahap et al., 2020). Whereas breads and cakes are relatively expensive, biscuits appear to be the most affordable bakery products in SSA. A Biscuit can be described as a dry bakery product made mainly from wheat flour, oil/fat with or without addition of other food ingredients (Okoye et al., 2008). However, Burrier et al. (2003) stated that, quality of biscuit depends on the type of ingredients and particular improvement needed. With a relative long shelf life, biscuits offer the possibility to be modified to suit specific nutritional needs of a target population (Elkhalifa and El-Tinay, 2002). Development of biscuits from composite flours has become the trend in the bakery industry (Hooda and Sudesh, 2005). Many studies were conducted to improve biscuit qualities using different ingredients including; soy flour or sesame flour to improve protein contents (Vitali, 2009; Gernah and Anyam, 2014); fiber from different cereals and fruits to improve fiber content (Sudha et al., 2007; Turksoy and Ozkaya, 2011) and red palm oil for formulating novel functional biscuit (El-Hadad et al., 2010).

Orange-Fleshed Sweet Potato (OFSP) and crude palm oil (CPO) are abundant food materials in SSA, rich in carotenoids also having properties and nutritional compositions that complete each other. These foods have been used in bakeries products (Jumirah, 2018). The OFSP is a potential source of dietary provitamin A. The predominant carotenoids is beta-carotene and it possesses the highest vitamin A activity among carotenoids (Khoo et al., 2011). The beta-carotene content of OFSP depends highly on the sweet potato variety and it may reach up to 20000 µg/100 g wet basis (Burri, 2011). On the other hand, palm oil is a rich source of carotenoids ranging from 13mg.Kg^{-1} to more than 1000 mg. Kg^{-1} (13 µg/g to more than 1000 µg/g) depending on the variety (Santos et al., 2015) of which more than 50% is beta-carotene (Benadé, 2000).

However, the retention or loss of carotenoids during processing and storage of food has been reported in numerous studies. Regardless of the processing method used, carotenoid retention decreases with longer processing periods, higher processing temperature, and cutting or pureeing of the food (Moura et al., 2015; Vimala et al., 2011).

The crude palm oil and OFSP flours were used separately in the biscuit formulation. However, combination of these two ingredients with the same formulation is expected to produce biscuits with higher carotenoid contents. The present study examined the effect of substitution of wheat flour with OFSP flour and margarine with crude palm oil for the carotenoid contents in biscuit. However, with regard to the high carotenoid degradation during processing, its retention was evaluated in the orange-fleshed sweet potato flour. Also, we evaluated the biscuit contribution in the diet of children 1-3 years old relating it to the energy and daily retinol equivalent supplied.

1.2 Objectives of study

1.2.1 General objective:

To produce the biscuits from composite wheat and orange-fleshed sweet potato flours, palm oil and eggs.

1.2.2 Specific objectives:

- To prepare orange-fleshed sweet potato flour and:
  - To evaluate the physicochemical properties
  - To determine beta-carotene retention;
- To prepare biscuits and:
  - To evaluate the physicochemical and functional properties (moisture, protein, lipid, carbohydrate, Ash, crude fiber)
  - To determine energetic value of the biscuit and beta-carotene content.
  - To determine the retinol equivalent supplied by hundred grams of biscuit
  - To realise a sensory evaluation of biscuit.

1.3 Problem statement and justification

Children under 3 years are more vulnerable to poor nutrition; the growth rate during this period is greater than at any other time, and thus, there exists an increased risk of growth retardation. Also, the immunological system is not fully mature at this age, this results in a risk of frequent and severs infections. Poor nutrition during the early years lead to profound deficit including delayed motor and cognitive development, learning deficiencies and lower education achievement (Kim et al., 2003).

The hidden hunger affects about one third of children living in low- and middle-income countries mainly in Sub-Saharan Africa and south Asia (FAO/WHO, 2013). To illustrate this, studies found the prevalence of VAD at 9.23% among Chinese children (Song et al., 2017), at 5.3% among Nigerian children (Abolurin et al., 2018), and at 20.3% among malnourished and healthy children in Kisangani, DRC (Mukunda et al., 2019). In addition, the prevalence of malnutrition was 8.5%; 43%; and 23% for acute, chronic malnutrition and weak weight of birth respectively (EDS, 2014).

Palm oil is an ingredient mostly used in culinary preparation in several households of Kisangani city, orange-fleshed sweet potatoes are cultivated and harvested in Kisangani. On the other hand, wheat flour is the main ingredient of cookies but is expensive and it is not affordable for most people. The substitution of wheat by OFSP flour may be more advantageous not only in terms of affordability of biscuits but also in terms of nutritional value (carotenoids rich biscuits).

1.4 Rationale of study

This study aims to highlight the importance of producing rich beta-carotene content biscuit from composite wheat and orange-fleshed sweet potato flours, crude palm oil and eggs. In this study, mixing of two rich carotenoid ingredients (OFSP flour and crude palm oil) appears as a new formulation for improving bakery products, since many researchers use it separately but the yield carotenoid in the final product requires a high level of substitution. The combination of these two ingredients would produce biscuit with a high carotenoid content, which requires a low level of substitution. In addition, the acceptability of the product may be better. Since the Vitamin A Deficiency is still a public health problem in Sub-Sahara Africa countries, especially in Democratic Republic of Congo, consumption of these rich carotenoid content biscuits would be an alternative strategy to provide the essential micronutrient to the population, particularly vulnerable groups like infants aged between 1-3 years, to result in a decreasing rate of VAD in the community.

1.5 Thesis' hypotheses

The present study investigated the effect of partial substitution of wheat flour with OFSP flour and margarine with crude palm oil on carotenoid content in biscuits.

H0: substitution of wheat flour by OFSP flour has no significant change in carotenoid content of biscuit when margarine was previously substitute by crude palm oil.

H1: substitution of wheat flour by OFSP flour has significant change in carotenoid content of biscuit when margarine was previously substitute by crude palm oil.

2. LITERATURE REVIEW

2.1 Sweet Potato

2.1.1 Background

Sweet potato (*Ipomea batata L. Lam*) is an herbaceous perennial vine in Convolvulaceae family, which produces storage roots and edible leaves and can grow on marginal lands. The sweet potato plants have been widely dispersed by humans throughout the world since its domestication in the New World (Drapal et al., 2019). The sweet potato is mainly cultivated in developing countries, where it serves as a principal nutritious food for low income people (Ruttarattanamongkol et al., 2016). In general, it is the sixth most important food crop after rice, wheat, potatoes, maize and cassava (Drapal et al., 2019). In Mozambique, it is the third most important food crop after maize and cassava (Alvaro et al., 2017).

Sweet potato cultivars with deep orange and purple-fleshed color are an excellent source of nutrients and natural health-promoting compounds, such as carotenoids, anthocyanin, polyphenols, ascorbic acid and dietary fiber (Ruttarattanamongkol et al., 2016). The crop has recently become the focus of the targeted bio-fortification for enhanced vitamin A. The orange fleshed varieties have more beta-carotene than standard varieties and these newly released varieties rank first among roots and tubers per their nutritional quality in Sub-Saharan Africa (Khoury et al., 2015). The beta-carotene content of OFSP depends highly on the sweet potato variety and it may reach up to 20000 μg/100 g wet basis (Burri, 2011). The root varies from various shades of white, cream, yellow to dark-orange depending upon the carotenoid content. The more orange color is, the higher carotenoid content (Vimala et al., 2011).

The biofortified varieties of sweet potato could help reduce the high rate of VAD prevalence among children 6-59 months (Moura et al., 2015). OFSP is one of the sweet potato varieties being promoted in Sub-Saharan Africa as a food-based measure to complement other efforts in reducing the occurrence of VAD in this region (Awuni et al., 2018). Some scholars suggest a food-based approach may be a successful way of reducing the prevalence of vitamin A deficiency. It is reported that one medium sized OFSP can provide about twice the β-carotene needed for the recommended daily requirement of vitamin A (Vimala et al., 2011).

2.1.2 Production of sweet potato

In the world, Asia records the biggest production with more of 75%, then Africa with 20%, America 4% and Europe 1%; the production of Oceania being very weak. In Africa, Tanzania is the first country producer (3,47 million of tons), followed by Nigeria (3,45 million of tons), Uganda (2.59 million of tons), Kenya (1,15 million), Madagascar (1.13 million) and Rwanda (1,08 million) (Songre et al. 2017). In 2015, 105 million tonnes of sweet potatoes were produced worldwide with China as the leading producer (Drapal et al., 2019).

2.1.3 Chemical composition of sweet potato

The chemical composition of sweet potato varies widely with sweet potato variety and environmental conditions. The moisture content range between 58.7 to 80.8% (18.8 to 34.6% of dry matter) was reported by Hagenimana et al. (1998). Sweet potatoes have high nutritional value, and they are source of carbohydrates, protein, vitamins, carotenoids and minerals. Carbohydrate content varies from 25% to 30%, from which 98% are easily digestible, besides the high content of carotenoids, they are also vitamin B, potassium, iron, and calcium in sweet potato (da Silva et al., 2017). Starch may represent up to 83.8% in dry bases for some varieties. The amylose fraction varies between 29.9 to 36.8 and resistant starch 5 to 17% depending on the sweet potato variety and starch treatments (Zheng et al., 2016). The mean beta-carotene content of sweet potato cultivars varies from 10 to 26600µg/100g (Rodriguez-Amaya and Kimura, 2004).

Fresh orange sweet potatoes are bulky, highly perishable, and therefore sweet potato roots can be sliced, dried and ground in order to produce flour which can be stored for 6 months in sealed containers and be used as a substitute for wheat flour at 25-50% in cookies, cakes and breads. It can be marked as a low cost alternative for imported wheat flour, especially for snacks and noodle producers (Srivastava et al., 2012). The picture of OFSP root we used in this research is presented in figure 1.

Figure 1. Orange-Fleshed Sweet Potato roots.

2.1.4 Bioavailability of Carotenoid of Orange-Fleshed Sweet Potato

Carotenoid bioavailability is defined as the fraction of carotenoids transferred by food to mixed micelles, therefore becoming accessible for subsequent uptake by the intestinal mucosa (Tumuhimbise et al., 2009). In the OFSP, the major carotenoid present is beta-carotene (Vimala et al. 2011). Studies have shown that the consumption of boiled OFSP roots improved the vitamin A marker in children and adults.

Despite being rich in beta-carotene, not all amounts are accessible. Carotenoid bio accessibility depends on food matrix, the type of fiber and fat in the food, and the heat and homogenization caused by food processing (Tumuhimbise et al., 2009). The increasing of carotenoid absorption by gradual increasing amount of fat (3%, 6% and 12%) in OFSP diet was observed at Mongolian people (Mills et al., 2009). In another study, humans feeding on sweet potato with fat, calculated bioavailability as 65% for beta-carotene beadlets and 37% for sweet potatoes (Huang et al. 2000).Thus the bioavailability of beta-carotene from sweet potato can be very low (<1%) if fed without fat. Even a small amount of fat appears to increase beta-carotene bioaccessibility in sweet potatoes by 2-to 20-fold. Even so, only 25% (11 to 48%) of the beta-carotene in sweet potatoes is bioaccessible, thus available to be absorbed into the intestine (Tanumihardjo et al., 2008).

2.2 Wheat

Wheat *(Triticum aestivum L.)* is the most important staple food crop for more than one third of the world population, wheat contributes more calories and proteins in the world dietary system than any other cereal crop. It is nutritious, easy to store and transport and can be processed into various types of foods. Wheat is considered a good source of protein, minerals, B-group vitamins and dietary fiber (Kumar et al., 2011).The wheat grain contains 2-3% germ, 13-17% bran and 80-85% mealy endosperm(Šramková et al., 2009)**.**. All constituents are presented in figure 2.

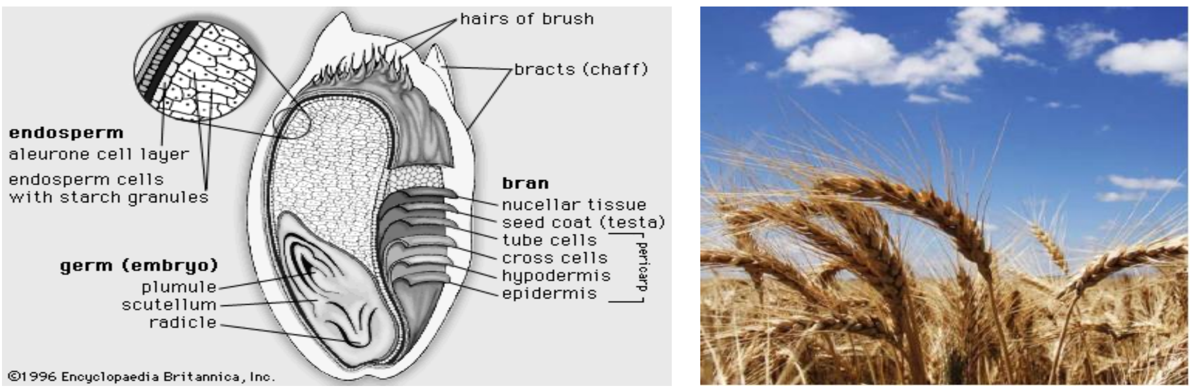


Figure 2. Wheat grain (from Encyclopaedia Britannica, *http://www.britannica.com*)

The bran (outer layers of wheat grain) is made up of several layers, which protects the main part of the grain. Bran is rich in B vitamins and minerals; it is separated from the starchy endosperm during the first stage of milling. In order to protect the grain and endosperm material, the bran comprises water-insoluble fibre. More than half of the bran consists of fiber components (53%). The endosperm mainly contains food reserves, needed for growth of the seedling, because it is rich in energy-yielding starch. Apart from carbohydrates, the mealy endosperm contains fats (1,5%) and proteins (13%): albumins, globulins and the major proteins of the gluten complex- glutenin and gliadins. Proteins that will form the gluten at dough making. The contents of minerals (ash) and of dietary fibres are low; 0,5% and 1,5%, respectively (Šramková et al., 2009). Wheat is the major ingredient in most breads, rolls, crackers, biscuits, cakes, doughnuts, macaroni, spaghetti, puddings, pizza, and many prepared hot and cold breakfast foods (David et al., 2015). In general, wheat contains carbohydrate 78.10%, protein 14.70%, fat 2.10%, minerals 2.10% and considerable proportions of vitamins (thiamine and vitamin-B) and minerals: zinc, iron, selenium and magnesium (Kumar et al., 2011).

2.2.1. Types of wheat flour and its uses

2.2.1.1 All- Purpose flour: All-purpose flour is the finely ground endosperm of the wheat kernel separated from the bran and germ during the milling process. All-purpose flour is made from hard wheat or a combination of soft and hard wheat from which the home baker can make a complete range of satisfactory baked products such as yeast breads, cakes, cookies, pastries and noodles (Kumar et al., 2011).

2.2.1.2 Bread flour: Bread flour, from the endosperm of the wheat kernel, is milled primarily for commercial bakers but is also available at retail outlets. Although similar to all-purpose flour, it has greater gluten strength and it is generally used for yeast breads (Kumar et al., 2011).

2.2.1.3 Whole wheat flour: Whole-wheat flour is a course-textured flour ground from the entire wheat kernel and thus contains the bran, germ and endosperm. The presence of bran reduces gluten development. Baked products made from whole-wheat flour tend to be heavier and denser than those made from white flour.

2.2.1.4 Others flours: Cake flours, Pastry flour, Gluten flour, Semolina, Farina, (Kumar et al., 2011).

The protein content of wheat grains may vary between 10% - 18% of the total dry matter. Wheat proteins are classified according to their extractability and solubility in various solvents. Albumins, which are soluble in water; globulins, which are insoluble in pure water and in dilute Sodium chloride (NaCl) solutions, and insoluble at high NaCl concentrations; gliadins, which are soluble in 70% ethyl alcohol, and; glutenins, which are soluble in dilute acid or sodium hydroxide solutions. Most of the physiologically active proteins (enzymes) in wheat grains are found in the albumin and globulin groups. The albumin and globulin fraction cover about 25% of the total grain proteins. Gliadins and glutenin are storage proteins and they cover about 75% of the total protein content. they are mainly located in the mealy endosperm and are not found in the seed coat layers nor in the germ. Storage proteins in wheat are unique because they are technologically active. They have no enzyme activity, but they have a function in the formation of dough as they retain gas, producing spongy baked products (Šramková et al., 2009).

Allergen: wheat is one of the most common allergenic food in the human diet and is mainly associated with asthma and atopic dermatitis.

Celiac diseases (Gluten- sensitive enteropathy) is a disorder caused by intolerance to the ingestion of cereal storage protein found in wheat, barley, rye and triticale. However, the disease does not affect people from all ethnic groups (Office of gene technology and regulation, 2008).

2.3 Palm Oil

Palm oil is produced from the fruit pulp of the oil palm tree *(Elaeis guineensis*), it grows in equatorial regions, such as in Indonesia and Malaysia (EPOA, 2016). *Elaeis. guineensis* originated in the humid tropical forests bordering the Gulf of Guinea in both West and Central Africa. The species is endemic to forests in the tropical zone countries such as Sierra Leone, Liberia, Ivoire-Cost , Ghana, Togo, Benin, Nigeria, Cameroon and the equatorial zones of Gabon, Republic of Congo, Democratic Republic of Congo and Angola (Rival and Levang, 2014).

Palm oil is considered one of the world's richest natural plant sources of carotenoids, which gives the oil and fruit their brilliant orange-red color. Due to their chemical composition, the oils obtained from native palm fruits are considered new sources of high-added value phytochemicals, since studies on certain species showed that CPO contains vitamin E (717-863ppm), Carotenoids (600–750 ppm), Phytosterols (325–365 ppm), Squalene (14–15 ppm), Ubiquinone (18–25 ppm) and unsaturated fatty acids. About 600 types of naturally occurring carotenoids are known, 13 are found in palm oil and the more dominated is beta-carotene, which is higher than 60% of the total fraction (Loganathan et al., 2017; Santos et al., 2015). It is an essential ingredient in much of traditional West African cuisine.

During the refining process, the carotenes become decomposed, resulting in a refined, bleached, and deodorized palm oil, which has a light yellow color and retains part of the other phytonutrients. On the other hand, red palm oil is obtained through the novel processes of pre-treatment, deacidification and deodorization using molecular distillation, this allows about 80% of the carotenes and vitamins present in the crude palm oil to be retained. Red palm oil contains 15 times more retinol (provitamin A) equivalents than carrots, 300 times more than tomatoes and 44 times more than leafy vegetables (Nagendran et al., 2000; Scrimshaw, 2000). The palm oil and the fruits are presented in figure 3. The nut referred to as a kernel at the centre of each piece of fruit, is where palm kernel oil is extracted from.

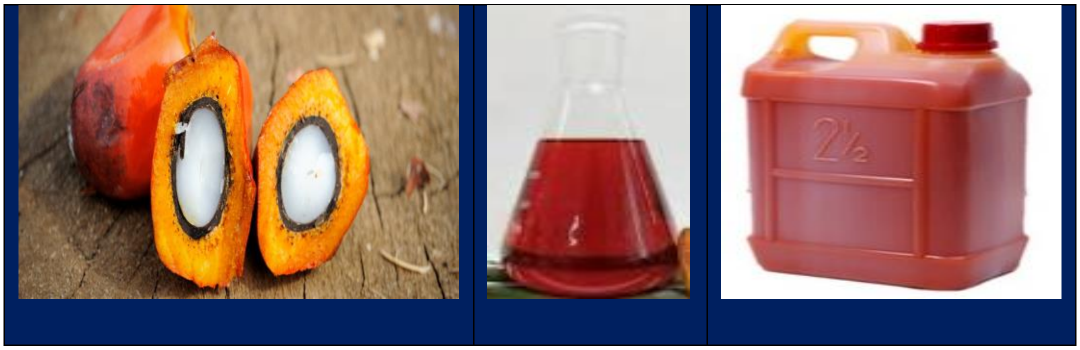


Figure 3. Fruits and palm oil (EPOA, 2016)

Bioavailability of carotenoid in palm oil was found to be 23% (You et al., 2002) and 37% (Huang et al., 2000) in healthy adult human subjects. Crude palm oil is an ingredient mostly used in culinary preparation in several households of Kisangani which provides more energy and fat-soluble vitamins.

2.4 Provitamin A Carotenoids

Carotenoids are usually C40 tetraterpenoids built from eight C5 isoprenoid units in an extensive conjugated double-bond system, which serves as the light-absorbing chromophore responsible for the yellow, orange, or red colour in fruits and vegetables (Moura, et al., 2015). The main condition for provitamin A activity is that the carotenoid must possess at least one beta-ionone ring residue (Figure. 4). Beta-carotene has two beta-ionone rings in its structure and is the most widespread of all carotenoids in food. Structurally, beta-carotene can generate two molecules of vitamin A (retinol) when centrally cleaved, while α-carotene and beta-cryptoxanthin can generate only one molecule. Approximately there are 600 carotenoids exist in nature, and only three are important precursors of vitamin A in human beings; beta-carotene, ά-carotene and beta-cryptoxanthin (Van Jaarsveld et al., 2005; Moura et al., 2015), and the beta-carotene is the major provitamin A of most carotenoids in food (Parker, 1996). Their structures are presented in figure 4.

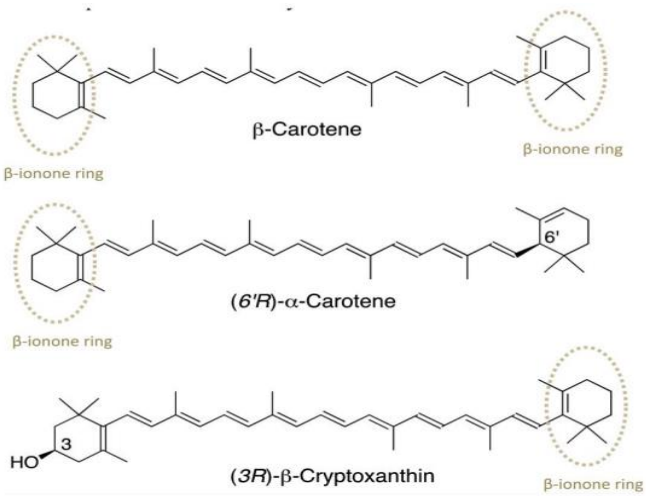


Figure 4. Chemical structures of provitamin A carotenoids (Moura et al., 2015)

2.4.1 Absorption and conversion of carotenoids to vitamin A

2.4.1.1 Absorption of carotenoids

Carotenoids appear to be absorbed in duodenal mucosal cells by the mechanism involving passive diffusion. The rate of diffusion is likely determined by the concentration gradient between the micelle and the plasma membrane of the enterocyte (Parker, 1996), similar to that of cholesterol and products of triglyceride lipolysis. Many factors known or believed to influence absorption of carotenoids have been summarized:

Dietary Fat: dietary vitamin A is digested in mixed micelles and absorbed with fat. In some studies, increasing the level of fat in a low-fat diet has been shown to improve retinol and carotene absorption (Jalal et al., 1998). For optimal carotenoid absorption, a number of research groups have demonstrated that dietary fat must be consumed along with carotenoids. An increase of carotenoid absorption from 5 to 25 percent was observed when 18g of olive oil was added in a diet (IOM, 2001). Moreover, Jayarajan et al. (2013) reported that the addition of 5 g fat to the diet significantly improved serum vitamin A concentrations among children after the consumption of a low-fat vegetable diet. Also, Deming (1999) did not observe any difference on improvement of serum vitamin A concentration by addition of 10g of fat and 5g in diet, and concluded that, a minimum of 5 g of fat in a diet is required for an optimal absorption of carotenoids.

Food matrix: the matrix foods affect the ability of carotenoids to be released and therefore affects intestinal absorption. In a study conducted in vitamin A equivalence of beta-carotene in Women as determined by a stable isotope reference method, the rise in serum beta-carotene concentration was significantly less when individuals consumed beta-carotene from carrots than they received a similar amount of beta-carotene supplement (Tang et al., 2000). The efficiency of release will be influenced by such factors as physical disposition of carotenoids in the food or supplement matrix, the achieved particle size that allows mastication and stomach action, and efficiency of digestive enzymes (Parker, 1996).

Food processing: the processing of foods affects the absorption of carotenoids. Heating of plant foods before ingestion improves the bioavailability of carotenoids pigments from many of such foods, probably as a result of dissociation or weaking of protein-carotenoid complexes. Such an effect has been clearly demonstrated for lycopene from tomato products (IOM, 2001).

Infections: malabsorption of vitamin A can occur with diarrhea and intestinal infections and infestations (IOM , 2001).

2.4.1.2 Conversion of carotenoids to vitamin A

In the test tube, 1 molecule of beta-carotene can be converted into 2 molecules of vitamin A (VA) by a simple one-step reaction via beta-carotene15, 15'-monooxygenase (CMBO1), which cleaves beta carotene centrally to 2 molecules of retinal. A secondary mechanism involves beta-carotene 9',10'-dioxygenase (CMDO2) which cleaves beta-carotene, alpha-carotene and beta-cryptoxanthin eccentrically to form 2 apo carotenals, the longer that can then be oxidised to 1 molecule of retinal (Lietz & Ange, 2010; Ho et al., 2007). In the body, an isotopic dilution study of beta carotene conversion in healthy well-nourished subjects show variable conversion ratio, forming negligible amounts of vitamin A (Burri, 2011). However, it was found in a study on bioconversion of plant carotenoids to Vitamin A that, the conversion ratio of beta-carotene to vitamin A in poorly nourished Filipino children varied inversely with their VA status; population and animal models with low VA status appear to convert a greater percentage of beta-carotene to VA (Ribaya-Mercado et al., 2000).

The conversion of carotenoid in the body was estimated to 6-µg beta-carotene: 1-µg of vitamin A (WHO and FAO, 2004) or 12-µg beta-carotene: 1-µg of vitamin A (IOM, 2001). The retinol activity equivalent (RAE) was established to replace the retinol equivalent (RE) as a measure of the vitamin A activity of dietary provitamin A carotenoids (IOM, 2001). One RAE is defined as 1 µg of all-trans-retinol= 12 µg of all-trans-beta-carotene and 24 µg of other provitamin A carotenoids (usually limited to α-carotene and beta-cryptoxanthin). International units (IU) One microgram of all-trans-retinol equals 3.33 IU of vitamin A activity.

Establishment of the RAE was based on the accepted carotene: retinol equivalency ratio (µg) of a low dose of purified beta-carotene in oil of 2:1, indicating that 2 µg of beta-carotene in oil yields 1 µg of retinol. Differences between the RE conversion factors and the RAE factors stem from absorption studies that show that 6 µg of dietary beta-carotene is equivalent to 1 µg of purified beta-carotene in oil (IOM, 2001). Since previous data indicated 3 µg of dietary beta-carotene was equal to 1 µg of beta-carotene in oil (6 x 2:1 = 12:1), the RAE calculation doubles the amounts of dietary provitamin A carotenoids required to provide 1 µg of retinol or 1 RAE as compared to calculation of RE values (IOM, 2001). The estimation of retinol equivalency compares favourably to retinol activity equivalencies calculated for fruits and vegetables (12 µg beta-carotene: 1 µg retinol and 24 µg alpha carotene: 1 µg retinol). They are more similar to retinol equivalencies calculated for fruits and vegetables (6 µg beta-carotene: 1 µg retinol and 12 alpha carotenes: 1 µg retinol). This ratio reflects a conversion efficiency that is about half that previously thought, leading to greater appreciation for why VAD may coexist in cultures that heavily depend on vegetables and fruits as their sole or main dietary source of vitamin A (WHO, 2009). The absorption and conversion of carotenoids are presented in figure 5.

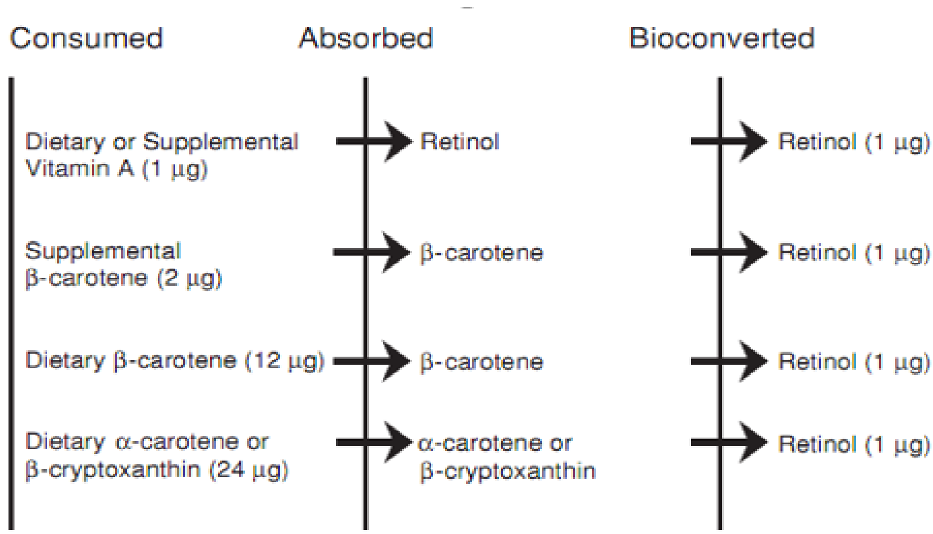


Figure 5. Absorption and bioconversion of ingested provitamin A carotenoids to retinol based on new equivalency factors (retinol activity equivalency ratio) (IOM, 2001)

2.4.2 Mechanisms of carotenoids degradation

The mechanisms of carotenoids degradation may involve the reaction of carotenoid with atmospheric oxygen (autooxidation), light (photodegradation), and heat (thermal degradation), as well as degradation by the interactions of carotenoids with singlet oxygen, acid, metals, and free radicals (Moura et al., 2015). However, in food systems, the degradation mechanism is complex. In the intact fruit, root or tuber, vegetable and grain, the carotenoid molecules are less susceptible to degradation because they are protected within tissues through molecular interactions. When the crop tissues are disrupted by cutting, chopping, shredding, cooking, or natural aging, these physical barriers are affected thus rendering the carotenoids open to exposure to oxygen and oxidizing enzymes (Britton and Khachik, 2009). The carotenoid retentions are largely different depending on the processing and the protective measures taken during processing. However, in a study evaluating sweet potato as an intervention food to prevent VAD, Burri (2011), reported that in sweet potato, the effects of cooking and storage methods were relatively negligible, causing losses of about 0% to 20%.

2.4.3 Daily requirement of vitamin A

Vitamin A is an essential nutrient needed in small amounts for the normal functioning of the visual system, and maintenance of cell function for growth, epithelia integrity, red blood cell production, immunity and reproduction (IOM, 2001). Dietary reference intake (DRI) values for vitamin A range from an adequate intake (AI) of 400µg RAE/day for 0 to 6 months infants to recommended dietary allowance (RDA) values of 700 µg RAE/day for adult women and 900 µg RAE/day for adult men. The RDA increases to 1300 µg RAE/day for lactating women (19-50 years) the tolerable upper intake level (UL) is 3000 µg RAE/day for adults (IOM, 2001).

In humans, the half-life of vitamin A is 200 to 300 days; hence, vitamin A status is not disturbed by slight fluctuations in vitamin A consumption. Ninety percent of vitamin A is stored in the liver, 1.5% is found in blood and the remainder is stored in other tissues. On the other hand, beta - carotene levels return to baseline levels within 20 to 30 days of the cessation of supplementation (Loganathan et al., 2017). The table 1 present the RDA for vitamin A and the daily requirement of energy for children aged from 0- 6 years is presented in table 2.

Table 1. Recommended dietary allowance for vitamin A

Life stage	Recommended nutrient intake (WHO and FAO 2004) (µg RE/d)	Dietary reference intake (USIOM 2000) (µg RAE/d)
Infant 0 to 6 mo	375	400 (adequate intake)
Children 7 to 12 mo	400	500
Children 1 to 3 y	400	300
Children 4 to 6 y	450	400
Children 7 to 8 y	500	400
Children 9 y	500	600
Children 10 to 13 y	600	600
Adolescents 14 to 18 y	600	Male 900; female 700
Adult males 19 to 65 y	600	900
Adult males 65 + y	600	900
Adult females 19 to 65 y	500	700
Adult females 65 + y	600	700
Pregnant females 14 to 18 y	800	750
Pregnant females 19 + y	800	770
Lactating females 14 to 18 y	850	1200
Lactating females 19 + years	850	1300

RE = Retinol equivalents; 1 retinal equivalent=6-µg β-carotene in food or 1-µg purified retinol. RE are the unit of measure used by the World Health Organization to describe the amount of vitamin A contributed to the diet by carotenoids

RAE= Retinol activity equivalents; 1 retinol activity equivalent= 12-µg β-carotene in food or 1-µg purified retinol. RAE are unit of measure Used by the United States Institute of Medicine (USIOM) to describe the amount of vitamin A contributed to the diet by carotenoids (Burri, 2011)

Table 2 Recommended nutrient in takes for energy MJ (Kcal)

Recommendation	European Union MJ (Kcal)		WHO MJ (Kcal)	
Age	Boys	girls	Boys	girls
0-3 months	2.2 (525)	2.1 (500)	2.3 (545)	2.2 (515)
4-6 months	3.0 (715)	2.8 (670)	2.9 (690)	2.7 (645)
7-9 months	3.5 (835)	3.3 (790)	3.4 (825)	3.2 (765)
10-12 months	3.9 (930)	3.7 (880)	3.9 (920)	3.6 (865)
1-3 years	5.1 (1215)	4.8 (1140)	5.2 (1230)	4.9 (1165)
4-6 years	7.1 (1690)	6.7 (1595)	7.2 (1715)	6.5 (1545)

Source: Kim et al. (2003).

2.4.4 Vitamin A deficiency

Infants and children have higher vitamin A requirements in order to promote rapid growth and better immunity to infections (Kim et al., 2013). Vitamin A deficiency is considered a serious public health problem in over 70 countries. This deficiency is the first reason for blindness in children. It is estimated that every year, about 300 000 children become blind because of the VAD, and about half among them die. However, the dimension of public health of the VAD does not limit itself to only ocular lesions because VAD is also a reason for morbidity of infectious origins and mortality in children, and even in pregnant women (WHO, 2001).

2.4.5 Health consequences of Vitamin A Deficiency

Vitamin A deficiency impairs numerous body functions which lead to many health consequences, putting young children and pregnant women at greatest risk (WHO, 2009):

a) Xerophthalmia is the most specific vitamin A deficiency disorder and is the leading preventable cause of blindness in children throughout the world.

b) During pregnancy, night blindness often appears as consequence of pre-existing marginal maternal vitamin A status superimposed by nutritional demands of pregnancy and intercurrent infections.

c) Anaemia can result from VAD in children and women, likely due to multiple apparent roles of vitamin A in supporting iron mobilization and transport, and haematopoiesis.

d) Pre-existing VAD appears to worsen infections and vitamin A supplementation has shown to reduce the risk of death in 6-59-months-old children by about 23-30% (WHO, 2009)

2.4.6 Control of vitamin A deficiency

When liver vitamin A reserves fall below a critical concentration, thought to be approximately 20 µg/g of liver, plasma retinol concentration declines. When dietary vitamin A is provided to VAD children, plasm retinol concentration increases rapidly, even before liver stores are restored (IOM, 2001). In malnourished populations, often 25 percent or more individuals exhibit a plasma retinol concentration below 0.70 µmol/l (20µg/dL), a level considered to reflect vitamin A inadequacy in a population (IOM 2001). The value of 0.70 µmol/l and 0.35 µmol/l represent VAD and severe VAD respectively. Although there is not yet an international consensus, WHO (2009), proposed a serum retinol concentration below a cut-off of 1.05 µmol/l to reflect low vitamin A status among pregnant and lactating women.

The average concentration of vitamin A in post-mortem livers of American and Canadian adults is reported to range from 10 to as high as 1400 µg/g liver (Furr et al., 1989). In developing countries where vitamin A deficiency is prevalent, the vitamin A concentration in liver biopsy sample is much lower (17 to 141 µg/g) (Haskell et al.,1997). The concentration of at least 20 µg retinol/g of liver in adults is suggested to be a minimal reserve. The mean liver stores of vitamin A in children (1 to 10 years of age) have been reported to range from 171 to 723 µg/g. whereas the mean liver vitamin A stores in apparently healthy infants is lower, ranging from 0 to 320 µg/g liver. The percentage of total body vitamin A stores lost per day was approximately 0.5 percent in adults consuming a vitamin A- free diet (IOM, 2001).

Liver (the reserve organ for vitamin A stored as retinyl esters) represents the richest food source of retinol from all animal species, containing from 810µg/100g of edible portion for cow liver to 8235 µg RE/100g for chicken liver. The preformed vitamin A sources are highly bioavailable and about 70 to 90% rapidly absorbed from the digestive tract. (Codjia, 2001; WHO and FAO, 2004).

The World Health Organization (2009), indicates that three types of community interventions can reduce the VAD in affected populations:

Firstly, improving the intake of vitamin A through dietary diversification should be viewed as an activity for all communities to enhance the overall nutritional status of the population. This requires nutrition education to change dietary habits, as well as providing better access to vitamin A or provitamin A-rich foods such as mangoes, papaya and dark green leafy vegetables. The second approach is through fortification of staple foods or condiments with vitamin A. In high income countries, many food items such as fats, oils, margarine and cereal products have long been fortified with vitamin A. Few national programmes exist in lower income countries. Lastly, the most widely practiced approach to controlling the VAD in most high-risk countries is the periodic delivery of high-potency supplements containing 200000 IU of vitamin A, to preschool-age children (< 5 years), with half this dose given to infants 6-11 months of age (WHO, 2009).

Global Hidden Hunger Index scores in pre-school (aged under-5) children (GHI-PD) over the period 1999-2009 calculated as the average of three deficiency prevalence estimates: pre-school children affected by stunting, anemia due to iron deficiency, and vitamin A deficiency. The scores ranged between the best and worst (0 and 100 respectively). Scores between 0 and 19.9 were considered as mild, 20-34.9 as moderate, 35-44.9 as severe, and 45-100 as alarmingly high (Muthayya et al., 2013). The reported scores are presented in figure 6 and the VAD (plasma retinol concentration below 0.70 µmol/l) prevalence according to WHO (2009) as a public health problem by country 1995-2005 preschool-age children is presented in figure 7.

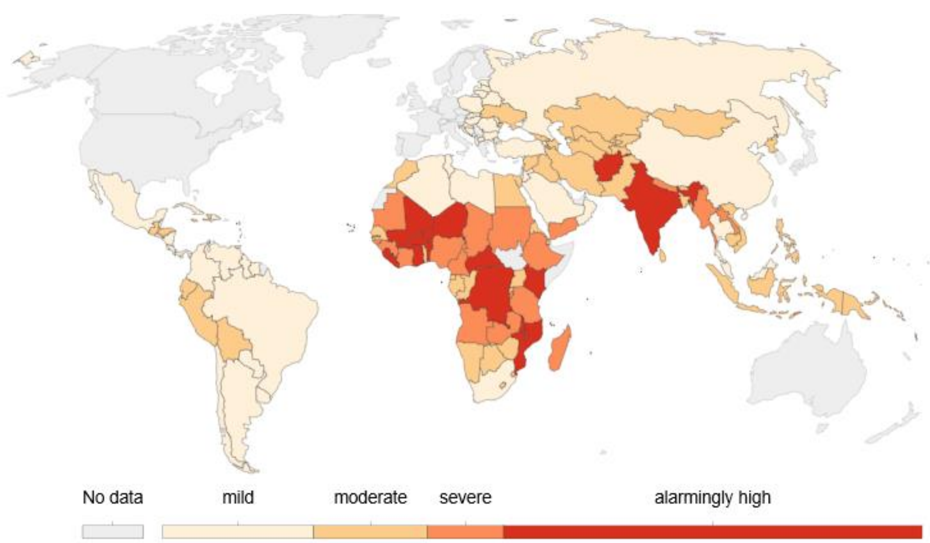


Figure 6. Hidden Hunger Index in pre-school children (Muthayya et al., 2013)

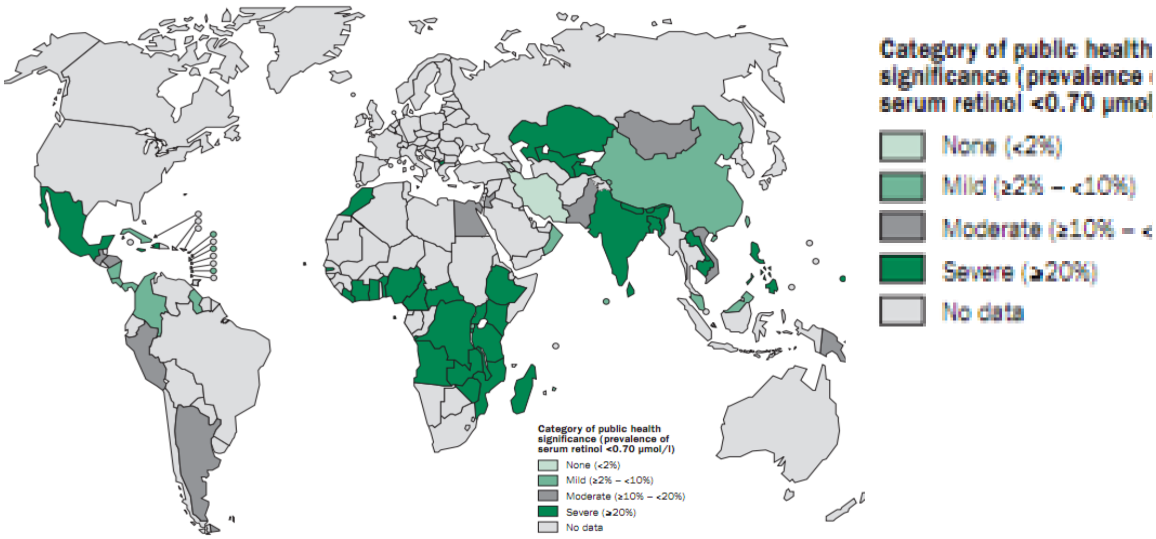


Figure 7. Vitamin A deficiency by country 1995-2005 preschool-age children (WHO, 2009)

2.4.7 Nutrient-nutrient interactions in relation with vitamin A

Iron: A direct correlation exists between haemoglobin and serum retinol concentrations. It was observed iron deficiency in young rats alters the distribution of vitamin A concentration between plasma and liver (Rosales et al., 1999). Intervention studies among Indonesian girls demonstrated that combining vitamin A with iron supplementation was more affective in increasing haemoglobin concentrations than was given iron alone (Suharno et al.,1993), suggested that VAD impaired iron mobilisation from stores and therefore vitamin A supplementation improves haemoglobin concentrations (IOM, 2001). Maximum haemoglobin response occurs when iron and vitamin A deficiencies are corrected together. The VAD appears to influence the availability of storage iron for use by haematopoietic tissue (WHO and FAO, 2004).

Zinc: Zinc is required for protein synthesis, including the hepatic synthesis and secretion of retinol binding protein (RBP) and transthyretin. Therefore, zinc deficiency influences the mobilisation of vitamin A from the liver and its transport by circulation. Because zinc is important in biosynthesis of the RBP, it has been suggested that zinc intake may positively affect vitamin A status only when individuals are moderate to severely protein-energy deficient (IOM, 2001).

Carotenoids: competitive interactions among different carotenoids have been observed when subjects were given purified beta-carotene and lutein in a combined dose, beta-carotene significantly reduced lutein absorption, and therefore serum lutein concentration, compared to when lutein was given alone. However, lutein given in combination with beta-carotene significantly increased beta-carotene serum concentration compared to when beta-carotene was given alone (IOM, 2001).

Alcohol: Ethanol consumption results in a depletion of hepatic vitamin A concentrations in animals. Ethanol mobilises vitamin A from the liver to other organs. A chronic ethanol intake result in increased destruction of retinoic acid through the induction of P450 enzymes, resulting in reduced hepatic retinoic acid concentrations (IOM, 2001).

2.4 Biscuits

Bakery products such as biscuits, breads, cakes are becoming a more important part of our daily diet (Harahap et al., 2020). Worldwide, biscuits are widely accepted and consumed due to its ready to eat nature, good nutritional quality, affordability and availability in different taste and longer shelf life (Turksoy and Özkaya, 2011). Traditionally, the process of cookie making is fairly simple with basic ingredients consisting of wheat flour, fat, eggs and sugar. Generally, biscuits are recognized as flat, hard and crunchy foods. They are classified according to their method of preparation such as drop, moulded, presses, refrigerated, bar or rolled. Apart from that, the dominant ingredients used in the formulation are also commonly being used to classify the cookies, for example, nut cookies, fruit cookies and chocolate cookies (Norhidayah et al., 2014)

Composite flour has the added advantages of improving the nutritious value of biscuits and other bakery products especially when cereals are blended with root like orange-fleshed sweet potato. They have become focus of interest as a source of incorporation of different ingredients to improve a desired quality. These characteristics make beta-carotene enriched biscuits attractive for target areas, such as child-feeding programmes, low-income group and disaster relief operations (Go and UE, 2015). Most of the research has been focused on the development of new products using sweet potato flour rather than on efficient methods to produce and store the flour that can be added in buns, chapattis and mandazis which can greatly increase the content of total carotenoids in these products. Addition of various proportions of sweet potato flour in wheat flour can increase the nutritive values in terms of fibre and carotenoids. This also helps to lower the gluten level and also prevent coeliac disease (Saeed et al., 2012).

3. MATERIAL AND METHODS

3.1 Study design

This was a quantitative experimental study designed in two steps. Firstly, the Orange-Fleshed Sweet Potato (OFSP) flour was prepared from fresh OFSP using two different drying methods; sun drying and oven drying. Secondly, one control biscuit was prepared from wheat flour and margarine (formulation A0), then six biscuit formulations were made by substitution of wheat flour by different amount of orange-fleshed sweet potato flour and margarine by crude palm oil in the first, the second and the third formulations named respectively (A, B, and C). The fourth, fifth and sixth formulations (AE, BE, CE) were same as the 1st, 2nd and the 3rd, but 20g of egg was added to each of them.

The proximate composition and carotenoid contents were analysed in the fresh OSFP; in the flour and in the biscuits, and the carotenoid retention was calculated in the OFSP flour. In addition, the RAE supply by 100g of biscuit and the amount of biscuit needed to cover the daily allowance of retinol equivalents (RE) for children aged 1-3-years were calculated.

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

3.2 Procurement of raw materials

Palm oil was brought from Kisangani, Democratic Republic of Congo, and kept in a well-sealed plastic box. All other materials were bought in local markets, that include orange-fleshed sweet potato (of an unknown variety), wheat flour and others ingredients such margarine, sugar, salt and baking powder.

3.3 Orange-Fleshed Sweet Potato Flour preparation

OFSP flour was processed according to the method reported by da Silva et al. (2017). 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 manually 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.

The oven dried flour showed a high carotenoid retention, then was used in biscuits formulations.

3.4 Elaboration of biscuits with different ingredients

3. 4.1 Preparation of wheat-sweet potato biscuit

The wheat-sweet potato flour composites were prepared at different ratios (**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 as shown in table 3.

Table 3. Ingredients for biscuits formulation

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

A0: Control= 100% Wheat Flour biscuits 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;

AE: biscuit100% 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;

3.4.2 Biscuit preparation

Biscuits based on Gernah et al. (2015), method but with some modifications in baking time and biscuit formulation. Instead of baking at 180 ^{o}C we baked at 150^{o}C. Fat (palm oil and margarine), sugar and salt were mixed together for three minutes, using a mixer (Kenwood chef serial N^{o} 0662544). After that, the flours thoroughly mixed with baking powder was added and mixed for further seven minutes to form dough in which moment we added water. 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^{0}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 (Gernah et al.,2015). The process is drecibed in figure 8.

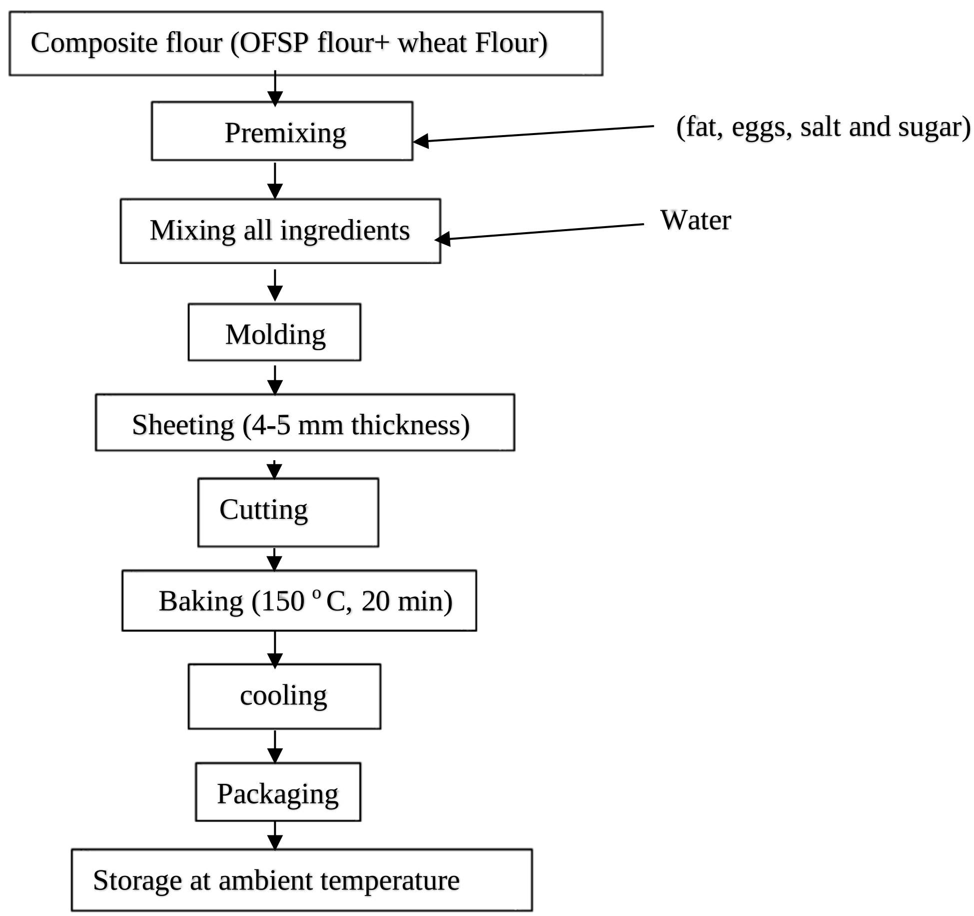


Figure 8. Flow chart for the preparation of biscuits

3.5 Physicochemical properties measurement of biscuit

3.5.1 Determination of Water activity

Determination of water activity (a_w) in the biscuit sample was performed by the water activity meter (AQUA Lab Serial 08038551B, USA) which was previously calibrated. The biscuit flour sample was put into a sample holder and inserted into the water activity meter; after which the reading displayed on the meter was recorded. The measurement was done in triplicate to get reliable results (Nielsen, 2010).

3.5.2 Determination of color

Determination of color in the biscuit samples was performed by the colorimeter device (CR-10, Minolta CO LTD, made in Japan). The colorimeter was first calibrated by using a plain white paper. 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 (Nielsen, 2010).

3.5.3 Weight Measurement

The weight (in grams) of biscuits was measured by a digital scale (Dalco chromtech ACADAM® n°, 17250), with max 250 grams. All the results were in triplicate.

3.5.4 Determination of Diameter

The diameter was measured in mm by digital calliper (ABSOLUTE DIGIMATIC, ref.3472, China). All the results were in triplicate.

3.6 Proximate composition determination

3.6.1 Determination of Moisture content

Moisture was determined according to the Association of Official Analytical Chemistries (AOAC, 2000). About 5g of samples were weighed and transferred to the pre-weighed crucibles. The crucible and their contents were placed in the drying oven for 3 hours at 105^{o}C before removing the crucibles 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.

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

3.6.2 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 Kjedahl tubes were placed in the digestion block and covered. The temperature of the digester was adjusted to 420^{o}C and digested for 45 minutes. After digestion, the solution obtained was taken off and allowed to cool to ambient temperature. About 70 ml 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-indictor 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 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.

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

2.6.3 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 to obtain a constant weight (W2) and expressed as g/100 g of sample. The formula 3, was used to express the percentage of total Ash. All the results were in triplicate.

$$\% \text{ Ash content} = \frac{(W1 - W2) \times 100}{\text{Weight of sample}} \quad (3)$$

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

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

3.6.4 Determination of Fat

Fat was determined by Soxhlet method (AOAC,1990). About 5g 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 105^{0}C for about 1h, for complete evaporation of the solvent and cooled to room temperature in the desiccators for about 30min and re-weighed. The results were obtained according to formula 4 and in triplicate.

$$\% \text{ Fat } = \left(\frac{\text{weight of fat in sample}}{\text{weigt of of dried sample}}\right) \times 100 \quad (4)$$

3.6.5 Determination of Crude fiber

Crude fiber was determined according to method described in AOAC (2000). About 1 g of sample was placed in beaker, 150ml of H_2SO_4 (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 was 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 105°C 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 550°C. The crucible was cooled in the desiccators and weighed (recorded as W2). Formula 5 was used to determine the crude fiber and in triplicate. $\% \, Crude \; fiber = \left(\frac{W1-w2 \; of \; ash}{Wt \; of \;\; sample}\right) \; x \, 100$ (5)

2.6.6 Determination of Carbohydrate

Carbohydrate (CHO) was determined by difference, according to reported formula by Afework et al.(2016), and the results were expressed according to formula 6.

%CHO = 100- (Moisture (%) + Ash (%) + fat (%) + protein (%) + fiber (%)) (6)

3.6.7 Determination of Gross energy

The value of the energy of OFSP flour and biscuits 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 (Haile et al. 2016), as showed in the formula 7.

Calorie (Kcal/100g) = (4 x carbohydrate) + (4 x protein) + (9 x fat) (7)

3.7. Carotenoid extraction and determination

3.7.1 Carotenoids extraction

Carotenoid extraction was carried out according to the method described by Rodriguez-Amaya and Kimura (2004). 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 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). All results were in triplicate. The analysis was carried out under low light conditions, using aluminium foil to cover the extract tubes to minimise carotenoids' oxidation.

3.7.2 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 8 described below:

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

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

We assumed that 80% of all carotenoids content in biscuits analysed were beta-carotene considering the previous published results on beta-carotene content in Orange-Fleshed Sweet Potato (Burri, 2011; Bengtsson et al., 2008 ; Takayata, 1993), and in palm oil (Manorama and Rukmini, 1991 ; Dong et al., 2017 ; Rao, 2000 ; Nokkaew et al., 2019).

3.8. Carotenoid retention

Retention of carotene was calculated using simplified formula 11 of the true retention in OFSP flour, assuming that the dry matter content was constant **(**Bechoff et al., 2015)**.**

Retention (%)= $\frac{carotenoid\ content\ in\ dry\ matter\ of\ flour}{carotenoid\ content\ in\ dry\ matter\ of\ fresh} x100$ (11)

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

Considering previous researches on carotenoid bioaccessibility in the OFSP, 25% (range from 11% to 48%) (Tanumihardjo et al., 2008), and between 33 and 75% in palm oil (Rao, 2000), in this study, we considered the bioaccessible fraction of the beta-carotene in biscuit as 0.25 (1/4) of total beta-carotene content as it was reported in orange-fleshed sweet potatoes (Burri, 2011). Bioaccessible beta-carotene of biscuit was calculated as: Total beta-carotene of biscuit x 0.25. This was used to calculated amount of biscuits needed to meet the daily retinol equivalent (RE) requires for infant with vitamin A deficiency, as grams per day of biscuit = ((µg RE/day (400))/ ((µg bioaccessible β-carotene/gram of biscuit)) (Burri, 2011). And for children with good vitamin A status, it was calculated using the Retinol Activity Equivalent (RAE) conversion factor 12 µg of β-carotene = 1 µg of trans-retinol (IOM, 2001), as grams per day of biscuit = ((µg RE/day (400))/ ((µg β-carotene/gram of biscuit) x (conversion ratio 12/1))) (Burri, 2011).

3.11 Sensory evaluation

The acceptability of the biscuits was evaluated using a 9-point hedonic scale (Muresan et al., 2012). Fifty untrained judges comprising of staff and students in the Faculty of Engineering 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=dislike extremely, 2=dislike very much, 3=dislike moderately, 4= dislike slightly, 5=neither like nor dislike, 6=like slightly, 7= like moderately, 8=like very much and 9= like extremely.

3.12 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.

4. RESULTS AND DISCUSSION

4.1 Results on Orange-Fleshed Sweet Potato

4.1.1 Proximate composition in Orange-Fleshed sweet potato fresh and flours

The results showed a high moisture content in the fresh sample: 79.97±0.26% (20.03% dry matter), 10.98±0.03% in oven dried flour and 12.41±0.47% in sun dried (table 4). The moisture ranged between 58.7 to 80.8% (19.2 to 41.3% of dry matter) were reported in sweet potatoes by Hagenimana et al.(1998). Moreover, the dry matter in OFSP cultivar in Gaza province (Mozambique) ranged from 20.1% and 27.2% were reported by Mazuze (2016). These previous 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 (8.24-12.86 %) reported of OFSP flour by Olatunde et al. (2016). It was stated that more factors could influence moisture content in sweet potato, including genetic composition, cultivation practices and the application of fertiliser (Takayata, 1993). Fat content was 0.52±0.03%, 1.51±0.05% and 1.96±0.04%, respectively in fresh, sun-dried and oven-dried (table 4). This result was similar to other study findings (Alam et al.,2016; Ruttarattanamongkol et al., 2016). Sweet potato is poor in fat as other roots and tubers.

Crude protein was 1.27±0.07%, 4.57±0.17% and 5.24±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. (2016), while da Silva et al. (2017), 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 (Ukom et al., 2009). Protein content in diets of the low-income population in developing countries is derived mostly from plant origin.

The crude fiber was 1.26±0.57%, 3.62±0.85% and 3.80±0.66%, respectively, for fresh, sun-dried and oven-dried flours. This finding result was high than the crude fiber (0.30% to 0.54%) reported in fresh sweet potato by Alam et al. (2016), but was in accordance with crude fiber(0.08 to 5.54%) reported in sweet potato flour (Olatunde et al., 2016). However the highest crude fiber ranged from 2.1% to 13.6% were reported in another study (Senanayake et al., 2013). 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 (Alam et al., 2016).

Ash content was 0.53±02%, 2.62±0.04 % and 2.86±0.01%, respectively, for fresh, sun-dried and oven-dried flour. This result was in the range of other reported ash content (0.15% to 3.52%) in OFSP tuber and flour (da Silva et al., 2017; Ruttarattanamongkol et al., 2016). 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 and sweet potato variety (Alam et al., 2016).

Carbohydrate obtained in this study was 16,45%, 75.07% and 75.16%, respectively for fresh, sun-dried and oven-dried flours. Carbohydrate content in fresh sample was lower than (18.86% and 22.33%) reported in other study (Ukom et al., 2006); 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 (Olatunde et al., 2016). 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 (Suparno et al., 2016).

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 (Nguyen Van Toan, 2018).

Table 4. Proximate composition of the fresh orange-fleshed sweet potato samples and flour

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 measures (n = 3)

4.1.2 Carotenoid contents in Orange-Fleshed Sweet Potato and its retention

In table 5, the total carotenoid contents in the fresh OFSP, the flours and its retention can be observed. The result showed that, the mean carotenoid content to be 5525.17±80µg/100g, 15970±45 µg/100g and 22287± 47 µg/100g (wet basis) respectively in the fresh, sun-dried and oven-dried sample. In dry matter basis it was 27584.61±41µg/100g, 18232.67±51µg/100g, and 25036.02±53µg/100g, respectively, for the fresh OFSP, sun-dried and oven-dried flours and were significantly different ($p<0.05$).

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.(2014), ranged between 0.38 to 7.34 mg/100g Alam et al.(2016), and between 0.01 and 26.6mg/100g Takahata et al.(1993). 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 factors that may influence variation of beta-carotene content in orange-fleshed sweet potato (Ukom et al., 2009; Nicanuru et al., 2015). Related to carotenoid in dry basis (d.b), our result was in agreement with (Bechoff 2010) study, where the carotenoids in dry basis of six varieties of OFSP ranged from 41.7 to 300.5 µg/g. The more predominant carotenoid in OFSP is the beta-carotene (Burri, 2011; Bengtsson et al., 2008). 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 (van Jaarsveld et al., 2005). Carotenoids whether provitamin A or not, have been credited to have a health-promoting effects; enhancement of immune response and reduction of risk of degenerative diseases (Rodriguez-Amaya and Kimura, 2004).

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 beta-carotene content. Sun drying was observed to retain 63-73%, oven drying 89-96%, boiling 84-90% and frying 72-86% of beta-carotene in OFSP varieties studied (Vimala et al., 2011). Carotenoid retention may be affected by different factors like slice thickness of pieces, temperature and stage of maturity (Adelaide et al., 2007). The influence of different procedures on carotene have been reported in sweet potato (Bengtsson et al., 2008), in cassava (Chavez et al., 2007), and in carrots (Dutta et al., 2005). 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 (Bengtsson et al., 2008). 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 (Rodriguez-Amaya and Kimura, 2004).

Table 5. Carotenoid contents in orange sweet potato and its retention

Parameter Sample	Moisture (g/100g)	Carotenoid µg/100g (w.b)	Carotenoid µg/100g (d.b)	Carotenoid loss (µg)	Carotenoid Retention %
Fresh	79.97 ± 0.26	5525.17±80^{c}	27584.61±41^{a}	-	100
Oven drying	10.98±0.03	22287± 47^{a}	25036.02±53^{b}	2548.59^{a}	90.76
Sun drying	12.41±0.47	15970±45^{b}	18232.67±51^{c}	9351.94^{b}	66.09

Means ± 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

4.2 Results of biscuits

Figure 9 shows biscuits produced from the experiment alongside their respective formulations

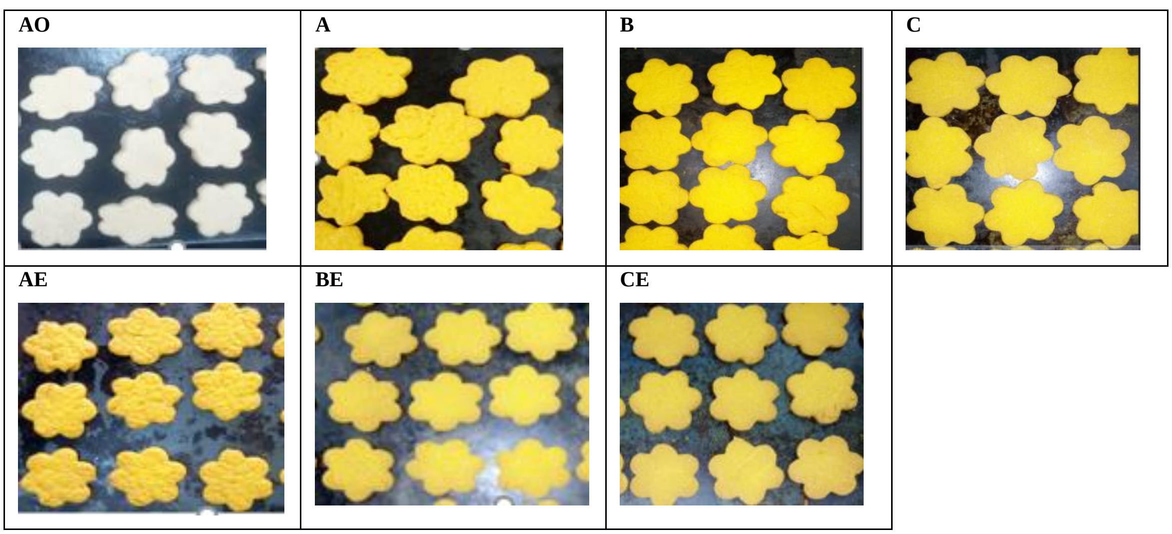


Figure 9. Biscuit samples for analysis.

A0: control= 100% of wheat flour + 30g of margarine; **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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.1 Physicochemical property of biscuits

The weight of the experimental biscuits was between 6.53±0.2g for formulation C to 6.98±0.1g for formulation A0 and AE (table 6). The results differed significantly among samples ($p<0.05$). These results were similar to those (6.07 to 6.62) reported on biscuits prepared from wheat substitute by sweet potato flour (Nguyen Van Toan, 2018). The weight of biscuits decreased with increasing level of replacement of wheat flour by the OFSP flour. Sweet potato flour had high water absorption capacity than the wheat flour but had higher loss of water during baking of biscuit (Nguyen Van Toan, 2018).

The water activity in the biscuits analysed ranged between 0.27±0.0 in formulation A and 0.38±0.0 for the control (A0). The results differed significantly among samples ($p<0.05$) (table 6). This may be due to the higher loss of water during baking of biscuit with sweet potato flour as well as red palm oil substitution. Biscuit is a baked product with moisture content less than 5% and low water activity which increases its shelf life (Hadnađev et al., 2015). The lower a_w (0.22 to 0.26) were observed in biscuits wheat flour and red palm oil (El-Hadad et al., 2010). 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.

The diameter of the biscuits ranged from 45.43±1 to 47.02±0.1 mm and was in accordance with values reported by Hadnađev et al. (2015). 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 (Ismail et al., 2018).

The color values of biscuits in this study was significantly different ($p<0.05$) for each sample (table 6): L* 40.10±0.2 to 49.9±0.6; a* from 4.86±0.4 to 13.66±0.2 and b* from 20.26±0.7 to 50.66±1. Only the formulations A and AE were 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. 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. 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 (Hadnađev et al., 2015). The similar results were observed in biscuits from sweet potato flour and wheat flour (Singh et al., 2008).

Table 6. Physical properties of biscuit

Physical properties	Formulations						
	A0	A	B	C	AE	BE	CE
Weight (g)	6.98±0.1^{a}	6.97±0.4^{a}	6.66±0.1^{b}	6.53±0.2^{c}	6.98±0.1^{a}	6.66±0.1^{b}	6.61±0.2^{bc}
Water activity	0.38±0.0^{a}	0.27±0.0^{e}	0.31±0.0^{b}	0.29±0.0^{c}	0.28±0.0^{d}	0.27±0.0^{e}	0.27±0.0^{e}
Diameter (mm)	47.02±0.1^{a}	46.76±0.4^{a}	45.82±1^{ab}	45.43±1^{b}	46.99±0.7^{a}	46.80±0.3^{a}	45.5±1.1^{b}
Color							
L*	47.96±3^{ab}	48.56±1^{ab}	43.23±0.5^{c}	41.6±0.1^{c}	49.9±0.6^{a}	40.10±0^{d}	41.53±1^{cd}
a*	4.86±0.4^{f}	9.43±0.5e	11.36±0.3^{c}	11.76±0.^{c}	10.86±1^{d}	13.66±0.2^{a}	12.70±0^{b}
b*	20.26±0.7^{f}	48.76±1^{b}	41.26±0.2^{c}	38.8±0.1^{d}	50.66±1^{a}	40.1±0.1^{d}	40.63±1^{cd}

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

A0: Biscuit control= 100% Wheat Flour biscuits with 30g margarine**; 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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.2 Proximate composition and energetic value of biscuits

4.2.2.1 Proximate composition

The proximate composition of biscuits showed significant change in the values of moisture, crude protein, crude fiber, ash and fat ($p<0.05$), except that of the control and the formulation A (table 7). The moisture content was between 3.21±0.21 to 4.37±0.6%. However, the lower moisture content in biscuits (1.329 % to 1.369%) and the higher content (4.3 to 5.9%) were reported by Srivastava et al. (2012) and Songre-Ouattara et al. (2017), respectively. Moisture content decreased significantly with the OFSP flour incorporation in the biscuit formulation. This effect was also observed by Nguyen Van Toan (2018) and Amir et al.(2015), 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 (Gernah and Anyam, 2014).

The protein content ranged from 4.83±0.03 to 7.91±0.61% and was significantly different between sample ($p<0.05$). The value of proteins (5.6 to 6.3%) and (5.14 to 7.21%) were observed in biscuit from the OFSP and sorghum flour (Songre-Ouattara et al., 2017), and Cassava starch, OFSP puree and cowpea flour (Oduro, 2017), respectively. The difference in protein content observed in this study could be due to substitution of wheat by the OFSP flour which decreased significantly the protein content. Moreover, addition of egg in biscuit formulation increased significantly the protein content of the formulations AE, BE and CE. Proteins are involved in numerous physiological functions. 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 33.3% (formulation C) to 54.8% (formulation AE) of dietary allowances of protein for children between the ages 1 and 3 years old. The recommended protein intake per day being 14.5g according to WHO reported by Kim et al. (2003).

Crude fiber ranged from 0.59±0.05 to 1.55±0.5% and ash from 1.25± 0.04 to 2.15± 0.01% and were significantly different. 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 by Nguyen Van Toan (2018). The observed difference of crude fiber and ash in different formulations could be due to OFSP substitution in the blend. The same observation was stated by Srivastava et al. (2012) and Songre-Ouattara et al. (2017). 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 (Gernah and Anyam, 2015).

The fat content found in experiment biscuits was between 18.66±0.6 to 22.48±0.3%, and is in the range of 9.72-26.69% reported by Nguyen Van Toan (2018), Oduro (2017), and Kolawole et al. (2020). The difference in fat content of biscuits may be attributed to the fat ability retention of the OFSP flour when comparing with that of wheat flour during the baking process. The higher fat retention may improve the mouth feel and retains the flavor of biscuits (Nguyen Van Toan, 2018). In addition, high fat content increases the energy content since fat provide more energy by gram compare to others macronutrients.

The results showed that the mean of the carbohydrate content ranged from 65.09 to 68.81% and was in the range (59.84 and 67.22%) of reported carbohydrate content in biscuits in numerous studies (Songre-Ouattara et al., 2017 ; da Silva et al., 2017). The control and the formulation A, exhibited high carbohydrate contents (68.78 and 68.81%) than 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. A similar finding was also stated when biscuit was produced from blends of wheat and sesame flour (Gernah and Anyam, 2015).

Table 7. Proximate composition of biscuits

Formulations	Proximate composition (%)					
	Moisture	Protein	Crud fiber	Ash	Fat	CHO.
A0	4.37±0.6[a]	6.59±0.05[b]	0.59±0.07[c]	1.25±0.04[c]	18.66±0.56[c]	68.78
A	4.32±0.26[a]	6.33±0.11[c]	0.58±0.05[c]	1.26±0.34[c]	18.70±0.27[c]	68.81
B	3.33±0.04[b]	5.67±0.1[d]	0.91±0.04[b]	1.56±0.04[b]	20.65±0.28[b]	67.88
C	3.21±0.21[c]	4.83±0,03[f]	1.55±0.04[a]	2.06±0.02[a]	22.46±0.25[a]	65.89
AE	4.37±0.33[a]	7.91±0.61[a]	0.61±0.06[d]	1.25±0.07[c]	20.46±0.3[b]	65.40
BE	3.37±0.1[b]	6.57±0.13[b]	1.01±0.08[b]	1.64±0.03[b]	20.66±0.1[b]	66.75
CE	3.25±0.14[c]	5.48±0.03[de]	1.55±0.5[a]	2.15±0.01[a]	22.48±0.3[a]	65.09

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

A0: Biscuit control= 100% Wheat Flour biscuits with 30g margarine**; 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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.2.2 Energy content in biscuits

The energy content ranged significantly between 468.46 to 485.38 Kcal/100g (figure.10). A similar energy content (469.0 to 474.6 Kcal/100g) was reported in biscuit from the OFSP and sorghum flour (Songre-Ouattara et al., 2017). 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 (Kim et al., 2003).

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 (Turksoy, S., & Özkaya, 2011). Hundred grams of biscuits could cover more than one-third of total daily energy required (1230 Kcal) for children aged 1-3 years. In Democratic Republic of Congo (DRC), the complementary foods for young children are made mainly from cereals and cassava flours, low in essential nutrients, therefore contributing less to the nutritional needs of young children. In contrast, the fortified biscuits have a potential role to play in a food vehicle to fight of malnutrition. Fortified biscuits (BP100) with minerals and vitamins were used in DRC as a therapeutic food for malnourished children under five years (Pronanut, 2002). The energetic values of the biscuits are presented in figure 10.

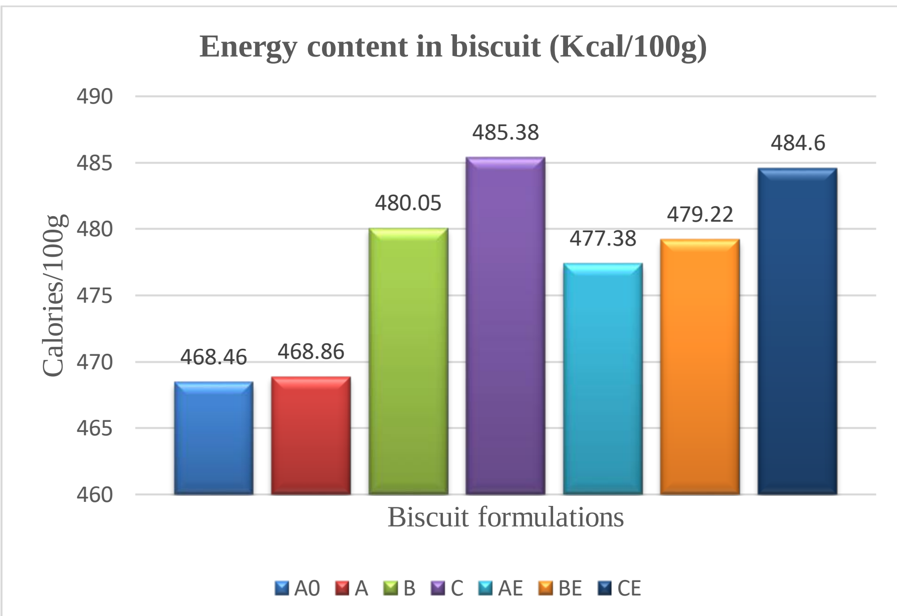


Figure 10. Energetic value of biscuits (Kcal/100g)

A0: Biscuit control= 100% Wheat Flour biscuits with 30g margarine**; 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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.3 Carotenoid content in biscuits

Figure 11, present the carotenoid contents in biscuits. Data observed was significantly different between biscuit formulations (*P* <0.05). Carotenoid content ranged between 0.96±0.46 µg/g for the control and 152.51±46 µg/g for the biscuit formulation CE. The similar low beta-carotene (0.54 µg/g) was found in control biscuit made from wheat flour and shortening (Afework et al., 2016). Moreover, no detectable amount of beta-carotene was stated in the control biscuits prepared with unspiked shortening (Rogers et al., 1993). It was stated that beta-carotene is only a trace constituent in wheat and other grains (Heinonen et al., 1989).

Incorporation of crude palm oil showed an increase of carotenoid from 0.96 µ/g in the control up to 55.77 µ/g in formulation A. This could be due to the high carotenoid content (720.23ppm) used in biscuit formulations (Lusamaki et al., 2021). Substitution of shortening by red palm oil increased carotenoids in biscuit from 25 µg/10g to 853.52 µg/10g (Butt et al., 2004). In addition, Gouado et al. (2008), reported the highest beta-carotene content (309.7 µg/g to 1624.3 µg/g) during the processing of cassava into yellow gari with addition of 2 ml to 8 ml of red palm oil for 210g of gari, and indicate that the analysis was done on the palm oil and not on the gari product itself.

Substitution of wheat flour by OFSP flour increased carotenoid from 55.77 µg/g up to 115.68 µ/g and 146.89 µ/g with 30% and 50% OFSP flour substitution respectively (formulation B and C). Moreover, addition of eggs in biscuit formulation increased significantly carotenoid in the formulations BE (120.01 µ/g) and CE (152.51 µ/g) compared to the formulations B and C. this effect could be due to the carotenoid content in eggs. The similar increasing of beta-carotene content (14.93 to 17.28 mg/100g) was observed in sweet potato biscuits and tempeh substtuted by red palm oil (Jumirah, 2018). Carotenoid contents in biscuit ranged from 0.54 µg/g to 6.01 µg/g and up to 9.44 µg/g were reported by Afework et al.(2016) and Oduro (2017), respectively.

Production of biscuit rich in beta-carotene from ingredient like crude palm oil, orange-fleshed sweet potato flour is more reported. However, most researchers, used these ingredients separately. The rate of substitution of wheat flour by OFSP flour or butter by palm oil is faced by the acceptability of product. It was stated that, incorporation of 15 to 45 % sweet potato flour yielded approximately simar results compared wheat flour cookies with improved nutritional value, texture and overall acceptability, but at 60% of incorporation, the sensory attributes score decreased (Singh et al., 2008; Harahap et al., 2020). On the other hand, biscuit made by replacing white shortening by 40% of red palm oil produced significantly superior biscuit based on overall acceptance (Butt et al., 2004; El-Hadad et al., 2010). In this experiment, a composite wheat-OFSP flour (30%) with substitution of margarine (1/3) by crude palm oil appears to be more efficient to produce biscuit with high carotenoid content and good acceptability.

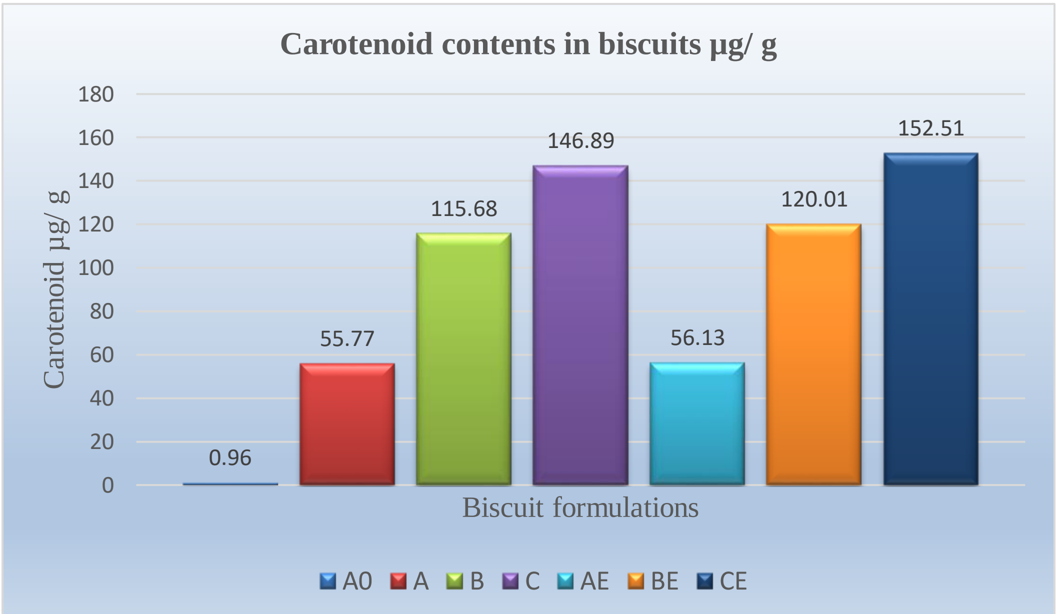


Figure 11. Carotenoid contents in biscuits (µg/ g)

A0: Biscuit control= 100% Wheat Flour biscuits with 30g margarine**; 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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.4 Beta-carotene and RAE of the biscuits

It was assumed that, the beta-carotene represented 80% of total carotenoid contents in biscuit, related to the previous study as described in material and methods. Table 8 present the carotenoid content, beta-carotene and the retinal activity equivalent for 100g of biscuits. Data observed was significantly different between formulations (*P* <0.05). Carotenoid content ranged between 96.03±0.46 µg/100g (86.82 µg/100g of β-carotene either 6.402 µg RAE) for the control and 15251±46 µg/100g (12200.8 µg/100g of β-carotene either 1016.722 µg RAE) for the formulation CE.

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 carotenoids. 1 µg RAE is defined as 1 µg of all-trans-retinol= 12 µg of all-trans-beta-carotene and 24 µg of other provitamin A carotenoids (usually limited to α-carotene and beta-cryptoxanthin) (IOM, 2001). They are more like retinol equivalencies (RE) calculated for fruits and vegetables (6 µg beta-carotene: 1 µg retinol and 12 alpha carotenes: 1 µg retinol) (WHO, 2009). The RAE being 300-400 µg RAE for children 1-3-years, 100g of biscuits could cover from 100% for formulation A to more than 200% for the formulation CE.

Table 8. 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)
AO	96.03±0.46^{f}	76.82	6.402
A	5576.8±65^{e}	4461.44	371.787
B	11568±111^{d}	9254.4	771.200
C	14689±18^{b}	11751.2	979.267
AE	5613.3±18^{e}	4490.64	374.220
BE	12001±36^{c}	9600.8	800.067
CE	15251±46^{a}	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: Biscuit control= 100% Wheat Flour biscuits with 30g margarine**; 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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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.

4.2.5 Amount of biscuit needed to cover retinol equivalent requires 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 (Ribaya- Mercado et al., 2000 ; Howe and Tanumihardjo, 2006). We estimated that children with good vitamin A status had a retinol equivalency ratio of 12- µg beta-carotene: 1- µg retinol (IOM, 2001). 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 (Tanumihardjo et al., 2008).

The amount of biscuit required to cover 400µgRE/day for a child aged between 1–3-year-old with VAD ranged from 13g for the biscuit (formulations CE) (2 biscuits) to 36g for the (formulation A) (5 biscuits) and from 39g for biscuit (formulation CE) (6 biscuits) to 108g for biscuit (formulation A) (16 biscuits) for well-nourished child (table 9).

This high retinol equivalent shown in this biscuit is mainly due to mixed ingredient rich in carotenoids (crude palm oil and OFSP flour). In fact, palm oil has been used for many bakery products to improve the carotenoid content (Jumirah, 2018). On the other hand, OFSP is rich in carotenoids and has been widely used in bakery for beta-carotene enrichment products (Afework et al., 2016). 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 (Rahmathullah et al., 1990). 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 (WHO and FAO, 2004). Since 2, 3 or 5 small biscuit, respectively, formulations C, B and A produced in this study would cover the daily requirement of retinol equivalent for a child with vitamin A deficiency, these biscuit formulations 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 DRC.

Table 9. Quantity of biscuits needed 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 (*)
A	44.6144	36 (5)	108(16)
B	92.544	17 (3)	51 (8)
C	117.512	14 (2)	41(6)
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. Grams per day of biscuit (**good vitamin A status**) = ((µg RAE/day(400))/((µg β-carotene/gram of biscuit)x(conversion ratio 12/1))) and **children with a marginal vitamin A deficiency**: Grams per day of biscuit = ((µg RE/day(400))/((µg bioaccessible β-carotene/gram of biscuit)) [6]. Bioaccessible β-carotene of biscuit was calculated as: total β-carotene of biscuit x 0.25 [6].

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. **AE**: biscuit100% Wheat Flour+10g of crude palm oil and 20g of margarine + 20g of egg; **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 egg.

4.3 Sensory evaluation

Measures of sensory characteristic of food products is important to determine the degree of acceptability of the final products (Muresan et al., 2012). In this study, the mean sensory score was presented in table 10. The biscuit control and the biscuit formulation (A) recorded higher scores for; color, flavor and acceptability with no significant difference between them. The attractive yellow color observed in (formulations A, AE and B) could be due to the combined effect of red palm oil and OFSP flour and eggs which gave an orange color and was probably improved by the maillard reactions due to the baking temperature.

The taste acceptability scores ranged between 6.29±1.6 and 7.74±0.1 with a significant difference between formulations C, with other formulations ($p<0.05$), this result could be due to high OFSP flour incorporated into the formulation whish gave a slight sweet potato taste.

The overall acceptability score of the biscuits was in the acceptable range (6.36 ± 1.4 to 7.63±0.94%). The result revealed that the flavor and the overall acceptability score decreased in the formulation C where 50% OFSP flour substituted wheat flour. 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. On the other, formulations where egg was added, the entire scores evaluated were low, compare to the same formulation without eggs.

Table 10. Sensory evaluation of biscuits

Formulations	Color	Taste	Flavour	Overall acceptability
A0	7.68 ± 0.89^{a}	7.74 ± 0.81^{a}	7.48 ± 1.14^{a}	7.63 ± 0.94^{a}
A	7.12 ± 1.65^{ab}	6.94 ± 1.23^{bc}	7.03 ± 1.24^{abc}	7.23 ± 1.03^{ab}
B	6.88 ± 1.52^{bc}	7.12 ± 1.30^{b}	7.09 ± 1.26^{abc}	7.24 ± 1.13^{ab}
C	6.44 ± 1.35^{d}	6.29 ± 1.64^{d}	6.46 ± 1.69^{c}	6.36 ± 1.44^{d}
AE	7.21 ± 1.5^{ab}	6.59 ± 1.7^{bcd}	6.74 ± 1.68^{bc}	6.87 ± 1.4^{bc}
BE	6.64 ± 1.6^{cd}	6.72 ± 1.6^{bcd}	6.71 ± 1.41^{bc}	6.81 ± 1.4^{cd}
CE	$6.80 \pm 1.4b^{cd}$	6.48 ± 1.6^{cd}	6.51 ± 1.75^{bc}	6.72 ± 1.55^{cd}

*Means ± standard deviation with different superscript letters in the same column are significantly different from each other.

5. CONCLUSION AND RECOMMENDATIONS

The conclusion and recommendations of this dissertation are presented in this chapter.

5.1 Conclusion

It was found in this study that processing of orange-fleshed sweet potato impacts significantly the carotenoid content in the end product. Carotenoid retention was significantly higher in oven-dried flour than in sun-dried. Suggesting that, the sun drying method of OFSP is more accessible method to produce flour but with high carotenoid lost.

Substitution of wheat flour by OFSP flour increased significantly the carotenoid contents of biscuit when margarine was previously substituted by crude palm oil which confirm our hypothesis.

Using composite wheat-orange-fleshed sweet potato flour, crude palm oil and eggs in biscuit formulations appear to be more efficient than OFSP flour or palm oil alone. It produces biscuits with high retinol activity equivalent which could prevent the VAD for young children, since 2 to 5 biscuits could cover the retinol equivalent for children aged 1-3 years- old. Moreover, addition of eggs in the formulations increased significantly the protein content in biscuit; thus, its nutritional value. It could be useful in a fortification program to manage vitamin A deficiency.

Hundred grams of these biscuits could cover up to 43% of dietary allowance energy requires for children aged 1–3-year-olds.

The entire sensory test score of the biscuits was in the acceptable range. The treatment A and B showed no significant difference with control, but treatment C recorded a low score in all parameter analysed including the overall acceptability compered to others.

5.2 Recommendations

Bakery products such as biscuits, breads, cakes are becoming a more important part of our daily diet worldwide. This formulation of composite flour using wheat and OFSP lour, palm oil and eggs produce a rich provitamin A biscuit, which could be useful to reduce prevalence of vitamin A deficiency to vulnerable population. We recommend the use of this formulation in all bakery products including, Chapati, cake, doughnut and biscuit which regularly consumption would improve the vitamin A status of people.

5.3 Limitation of study

More was supposed to be accurately done on carotenoid contents in Orange-Fleshed- Sweet Potato and in biscuit related to the beta-carotene content but wasn’t possible as the limitations were above the efforts invested. The High-Performance Liquid Chromatography device (HPLC) which could be used to separate the individual carotene fraction was broken. This could justify why we assumed the beta-carotene contents in biscuit using the previous published studies and allowed us to calculate the retinol equivalent supply by of biscuits.

5.4 Future researches focus composite wheat-OFSP flour and palm oil in biscuits

- Biscuit produced from composite wheat and OFSP flour with palm oil and milk powder to improve the protein content in biscuits;

- Enrichment of biscuit from wheat and OFSP flour and palm oil with multiple micronutrients powder to improve the micronutrients content in biscuits;

- The composite wheat, OFSP and soybeans flour with palm oil as main ingredients for rich energy, protein and beta-carotene biscuits.

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APPENDIXES

Appendix I.

Physicochemical composition of OFSP, and biscuit and the carotenoid extraction and determination in both OFSP and biscuits.

Table 1. 1. Physicochemical composition of OFSP fresh and flour

Fresh sample	Moisture	Fat	Crude fiber	Crude protein	Ash
S1	80.2769	0.521	1.189	1.1875	0.554
S2	79.8222	0.492	1.381	1.3125	0.541
S3	79.8397	0.550	1.221	1.3125	0.5125
Mean±SD	**79.97±0.26**	**0.52±0.03**	**1.26±0.57**	**1.27±0.07**	**0.53±0.02**
Sun drying					
S1	12.37959	1.520	3.692	4.681	2.6144
S2	12.40619	1.451	3.671	4.672	2.5915
S3	12.43522	1.552	3.499	4.375	2.6693
Mean±SD	**12.41±0.47**	**1.51±0.05**	**3.62±0.85**	**4.57±0.17**	**2.62±0.04**
Oven drying					
S1	10.49722	2.011	3.811	5.375	2.850
S2	11.0069	1.950	3.703	5.812	2.869
S3	11.43752	1.922	3.911	4.562	2.8654
Mean±SD	**10.98±0.03**	**1.96±0.04**	**3.80±0.66**	**5.24±0.63**	**2.86±0.01**

S1= sample 1, S2= sample 2; S3= Sample 3

Table 1. 2. Carotenoid content in Fresh and OFSP flour (wet basis)

Sample	Fresh	Sun drying	Oven drying
S1	5467.3	15929.11	22229.29
S2	5469.22	15947.05	22286.49
S3	5638.98	16033.83	22345.37
Mean ± **SD**	**5525.17±80**	**15970±45**	**22287± 47**

Table 1. 3. Carotenoid content in Fresh and OFSP flour (dry basis)

Sample	Fresh	Sun drying	Oven drying
S1	27295.66	18186.55	24971.01
S2	27305.47	18206.24	25035.04
S3	28152.71	18305.23	25102.01
Mean ± **SD**	**27584.61±41**	**18232.67±51**	**25036.02±53**

Table 1. 4. Physical properties of Biscuits

	Formulations						
Water activity	A0	A	B	C	AE	BE	CE
S1	0.381	0.271	0.311	0.292	0.281	0.273	0.271
S2	0.380	0.270	0.314	0.290	0.280	0.269	0.270
S3	0.380	0.270	0.306	0.290	0.280	0.268	0.269
Mean ± SD	**0.38±00**	**0.27±0.00**	**0.31±0.00**	**0.29±0.00**	**0.28±0.00**	**0.27±0.00**	**0.27±0.00**
Weight							
S1	6.8423	7.0088	6.7831	6.7121	7.206	6.6094	6.8012
S2	7.0121	6.9156	6.5101	6.5001	6.8829	6.7701	6.6001
S3	7.1001	6.9901	6.6904	6.3801	6.8524	6.6114	6.4379
Mean ± SD	**6.98±0.1**	**6.97±0.4**	**6.66±0.1**	**6.53±0.2**	**6.98±0.1**	**6.66±0.1**	**6.61±0.2**
Diameter							
S1	47.010	46.51	45.44	45.52	47.77	46.77	46.54
S2	47.210	47.20	46.44	46.50	46.62	46.83	45.52
S3	46.840	46.59	45.59	44.29	46.58	46.82	44.45
Mean ± SD	**47.02±0.1**	**46.76±0.4**	**45.82±1**	**45.43±1**	**46.99±1**	**46.80±0.3**	**45.50±1**
Color							
L*							
S1	47.55	48.00	42.80	41.60	50.60	39.90	40.60
S2	51.93	49.50	43.11	41.70	49.60	40.30	42.00
S3	44.42	48.20	43.80	41.50	49.50	40.10	42.00
Mean ± SD	**47.96±3**	**48.56±1**	**43.23±0.5**	**41.6±0.1**	**49.9±0.6**	**40.10±01**	**41.53±1**
a*							
S1	4.50	10.12	11.69	11.60	11.98	13.40	12.50
S2	5.39	9.09	11.53	11.80	11.09	13.80	12.81
S3	4.70	9.09	10.85	11.90	9.51	13.80	12.80
Mean ± SD	**4.86±0.4**	**9.43±0.5**	**11.36±0.3**	**11.76±0.1**	**10.86±1**	**13.66±0.2**	**12.70±0.1**
b*							
S1	19.81	48.1	41.09	38.90	51.39	40.20	41.45
S2	19.89	50	41.29	38.80	50.22	40.10	40.95
S3	21.07	48.2	41.41	38.70	50.38	40.00	39.51
Mean ± SD	**20.26±0.7**	**48.76±1**	**41.26±0.2**	**38.8±0.1**	**50.66±0.6**	**40.1±0.1**	**40.63±1**

Table 1. 5. Proximate composition of biscuit

	Formulations						
Moisture	A0	A	B	C	AE	BE	CE
S1	4.9978	4.1590	3.3282	3.1787	4.8115	3.5433	3.4158
S2	4.6099	4.1010	3.2905	2.9726	4.1814	3.1692	3.1093
S3	3.505	4.6909	3.3724	3.4796	4.1093	3.3872	3.2228
Mean ± SD	**4.37±0.6**	**4.32±0.26**	**3.33±0.04**	**3.21±0.21**	**4.37±0.33**	**3.37±0.1**	**3.25±0.14**
Crude Protein							
S1	6.531	6.501	5.56	4.85	7.855	6.672	5.49
S2	6.592	6.214	5.75	4.86	7.731	6.622	5.52
S3	6.642	6.303	5.72	4.79	8.155	6.424	5.45
Mean ± SD	**6.59±0.05**	**6.33±0.11**	**5.67±0.1**	**4.83±0,03**	**7.91±0.61**	**6.57±0.13**	**5.48±0.03**
Crude fiber							
S1	0.510	0.520	0.890	1.510	0.643	0.952	1.551
S2	0.665	0.610	0.960	1.600	0.671	0.990	1.612
S3	0.621	0.630	0.880	1.550	0.531	1.112	1.501
Mean ± SD	**0.59±0.07**	**0.58±0.05**	**0.91±0.04**	**1.55±0.04**	**0.61±0.06**	**1.01±0.08**	**1.55±0.5**
Ash							
S1	1.289	1.021	1.527	2.087	1.162	1.619	2.194
S2	1.279	1.002	1.612	2.043	1.277	1.624	2.148
S3	1.209	1.745	1.568	2.039	1.315	1.684	2.112
Mean ± SD	**1.25±0.04**	**1.26±0.34**	**1.56±0.04**	**2.06±0.02**	**1.25±0.07**[c]	**1.64±0.03**	**2.15±0.01**
Fat							
S1	19.381	19.003	20.798	22.171	20.861	20.581	22.11
S2	18.622	18.351	20.910	22.790	20.532	20.689	22.572
S3	17.991	18.727	20.256	22.430	19.994	20.722	22.751
Mean ± SD	18.66±0.56	18.70±0.27	20.65±0.28	22.46±0.25	20.46±0.3	20.66±0.1	22.48±0.3
Carbohydrate	**68.78**	**68.81**	**67.88**	**65.89**	**65.40**	**66.75**	**65.09**
Gross energy							

Table 1. 6. Carotenoid content in biscuits

	Formulations						
Treatments	A0	A	B	C	AE	BE	CE
S1	96.08	5612.412	11632.17	14709.16	5595.388	11958.68	15425.21
S2	95.44	5501.522	11439.75	14673.85	5612.231	12022.57	15475.1
S3	96.57	5616.539	11632.17	14683.48	5632.301	12022.57	14852.11
Mean ± SD	96.03±0.46	5576.8±53	11568±111	14689±18	5613.3±18	12001±36	15251±46

Appendix II.

Sensory evaluation of biscuits

The acceptability of the biscuits was evaluated using a 9-point hedonic scale. Quality parameters of the biscuits (color, taste, flavour, and the overall acceptability) were evaluated. Judges were requested to indicate their preference using the panelist method for a nine-point hedonic scale presented below.

Appreciation	codes
Dislike extremely	1
Dislike very much	2
Dislike moderately	3
Dislike slightly,	4
Neither like nor dislike,	5
Like slightly,	6
Like moderately,	7
Like very much	8
Like extremely.	9

Food sensory analysis data score (mean ± standard deviation for fifty judges)were reported in the following sheet.

Table 2. 1. Reported data in sensory analysis of biscuits

Sample code	Sensory attributes / 9 points			
	Color	Taste	Flavor	Overall acceptability
A0	7.68±0.89	7.74±0.81	7.48±1.14	7.63±0.94
A	7.12±1.65	6.94±1.23	7.03±1.24	7.23±1.03
B	6.88±1.52	7.12±1.30	7.09±1.26	7.24±1.13
C	6.44±1.35	6.29±1.64	6.46±1.69	6.36±1.44
AE	7.21±1.5	6.59±1.7	6.74±1.68	6.87±1.4
BE	6.64±1.6	6.72±1.6	6.71±1.41	6.81±1.4
CE	6.80±1.4b	6.48±1.6	6.51±1.75	6.72±1.55

Appedix III.

3.1 Processessing of Orange-Fleshed Sweet Potato and catotenoid extraction

The processing of fresh OFSP to flour and the extraction of carotenoid are presented in different figures below.

<table>
<tr><td>
Figure 3.3. 1 Washed OFSP roots</td><td>
Figure 3.3.2 OFSP washed ready for peeling</td></tr>
<tr><td>
Figure 3.3.3 peeled and sliced of OFSP</td><td>
Figure 3.3.4 Oven Drying OFSP sample</td></tr>
<tr><td>
Figure 3.3.5 Sun drying OFSP sample</td><td>
Figure 3.2 raw material crude palm oil</td></tr>
</table>

Figure 3.3. 1 Washed OFSP roots   washed ready for peeling

Figure 3.3. 2 OFSP washed ready for peeling

Figure 3.3. 3 Peeled and sliced of OFSP 2-3 mm

Figure 3.3. 4 Oven Drying OFSP sample

Figure 3.3. 5 Sun drying OFSP sample

Figure 3.2 raw material crude palm oil

3.7 Carotenoid extraction and determination

The process of carotenoid extraction and determination are presented in figures billow.

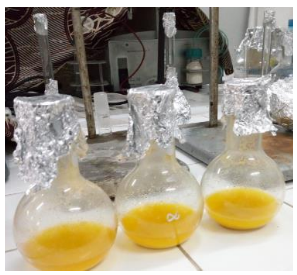
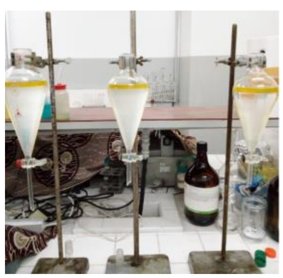
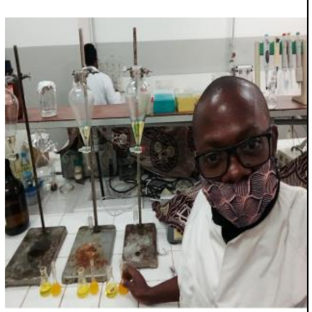
 Figure 3.7.1	 Figure .3.7.2	 Figure 3.7.3	 Figure 3.7.4
 Figure 3.7.5	 Figure 3.7.6	 Figure 3.7.7	 Figure 3.7.8
 Figure 3.7.9	 Figure 3.7.10	 Figure 3.7.11	 Figure 3.7.12

Figure 3.7.1 Crunched of fresh OFSP

Figure 3.7.2 to 3.7.4 Carotenoids extraction

Figure 3.7. 5 to 3.7.8 Washing and partition phase

Figure 3.7. 9 to 3.7.10 dilution and determination of carotenoid

Figure 3.7. 11 to 3.7.12 Some equipment we used (centrifuges and Spectrophotometer)

3.4. 2 Biscuit preparation and materials used

The figures billow is related to the biscuit's preparation and sensory evaluation


Figure 3.4.1


Figure 3.4.2


Figure 3.4.3

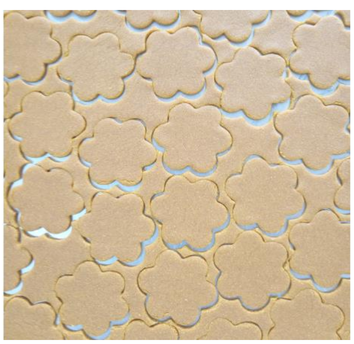


Figure 3.4.5


Figure 3.4.6


Figure 3.4.7


Figure 3.4.8


Figure 3.4.9


Figure 3.4.10

Figure 3.4.1; Raw material used for biscuit preparation

Figure 3.4.2; Mixing of palm oil, margarine, sugar, salt

Figure 3.4.3; Figure 3.4.4 Mixing and sheeting after molding

Figure 3.4.7 to Figure 3.4.9 biscuits sample after baking

Figure 4.3.10 sensory evaluation of biscuits

Figure 3.4.6 the Oven used for biscuit preparation