Composition of Oil from selected Traditional and improved Groundnuts (*Arachis hypogea*) and Oyster nuts (*Telfairia pedata*) grown in Uganda

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Declaration and Approval

I, Juliet Hatoho Musalima, declare that this thesis is original and has never been submitted to any University or any other institution for the award of a Doctor of Philosophy or any other award.

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Dedication

This work is dedicated to my children Amwiine Bella, Mwiine Ryan and Muhaise Elber as a future inspiration.

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Abbreviations and Acronyms

AACC	American Association of Cereal Chemists
AAS	Atomic Absorption Spectrophotometer
ALA	Alpha Linolenic Acid (C18.3ω3)
AV	Acid Value
EFSA	European Food Safety Authority
FA	Fatty Acids
FAME	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization of the United Nations
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IV	Iodine Value
Kg	Kilogram
LA	Linoleic Acid (C18.2ω6)
µg	Microgram
MUFA	Monounsaturated Fatty Acids
NaSARRI	National Arid and Semi-Arid Research Resources Institute
PUFA	Polyunsaturated Fatty Acids
PV	Peroxide Value
RDI	Recommended Daily Intake
SFA	Saturated Fatty Acids
USA	United States of America
Uv-vis	*Ultraviolet–visible* Spectroscopy
ω3	Omega-3
ω6	Omega-6

Abstract

Groundnuts and oyster nuts are important sources of edible oil, protein and micronutrients. Chemical composition of these nuts is affected by genetic and environmental factors as well as thermal treatment. The study determined chemical composition and oxidative stability of oil from groundnuts and oyster nuts grown in Uganda. Six traditional groundnut cultivars; Acholi white, *Igola*, *Egoromoit*, *Rudu* red, *Rudu* white, Red beauty and 14 improved cultivars; Serenut 1 to 14 were examined. Serenut cultivars were obtained from the National Semi-Arid Research Resources Institute in Serere District, Uganda while local cultivars were obtained from Soroti and Katakwi Districts in Eastern Uganda. Oyster nuts were obtained from three districts; Dokolo in North, Luwero in Central and Kamuli in Eastern Uganda. Oil from raw samples and heat treated nuts was analysed for fatty acid (FA) composition. Oil from raw nuts was analysed for antioxidant vitamins, minerals and tannins. Fatty acids were determined by Gas Chromatography-Mass Spectrometry with a Flame Ionisation Detector. Iodine value and lipid health indices were calculated from FA composition data. Vitamin A and E were analysed by High Performance Liquid Chromatography, beta-carotene and tannins by Visual Spectrometry while minerals by Atomic Absorption Spectrometry. All assays were done in triplicate. Analysis of variance was used to determine differences in composition was done using SPSS version 23. Traditional and improved groundnut cultivars had high oil yield ranging from 26.63 to 54.60%. Significant variability ($p<0.05$) was detected in oil yield among all cultivars. Oyster nuts yielded 45.32 to 56.14% oil. Acid value, peroxide value and iodine value of raw groundnut and oyster nut oil were within the recommended levels. Peroxide value of oil from stewed nuts was within recommendations. Raw groundnut oil contained 39.83 to 55.89% oleic, 20.23 to 35.59% linoleic and 11.90 to 17.17% palmitic acid as the major FA. Oil from Acholi white had high level of saturated and polyunsaturated FA compared to other cultivars. Raw oyster nut oil contained 41.50 to 44.87% linoleic, 33.58 and 38.75% palmitic and 9.48 to 13.65% stearic acid. Oleic, alpha linolenic, gamma linolenic and eicosenoic acid occurred in trace amounts. Polyunsaturated to saturated FA ratios were greater than the minimum level; 0.45 established by FAO/WHO. Omega-6 to ω3 ratio was beyond the recommended level of 4:1. Atherogenic and thrombogenic indices were <1 while hypocholesterolemic to hypercholesterolemic index was >1. Iodine decreased while peroxide value increased significantly ($p<0.05$) during processing. There was only slight modification (<10%) of the fatty acids composition during heat treatment and roasting at 179.3°C for 15min mostly caused decrease in polyunsaturated fatty acids. Stewing at 92°C for 15min appeared to be the better processing method for cooking of groundnuts and oyster nuts regarding conservation of linoleic acid and stability to oxidation. Vitamin A content of oil varied from not detected to 559 µg Retinol Activity Equivalents (RAE)/100 g. The highest level was detected in oil from Acholi white while none was detected in *Igola*, *Rudu* red and Red beauty; all traditional cultivars. Vitamin A in oil from improved cultivars ranged from 1.02 to 208.8 µg RAE/100 g in oil from Serenut 4Tan and 9Tan, respectively. Beta-carotene in oil ranged from 0.21 mg/100 g in *Egoromoit* to 1.72 mg/100 g in *Rudu* red while α-tocopherol ranged from 0.88 mg/100 g in Acholi white to 7.19 mg/100 g Serenut 13 Tan. Vitamin A in oyster nut oil ranged from 23.84 to 29.17 µg RAE/100 g. Oil from Kamuli nuts had the highest yield and nuts from Dokolo had the lowest. Beta-carotene in oil ranged from 2.65 to 3.69 mg/ 100 g. Nuts from Kamuli had the highest levels. Vitamin E (α-tocopherol) in oil ranged from 1.03 to 1.77 mg/100 g. The highest level of vitamin E was found in nuts from Dokolo. Calcium content ranged from, 0.05 to 3.41 mg/100 g; magnesium from 0.66 to 3.49 mg/100 g, iron from 0.02 to 0.35 mg/100 g and 0.05 to 0.55 mg/100 g zinc. Calcium content was low in traditional cultivars and the reverse was true for magnesium in comparison to improved cultivars. Iron and zinc occurred in trace

amounts. Levels of calcium, magnesium, iron and zinc in oyster nut oil varied from 16.55 to 31.51 mg/100 g, 42.15 to 64.04 mg/100 g, 0.16 to 0.22 mg/100 g and 0.23 to 0.43 mg/100 g, respectively. Calcium was highest in oil extracted from Luwero nuts while nuts from Kamuli had the highest magnesium. Iron and zinc occurred in trace amounts (<1.0 mg/100 g) in oil. Levels of minerals were low compared to the Recommended Daily Intakes of; 1000 to 1300 mg, 190 to 260 mg, 7.5 to 19.6 mg and 3 to 6 mg for calcium, magnesium, iron and zinc, respectively. Tannin levels in groundnut oil ranged from 531.80 to 852.25 µg/100 g. The highest levels were observed in *Rudu* red, *Rudu* white, *Egoromoit* and Red beauty. Tannins in oyster nut oil ranged from 340.85 to 440.06 µg/100 g. Oyster nut and groundnuts had high oil yield comparable to other commercial oil seeds. The oils were rich in the essential FA linoleic acid but alpha linolenic was a limiting FA in both oil types. Oil was low in beta-carotene, vitamin A and minerals. Oyster nut was low in vitamin E while consuming 200 to 300 g of oil groundnut oil could provide considerable level of vitamin E. Tannins in oyster nut and groundnut oil were within the safe range of <1500 µg/100 g established by the European Food Safety Authority.

Keywords: composition, groundnuts, heat treatment, linoleic acid, oil, oleic acid, oyster nut, Uganda

CHAPTER ONE

1 Introduction

1.1 Background

Nuts are important sources of nutrients for humans and animals. Major producers of nuts are China, India, Iran, North America and Turkey, (FAOSTAT, 2018). Groundnut is the 6th most important oil seed crop in the world (Usman et al., 2013). World production of groundnuts in 2018 was estimated at 45.95 million metric tons (FAOSTAT, 2018). China is the largest producer accounting for 37.85% of World production followed by India with 14.57% of the total production. Top groundnut producers in Africa include Nigeria and Sudan accounting for 20.18% and 20.15% of total groundnut crop (FAOSTAT, 2018). In Uganda, groundnut is the second most widely grown legume after the common bean, *Phaseolus vulgaris* (Mugisha, Lwasa, and Mausch, 2014). Annual groundnut production was estimated at 242,243 metric tons in 2018 (FAOSTAT, 2018). Groundnuts, in Uganda, are mostly consumed as a stew popularly referred to as '*ebinyebwa*' accompanying a starchy meal or roasted as snacks and to a small extent used to produce oil.

Groundnuts contain about 44 to 56% edible oil that is low in saturated fatty acids (SFA) and high in unsaturated fatty acids (Achola et al., 2017). The dominant polyunsaturated fatty acid (PUFA) is linoleic acid (C18.2 omega-6) while the abundant monounsaturated fatty acid (MUFA) is oleic acid (C18.3 omega-3) (Kris-Etherton, 1999; Ros and Mataix, 2006; Kris-Etherton et al., 2008). Essential fatty acids (linoleic acid (C18.2ω6) and linolenic acid (C18.3ω3) in the oil influence its health benefits. Regular consumption of oil from groundnuts is associated with improved anti-oxidant potential, hypo-cholesterolemic, cardio-protective, anti-cancer, anti-inflammatory, and anti-diabetic benefits (Ros, 2010; Vadivel et al., 2012). Due to their lack of cholesterol, groundnuts are promoted as healthy foods which contribute to reduction of low density lipoprotein cholesterol (LDL) in the body

(Orsavova et al., 2015). Nutritional and cardio-protective roles are based on lipid health indices such as polyunsaturated to saturated FA ratio, atherogenic, thrombogenic and hypocholesterolemic to hypocholesterolemic indices of oil. Groundnut oil contains considerable amounts of vitamin E that plays a role in retardation of oxidative deterioration of edible oil. In the body, vitamin E slows the ageing process and inflammation (Eitenmiller and Lee, 2004).

Groundnuts contribute to diets as a protein source and as a source of income especially in Eastern and Northern Uganda. The groundnut research program at the National Arid and Semi-Arid Research Resources Institute (NaSARRI) in Serere is focused on producing varieties with high yielding potential, high quality, resistance to disease and quick maturity period (Okello and Biruma, 2010). The improved cultivars presently promoted in Uganda include Serenut 1 to 14. Although groundnuts have received a lot of research attention with regard to agronomy, little information is available on the nutritional profile of their oil.

On the other hand, oyster nuts are grown in East Africa (Uganda, Kenya, and Tanzania) and Northern Mozambique (Ajayi et al., 2004; van der Vossen and Mkamilo, 2007). The plant bears long pods containing many sand coloured, flat seeds. The seeds are harvested when pods fall and split open releasing the seeds. Oyster nuts are grown for their seed and oil. They contain 40 to 60% extractable oil and 25 to 30% protein (Pareek and Sharma, 2009). Oyster nut oil is regarded as good quality cooking oil which can also be used for salad dressing in the confectionary. Oyster nut oil also has industrial uses in cosmetics as well as soap making (Pareek and Sharma, 2009). Oyster nuts are consumed raw, roasted or crushed into a paste and mixed with stew. Oyster nuts are grown mainly for subsistence and for the local market. Data on production of and statistics of consumption in Uganda are limited.

This study characterized oil from *Arachis hypogea* and *Telfairia pedata* grown in Uganda, on the basis of its fatty acids, anti-oxidant vitamins, minerals and tannins composition.

1.2. Problem Statement

Nuts contain substantial amounts of oil, proteins, essential fatty acids, antioxidant vitamins and minerals. They are also an important source of income for rural communities in Uganda at subsistence level. Until recently, research on groundnuts in Uganda was biased on agronomic studies. There is scanty information in literiture on the nutrient profile of groundnut or its products. In addition, the National Semi-Arid Research Resources Institute (NaSARRI) in Serere undertook to improve groundnuts leading to the release of improved groundnuts (Serenut 1 to 14). Little information is available on improved groundnut oil in comparison with the local groundnut landraces. Similarly, there is inadequate information in literature about the nutrient profile of oyster nut oil. Nuts are cooked mostly by roasting or stewing to improve their digestibility and to reduce the level of anti-nutrients in them. There is little available data on the effect of thermal treatments such as roasting and stewing on nutrients in oil derived from oyster nuts and groundnuts grown in Uganda. Lack of such information limits optimal utilisation and marketing of groundnuts and oyster nuts. This study examined the yield and stability of oil, the fatty acid composition, antioxidant vitamins, minerals and tannins in groundnut and oyster nut oil.

1.3 Objectives

1.3.1 General Objective

To determine chemical composition of oil extracted from traditional and improved groundnuts (*Arachis hypogea*) and oyster nuts (*Telfairia pedata*) grown in Uganda with the view of promoting better cultivars for economic and nutritional exploitation from an informed point of view.

1.3.2 Specific Objectives

The specific objectives were;

1. to determine yield and stability of oil extracted of oil extracted from traditional (Acholi white, *Rudu* white, *Rudu* red, *Egoromoit* and Red beauty) and improved (Serenut 1 to 14) groundnuts as well as oyster nuts grown in Uganda.
2. to determine the fatty acid composition of oil extracted from groundnuts and oyster nuts before and after heat treatment.
3. to determine health lipid indices of oil extracted from groundnuts as well as oyster nuts.
4. to determine content of anti-oxidant vitamins (A, E and beta-carotene) in oil extracted from groundnuts as well as oyster nuts.
5. to determine levels of magnesium, calcium, iron, zinc present in oil extracted from traditional and improved groundnuts as well as oyster nuts.
6. to analyze levels of tannins present in oil extracted from traditional and improved groundnuts as well as oyster nuts.

1.4 Justification

Groundnut production in Uganda was about 242,243 metric tons in 2018 (FAOSTAT, 2018). This was a leap production compared to 216,828 metric tons in 2017 and 210,000 metric tons in 2016 (FAOSTAT, 2018). Soroti District presented the highest groundnut production estimated at 19,599 tonnes per annum. In the National Development Plan for Uganda NDP II 2015/16 – 2020/21, the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) aimed at increasing sustainable production, productivity and value addition in key growth opportunities including the edible oil sector (MAAIF, 2015). Currently, however, Uganda imports about 70% of its edible oil from Malaysia and Indonesia (MAAIF, 2015). Groundnuts and oyster nuts are potential sources of edible oil. Despite being major oil seeds, there is little exploitation of groundnuts and oyster nuts in Uganda for oil production.

Oil production from oyster nuts and groundnuts could boost the Uganda edible oil industry and ultimately reduce the edible oil importation through promotion of the local industry which is currently at 70% (MAAIF, 2015). This includes; growers, breeders and industrialists who could earmark and promote particular cultivars for oil production based on results of this study. Composition of edible oil is affected by factors such as maturity, environment, cultural practices, variety and soil temperature (Shahidi, 2005; Jonnala, Dunford and Dashield, 2006). Fatty acids such as oleic (C18.1) and linoleic (C18.2ω6) acid have been reported to lower total cholesterol in the body and may therefore reduce cardiovascular risk (Akhtar et al., 2014; Özcan, 2010). Information about nutrient profile of groundnut and oyster nut oil would be of benefit nutritionists to know cultivars with better nutrient profile and breeders to identify potential donors of desirable traits for breeding. Information about heat treatment would provide baseline information about heat stability of fatty acids in groundnut and oyster nut oil. Findings of this study would contribute to the

database of Ugandan crops and their products. Ultimately this would benefit consumers and users of groundnut oil to consume from an informed perspective.

1.5 Hypotheses

1. There is no difference in oil yield, stability and essential fatty acid composition in oil from traditional and improved groundnuts or oyster nuts.
2. There is no difference in anti-oxidant vitamins, minerals and tannins in oil from traditional and improved groundnuts or oyster nuts.
3. There is no difference in the thermal response of essential fatty acid and stability of oil from groundnuts and oyster nuts.

CHAPTER TWO

2 Literature Review

2.1 Background on Nuts and seeds

Edible nuts include tree nuts, seeds and certain legumes. Botanically, the term "nut" refers to an indehiscent fruit that is usually shed as a one seeded unit. The pericarp is usually lignified (McNeil, Jackson, and Morley-Bunker, 2017). They are valued for their sensory, nutritional, and health attributes (Ventachalam and Sathe, 2006). Nuts that are consumed include; tree nuts such as hazelnuts (*Corylus avellana*), macadamias (*Macadamia integrifolia*) and pecans (*Carya illinionensis*). Seeds include Brazil nuts (*Bertholletia excelsa*), almonds (*Prunus amigidalis*), pistachios (*Pistachia vera*), oyster nuts (*Telfairia pedata*) and cashew nuts (*Anacardium occidentale*). Legumes in the nut category are represented by groundnuts (*Arachis hypogea*) and bambara nuts (*Vigna subterranean*). In Uganda groundnuts, shea nuts, oyster nuts are popular sources of oil (Okullo et al., 2010; Mugisha, Lwasa, and Mausch, 2014).

2.1.1 Production and Consumption of Nuts and Seeds

Production and consumption data vary according to particular nut. World production of tree nuts is highest in the United States, India, Iran, Turkey, and China. Major producers of groundnuts are China, India, and the United States (Varshney, Pandey, and Puppala, 2017). In 2017, almond were the most produced and consumed nuts globally at 2,161,199 and 2,032,227 metric tons respectively (McNeil, Jackson, and Morley-Bunker, 2017). This was followed by walnuts and pistachios with respective production levels of 1,214,990 and 508,270 metric tons (McNeil et al., 2017). World over, the per capita consumption of nuts is low but is improving due to inclusion of nuts in guidelines for healthy eating in some

Western countries (Ros, 2010). Nuts are part of the healthy Mediterranean diet but their per capita consumption is as low as 2 to 9 kg (Brufau et al., 2006). The largest consumers of nuts in Africa include; South Africa, Morocco, Nigeria and Egypt where macadamias, cashew nuts and shea nuts are the most popularly consumed nuts. Groundnuts are important oil seed grown and consumed world-wide with annual production levels in excess of 45 million metric tons (FAOSTAT, 2018). Groundnuts make a significant contribution to nutrition and livelihood improvement in sub-Saharan Africa (Okello, Biruma, and Deom, 2010).

2.1.2 Nutrient Composition of Nuts and Seeds

In developing countries, nuts and groundnuts are the primary source of digestible protein with amounts varying between 25 and 34%, cooking oil with yield ranging from 44 to 56%, and then vitamins in relatively high proportions. Importantly, nuts constitute complex matrices rich in unsaturated FA and bio-active compounds such as tocopherols, phytosterols and phenolic compounds. They contain proteins, carbohydrates and fibre (Brufau et al., 2006; Ros and Mataix, 2006; Carughi et al., 2015). Total fat content of nuts ranges from 46% in cashew nuts and pistachios to 76% in macadamia nuts (Carughi et al., 2015; Table 1). Oil from nuts are constituted of low levels of saturated fatty acids (SFA) and rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Kris-Etherton et al., 1999; Ros and Mataix, 2006). Groundnuts are particularly rich in MUFA (Mckevith, 2005). These properties of the nuts make them reliable raw material for enhancing nutrients in mainly cereal diets of maize, millet and sorghum in product development.

Nuts are cholesterol free but contain sizable amounts of phytosterols that are essential in membrane structure (Yang, Liu, and Halim, 2009). When present in sufficient amounts in

the intestinal lumen, phytosterols interfere with cholesterol absorption and hence lower blood cholesterol (Ros, 2010). Nuts contain substantial amounts of calcium, potassium and magnesium but their sodium content is low (Carughi et al., 2015).

2.2 Groundnuts

Groundnut, *Arachis hypogea*, is the 6th most important crop in the world valued by both small holders and commercial traders (Usman, Taiwo, Haratu, and Abubakar, 2013). Groundnut is an annual self-pollinating herbaceous legume that grows to a maximum height of 60 cm (Okello et al., 2015). This annual herbaceous legume is also known as groundnut, monkey nut, earthnut, gobber, gobber pea, groundnut vine, potato bean, wild bean, earth-ball, *Chang Sheng Guo* (Long-life nuts), pygmy nut and pig nut (Özcan, 2010). Groundnuts are distinguished from other plants by flowering above the ground but producing fruits below the surface (Okello et al., 2014). Groundnut cultivation was also active in Ecuador, Bolivia, Brazil, and the Caribbean.

In Uganda, groundnut is among the main staple crops because it contains high amounts of oil widely used in food preparation. It is becoming a cash crop with notable increase in both production area and productivity. This is evident by the significant expansion of the industry in Uganda and with spillovers in the neighboring countries (Okello, Monyo, Deom, Ininda, and Oloka, 2013). The National Groundnuts Improvement Programme has developed groundnuts varieties to meet the challenge of persistent pests and diseases (Okello, Biruma, and Deom, 2010).

Table 1: Nutrient composition of common nuts per 100 g

Nut	Energy (kcal)	Fat (g)	SFA (g)	MUFA (g)	PUFA (g)	Protein (g)	Fibre (g)	Tocopherols (mg)	Carotenoids (mg)	Calcium (mg)	Magnesium (mg)	Iron (mg)
Almonds	575	50.6	3.9	32.2	12.2	21.3	8.8	25.0	2.0	248	275	1.4
Brazil nuts	656	66.4	15.1	24.5	20.6	14.3	8.5	4.0	nd	160	376	2.7
Cashews	553	46.4	9.2	27.3	7.8	18.2	5.9	1.0	nd	37	292	3.3
Hazel nuts	628	60.8	4.5	45.7	7.9	15.0	10.4	33.0	106.0	114	163	1.0
Macadamias	718	75.8	12.1	58.9	1.5	7.9	6.0	4.0	nd	85	130	0.9
Groundnuts	**567**	**49.7**	**6.9**	**24.6**	**15.7**	**23.7**	**3.1**	**8.0**	**nd**	**92**	**168**	**2.3**
Pecans	691	72.0	6.2	40.8	21.6	9.2	8.4	4.0	55.0	70	121	4.0
Pine nuts	673	68.4	4.9	18.8	34.1	13.7	3.7	6.0	nd	16	251	2.1
Pistachios	557	44.4	5.4	23.3	13.5	20.6	9.0	7.0	332.0	107	121	0.9
Walnuts	654	65.2	6.1	8.9	47.2	15.2	6.4	6.0	nd	98	158	1.9

Adopted from Carughi et al. (2015), nd: not determined;SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs.

Although, several varieties have been introduced and are currently being experimented in Eastern Uganda and other parts of the country, limited information is available on the nutrients.

2.2.1 Agronomy

Groundnuts are divided into two subspecies, *hypogaea* and *fastigiata*. *Arachis hypogaea* subspecies *hypogaea* has two varieties *hypogaea* and *hirsuta* while *Arachis hypogaea* subspecies *fastigiata* has four varieties i.e. *fastigiata, vulgaris, peruviana and aequatonaria*. These differ in plant, seed and pod characteristics (Okello et al., 2016). Commercial varieties include *Virginia* or *runner* (subspecies *hypogea*), Valencia (subspecies *fastigiata*) and *Spanish* (subspecies *vulgaris*). Ninety percent of the Ugandan market is dominated by *Virginia* and Spanish varieties (Table 2). Most *Valencias* are landraces which are superior in taste and aroma to other market types. However, they are low yielding, and susceptible to the major diseases of groundnuts (Okello et al., 2014). Commercial groundnut varieties released in Uganda from 1966 to 2011 include; *Virginia* cultivars such as Serenut 1Red, 2, 5Red, 7Tan, 8Red, 9Tan, 10Red, 11Tan, 12Red, 13Tan, 14Red. Serenut 3Red, 4Tan and 6Tan belong to the Spanish variety while Red beauty, Acholi white, *Rudu* red and *Rudu* white are Valencias. These varieties are the most popularly grown in the East, North and North Eastern districts of Uganda (Okello, Monyo, Deom, Ininda, and Oloka, 2013).

2.2.2 Production of Groundnuts

Groundnut is the 6th most important oil seed crop in the world (Usman et al., 2013). The World production of groundnuts in 2018 was estimated at 45.95 million metric tons (FAOSTAT, 2018;

Table 3). China is the largest producer accounting for 37.85% of World production. This is followed by India with 14.57% of the total production.

The top groundnut producers in Africa include Nigeria and Sudan accounting for 20.18% and 20.15% of total groundnut crop (FAOSTAT, 2018).

Table 2: Botanical characteristics of improved and traditional groundnut cultivars in Uganda

No.	Cultivar	Release year	Colour	Seed size	Variety
Improved Cultivars					
1.	Serenut 1Red	1998	Red	Large	Virginia
2.	Serenut 2	1998	Tan	Large	Virginia
3.	Serenut 3Red	2002	Red	Medium	Spanish
4.	Serenut 4Tan	2002	Tan	Small	Spanish
5.	Serenut 5Red	2010	Red	Large	Virginia
6.	Serenut 6Tan	2010	Tan	Small	Spanish
7.	Serenut 7Tan	2011	Tan	Large	Virginia
8.	Serenut 8Red	2011	Red	Medium	Virginia
9.	Serenut 9Tan	2011	Tan	Large	Virginia
10.	Serenut 10Red	2011	Red	Medium	Virginia
11.	Serenut 11Tan	2011	Tan	Large	Virginia
12.	Serenut 12Red	2011	Red	Medium	Virginia
13.	Serenut 13Tan	2011	Tan	Large	Virginia
14.	Serenut 14Red	2011	Red	Medium	Virginia
Traditional Cultivars					
15	Acholi white	1966	Off white	Medium	Valencia
16	*Egoromoit*	na	Tan	Large	Local
17	*Rudu* white	na	White	Medium	Local
18	*Rudu* red	na	Red	Medium	Local
19	Red beauty	1966	Red	Medium	Valencia
20	*Igola*	1995	Red/white striped	Large	Virginia

Source: Okello et al. (2012; 2013; 2016); na, not available.

In Uganda, groundnut is the second most widely grown legume after *Phaseolus vulgaris*, the common bean (Apalia, Alia, and Namera, 2006; Mugisha et al., 2014). The annual groundnut production was estimated at 242,243 metric tons in 2018 (FAOSTAT, 2018; Table 4).

Groundnut production has been gradually growing since 1991 (Shiferaw, Muricho, Okello, Kebede, and Okecho, 2010). From 2012 to 2014, Uganda groundnut production remained almost constant ≈ 295,000 metric tons from nearly 422,000 hectares mostly in the Northern and Eastern regions where it is now part of peoples culture (Okello et al., 2010, FAOSTAT, 2017).

Table 3: Groundnut production trends among top producers in million metric tons

Ranking	Country	2014	2015	2016	2017	2018
1	China	16.55	16.02	16.42	17.12	17.39
2	India	9.25	7.46	6.73	7.40	6.70
3	Nigeria	3.40	3.47	3.58	2.42	2.87
4	USA	3.23	2.53	2.81	2.35	2.48
5	Argentina	1.03	1.00	1.01	1.12	0.92
6	Indonesia	0.50	0.57	0.61	0.64	0.46
7	Senegal	0.67	1.05	0.72	0.92	0.85
	Others	10.97	12.12	12.66	15.47	14.28
	World	45.60	44.22	44.54	47.44	45.95

Source*:* FAOSTAT (2018)

The districts where groundnut production is predominant include; Nakasongola in Central, Soroti in Eastern, Amuru in Northern and Kibaale in Western regions. Data collected by Uganda Bureau of Statistics and Ministry of Agriculture, Animal Industry and Fisheries in 2008/2009 indicated that the levels of production were; 19.183, 19.599, 14.375 and 124.73 tons, respectively, for the above mentioned districts. The corresponding production totals for the

regions were; 32.757, 77.247, 83.182 and 51.497 tons of groundnuts, respectively (UBOS, 2017).

Table 4: Groundnut production in Uganda

	Year	Production in metric tons
1	2014	295,601
2	2015	210,000
3	2016	210,000
4	2017	216,828
5	2018	242,243

Source: FAOSTAT (2018)

2.2.3 Nutrient composition of groundnuts

Groundnuts are an important source of nourishment providing several long term health benefits due to the presence of essential nutrients and antioxidants (Bishi et al., 2013). As shown in Table 5, groundnuts are rich in macro and micro nutrients. Groundnut composition may vary depending on cultivar, maturity, growing location, stress factors, herbicides, pesticides, infection and atmospheric factors (Rodrigues et al., 2011; 2013).

2.2.3.1 Fatty Acids

Groundnut kernels contain 40 to 50% oil (Asibuo et al., 2008; Gunstone, 2002; Mukri et al., 2012). The oil contains 80% unsaturated FA particularly oleic (C18.1) and linoleic acid (C18.2ω6) that are beneficial to cardiovascular health. The ratio of oleic to linoleic acid is an

important indicator of nutritional quality and stability of groundnuts and their products (Gulluoglu et al., 2016). Groundnut oil contains both saturated and unsaturated FA (Gunstone and Sanders, 2002). Levels of these FA vary with variety, maturity of seed and environmental conditions (Reddy, 1988; Singh and Singh,1991).

Table 5: Nutrient composition of groundnuts in 100 g

Component	Nutritional value	% RDI
Carbohydrates	16.13 g	12
Protein	25.80 g	46
Total fat	49.24 g	165
Fibre	8.5 g	22
Energy	567 kcal	29
Vitamin A	0 IU	0
Vitamin E	8.33 mg	55.5
Potassium	705 mg	15
Calcium	92 mg	9
Iron	4.58 mg	57
Magnesium	168 mg	42
Selenium	7.2 µg	13
Zinc	3.27 mg	30

Source: USDA, National Nutrient Database for Standard Reference. Release 24, 2011; RDI: Recommended Daily Intake.

Dominant FA in groundnut oil are; oleic (C18.1), linoleic (C18.2ω6) and palmitic (C16.0) constituting about 80% of the total FA composition. Stearic (C18.0) , arachidic (20.0), behenic (C22.0), lignoceric (C24.0) acids occur in less amounts (Savage, and Keenan, 1994; Zahran, and Tawfeuk, 2019). Arachidic acid (C20.0) is the characteristic FA in groundnuts. However, levels beyond 4.8 mg/100 g are indicative of adulteration (Carrín and Carelli, 2010). In addition, C20.0 and C22.0 are useful in the stabilisation and emulsification of peanut butter but are reported to be atherogenic (Savage, and Keenan, 1994). Total FA in groundnut oil vary with variety and other factors. Groundnut oil comprises 80% unsaturated FA and 20% saturated FA (Shasidhar, Vishwakarma, and Pandey, 2017). Typical proportions of oleic acids vary between 21 and 85% while linoleic acid ranges from 2 to 43% in groundnut oil. Linolenic acid (C18.3ω3) content in groundnut oil has been reported to be as low as <1% (Savage, and Keenan, 1994; Rodrigues, Ströher, Freitas, Visentainer, Oliveira, and Souza, 2011; Wang et al., 2012).

Linoleic acid and Alpha-linolenic acids are essential for humans while docosahexaenoic and gamma-linolenic acids are conditionally essential under some developmental or disease conditions (Whitney and Rolfes, 2008). Essential fatty acids (EFA) are beneficial to cardiovascular health, cognitive health (proper impulse transmission), pregnancy, lactation and infancy to ensure proper development of the brain and nervous system (Jumbe et al., 2016). Different EFA pose varying effects in the body for instance; omega-6 FA encourage blood clot formation whereas omega-3 FA reduce clotting. The ideal is to achieve a balance between omega-6 and omega-3 FA (Arbex et al., 2015). According to Simopoulos (2010), the ratio of omega-6 to omega-3 should be 4:1 for good health.

2.2.3.2 Vitamins

According to Krautgartner et al., 2016, groundnuts consists of varying amounts of vitamins A, E, thiamine, riboflavin, niacin, pantothenic, folate, B6 and C (Inuwa, Aina, Gabi, Aimola, and Toyin, 2011; Reddy, 1988; Settaluri, Kandala, Puppala, and Sundaram, 2012; Wang, 2018). However, Reddy (1988) described vitamins A, C and D as limiting vitamins in groundnut. Beta-carotene is the most prevalent carotenoid in groundnut oil and can be detected at a level of 60 µg/l in oil from immature groundnuts. Literature about actual amounts of beta-carotene in mature nuts is scanty, however, the levels are reported to decrease with maturity (Gunstone and Sanders, 2002; Nautiyal, 2002). Other carotenoids including alpha and beta are present in lower amounts (Cobb and Johnson, 1973). Groundnuts also contain tocopherols that act as anti-oxidants in groundnut oil (Wasowicz et al., 2004). Vitamin E occurs in four main isomers in foods α-, β-, γ-, and δ-tocopherols. Average amounts of the above tocopherols in groundnut oil were reported as 6.1 mg/100 g alpha tocopherol, 8.1 mg/100 g β and γ- tocopherol and 1.8 mg/100 g δ-tocopherol (Kornsteiner, Wagner, and Elmadfa, 2006). According to Grilo et al. (2014), α-tocopherol has the highest biological potency. Alpha tocopherol improves immunity, delays ageing, reduces risk of cardiovascular disease and enhances stability of groundnut oil (Wang, 2018).

2.2.3.3 Minerals

Groundnuts contain a wide range of minerals that contribute to health and well-being (Asibuo et al., 2008; Rodrigues et al., 2013; Savage, 2014). The above scholars reported high levels of potassium (600 to 1,693 mg/100 g), magnesium (250 to 308 mg/100 g) and moderate levels of calcium (65 to 134 mg/100 g) in whole groundnut kernels. The amounts of iron, zinc,

manganese, selenium and copper were <10 mg /100 g of groundnut kernels. Asibuo et al. (2008) asserted that cultivar and genotype may affect mineral content of groundnuts. Savage (2014) noted that despite the substantial levels of minerals in groundnuts, there can be losses during handling and processing. Processing on the other hand may add to the mineral content due to tear and wear of machinery. Groundnuts are an excellent source of phosphorous providing about 50% of our daily needs. Minerals maintain healthy fluid and electrolyte balance in the human body while others work as precursors for enzymes, blood and bones. Zinc is a co-factor in many body processes (FAO/WHO, 2010; Jumbe et al., 2016). Raw groundnuts provide 4.53 mg/100 g of zinc (Mustapha et al., 2015). Although appreciable amounts of mineral elements in whole kernels, Mckevith (2005) asserted that vegetable oil does not contain the same amount of macronutrients, vitamins and minerals as whole oil seeds. Detailed information about mineral content of groundnut oil is limited, however, levels of metal ions should be low to minimize chances of oil oxidation (Frankel, 1980).

2.2.3.4 Protein

Groundnuts contain up to 24% protein and they make a significant contribution to nutrition when consumed (Asibuo, 2008; Ayoola and Adeyeye, 2012; Bishi, et al., 2013). Groundnuts contain eleven essential amino acids (Latif, Pfannstiel, Makkar, and Becker, 2013; Table 6), These include; leucine, iso-leucine, tryptophan, lysine, phenylalanine, cysteine, threonine, methionine, tyrosine, histidine and valine. Although it is non-essential, groundnuts contain significant amounts of arginine which is a precursor to nitric oxide that helps to keep arteries relaxed, improving blood flow. Arginine also improves healing time in tissues in the body (Gornik and Creager, 2004).

Table 6: Amino acid composition of groundnuts in g/100 g of crude protein

S.No.	Amino acid	Composition	S.No.	Amino acid	composition
1	Methionine	1.09	10	threonine	2.67
2	Cystine	1.20	11	tryptophan	0.86
3	valine	3.83	12	Serine	4.92
4	Isoleucine	3.21	13	Arginine	11.32
5	Leucine	6.24	14	Glutamic acid	18.72
6	Phenylalanine	5.04	15	Aspartic acid	11.17
7	Tyrosine	3.65	16	Proline	4.32
8	histidine	2.33	17	glycine	5.49
9	lysine	3.35	18	Alanine	3.98

Source :Latif et al. (2013)

2.2.3.5 Anti-nutritional Factors

Anti-nutritional factors present in human or animal foods tend to reduce nutrient utilization or food intake, thereby contributing to impaired gastrointestinal and metabolic performance. Anti-nutritional factors in groundnuts include; trypsin inhibitors, agglutinin, phytates, condensed tannins and α-amylase inhibitors (Qiang et al., 2016). Phytates act as chelating agents that combine with minerals in groundnut oil and reduce chances of its oxidation. A distinguishing factor between tannins and other anti-nutrients is their high molecular weight and ability to function as anti-oxidants (Okuda and Ito, 2011). Tannins like the above mentioned compounds form cross links with proteins and other molecules (West, Hill, and Utley, 1993). Tannins are widespread in foods of plant origin, particularly in legume seeds, cereal grains, fruits,

vegetables, and different beverages such as wine, tea, cocoa, as well as cider (Kunyanga et al., 2011). Research has shown that groundnut skin contain high levels of tannins (Isanga and Zhang, 2007). Given their astringency, tannins in peanut skin may have an influence on the sensory properties of peanut and its products (Ashok and Upadhyaya, 2012). Groundnut skins contain catechol tannins which influence testa colour (Nautiyal, 2002; Woodroof, 1983). Tannins content in groundnut skin vary from 20.5 to 23.8% (West et al., 1993). Amounts of tannins tend to decrease with de-hulling and normal cooking procedures of boiling and roasting (Anuradha, Pradhuman, and Lakshmi, 2017).

2.2.4 Health Benefits of Groundnuts

Groundnut consumption is associated with several health benefits, such as antioxidant, hypocholesterolemic, cardio-protective, anti-cancer, anti-inflammatory and anti-diabetic benefits (Davis et al., 2008; Justyna and Waldemar-Wardencki, 2011; Vadivel et al., 2012). They are good sources of manganese, copper, zinc and phosphorous. Most nuts are rich in magnesium providing 8 to 20% of daily recommended in take of 400 mg in a serving of 28 g (Kris-Etherton et al., 1999). Nuts are excellent sources of vitamin E (King et al., 2008).

Research by Reis et al. (2011) demonstrated that ingestion of 63 g of roasted peanuts per day at breakfast lowered carbohydrate intake and ultimately reduced postprandial glycemic response, which might help improve glycemic control and reduce the diabetes risk. According to the above mentioned scholar, roasting resulted into cleavage of the cell walls after that released the fat content of the peanuts, resulting in the lower glycemic response observed. It is therefore implied that peanut consumption (42.5 to 75 g/day) in a period of three weeks, independent of body

composition alteration, improves glycemic control, induces satiety, and attenuates non-esterified fatty acids (NEFA) concentration. On the whole, inclusion of peanuts in the diet does not only improves the quality of the diet, but stimulates satiety and reduces glycemic response (Reis et al., 2011; Wien, Oda, and Sabaté, 2014). Further observations suggest that regular consumption of peanuts in adequate portions may result in weight control (Wien et al., 2014).

2.2.5 Forms of Utilization

2.2.5.1 Seeds

Groundnuts seeds are consumed directly either raw or roasted. Young pods may be consumed as a vegetable (Martin and Ruberte, 1975). Roasted groundnuts make good accompaniment to coffee and tea and eaten alone even without any beverage. The roasted groundnuts are becoming common in large supermarkets packed in polyethene materials usually in 250 g and in small plastic cups in measures of 250 to 500 g. Raw groundnuts are also sold in its primary forms for further processing. The consumption of raw groundnuts is nowadays rare.

2.2.5.2 Flour

Edible-grade groundnuts with low oil content may be milled into flour and boiled with onions, tomatoes, salt, mushrooms, dried fish, meat or chicken and hot water. It may also be mixed with green vegetables such as cowpea leaves, amaranthus or beans, mushrooms to make a stew. Stew may be vegetarian or prepared with meat.

2.2.5.3. Paste

Groundnut butter sauces are made in Uganda and eaten as a side dish to a starchy staple food e.g. *Matooke* (green bananas), rice, sweet potatoes, cassava, millet/maize bread (*kalo/kawunga*) and yams (Mugisha, Lwasa, and Mausch, 2014). The paste popularly known as *odii* or *kipooli*, is used as a sauce mixed with fish, meat or vegetables to accompany the main dish. It is important in children's diet especially in East, North and North Eastern Uganda. It is also popular with children in boarding schools where it is termed as an appetizer for meals and as a spread.

2.2.5.4 Ingredient

Groundnuts are used as ingredients in various bakery products such as cakes, cookies and confectionaries. Groundnuts used in confectionary are large-seeded, have high sucrose and protein content. They are low in oil and raffinose family oligosaccharides which are known for causing flatulence, bloating and abdominal discomfort (Bishi et al., 2013).

2.2.5.5 Oil

World over, the biggest percentage of groundnuts is used for oil production. Oil may be used in domestic culinary operations or in industry. China and India are the largest producers and consumers of groundnut oil (Arya, Salve, and Chauhan, 2016; Kaiser and Ernst, 2012; Nautiyal, 2002; Salve and Arya, 2018). Oil from groundnuts is considered a premium cooking and frying oil due to its excellent oxidative stability (Varshney, Pandey, and Puppala, 2017). Groundnut oil is considered to be superior to soybean oil during frying because it develops fewer flavour

defects with long-term use (Štokovi et al., 2013; Young, 1996). The oily cake which is a bi-product of the groundnut oil extraction process serves as high protein livestock feed.

2.2.5.6. Other Uses of Groundnuts

Products such as soaps, medicines, cosmetics, pharmaceuticals, emulsions for insect control, lubricants and fuel for diesel engines can be made from groundnut oil (Reddy, 1988; .Wang, Raymer, Chinnan, and Pittman, 2012; Wilson et al., 2013). Groundnut haulms are excellent high protein hay for horses and ruminant livestock (Reddy, 1988; Shiferaw, Muricho, Okello, Kebede, and Okecho, 2010). Groundnut shells are used for fuel (fireplace "logs"), as a soil conditioner, for sweeping compounds, as a filler in cattle feed, as a raw source of organic chemicals (Usman et al., 2013).

2.3 Oyster Nut

Oyster nut is the seed of the climbing African liana tree, *Telfairia pedata.* (Latham, 2008; Pareek and Sharma, 2009). *Telfairia* is classified in the tribe of Joliffieae of the cucurbitaceae family. It comprises three species of which oyster nut is much cultivated for its oil in East Africa while *Telfairia occidentalis* (fluted pumpkin) is grown in West Africa as a vegetable (Lock, Grubben, and Denton, 2004). Oyster nut is native to Tanzania and Northern Mozambique. It is found in the hot and humid areas of Central and Eastern Uganda, Pemba, Mozambique, and Kenya (Ajayi et al., 2004). Oyster nut is referred to as *Mkweme* in Tanzania, *Murekula*, *Mkwini* in Kenya (Nyagah, 2016) and *Kulekula* in Central and *Mulekula* in Eastern Uganda. Other names include, Zanzibar oil vine, queen's nut or *Telfairia* nut.

2.3.1 Agronomy

The genus *Telfairia* consists of two main species, *Telfairia occidentalis* (fluted pumpkin) which is grown in West Africa and *Telfairia pedata* (oyster nut) which is grown in East Africa (Ajayi et al., 2004). Oyster nut plant can be grown from seeds or cuttings. The seeds have short viability and may grow within 1-2 weeks of planting (Owra, Mutua, Kindt, Jamnadass, and Anthony, 2009). Oyster nut grows best in lowland, humid tropical areas at elevations up to 1,000 metres. The plant can grow in light sandy and heavy clay soil but thrives best in in well drained loam soil and tolerate a pH range of 5.5 to 7.0 (van der Vossen and Mkamilo, 2007). It grows best in areas where the annual average temperature are between 14 and 38^{o}C. Oyster nut is favoured by a mean annual rain fall in the range 1,500 to 2000 mm. Oyster nut plants bear a deep tap root and are very drought resistant. Oyster nut plants thrives well in plenty of sunlight but can grow in light shade forests. Oyster nut is dioecious (produces male and female flowers on separate vines) and pollination is by insects although production of seed is possible without the male flower (van der Vossen and Mkamilo, 2007).

2.3.2 Production of Oyster Nuts

Data on production of oyster nuts in Uganda is scarce (Okoli, 2007), however, in 2015, Sseremba, Kabod, and Kasharu (2017), carried out a survey on African indigenous vegetables and reported that the abundance of oyster nut in Uganda was 1%. Oyster nut is a crop with potential for use since it survives harsh conditions of drought, however, according to Latham (2008), most production is done for domestic consumption and local markets. One farmer can produce about 100 gourds per plant per season (Ajayi et al., 2004). The oyster nut plant can produce 10 to 30 pods in its 3rd year and may have an annual yield of 3 to7 tons per hectare

(Lock et al., 2004; van der Vossen and Mkamilo, 2007). The oyster nut plant starts flowering in 15 to 18 months after planting and the fruit ripens 5 to 6 months later. The oyster nut bears long fruits which are about the size of an average watermelon (Figure 1). The fruit weighs about 15 kg; it is 45 to 60 cm long and 20 cm in diameter, and contains between 70 to 150 seeds/fruit (Kinsey, Berkelaar, and Motis, 2013). Seeds are oyster-shaped (Figure 2), flattened, 33 to 40 mm in diameter, 10 to 13 mm thick.

Figure 1: Oyster nut pod

Figure 2: Oyster nuts

2.3.3 Nutritional Composition

Oyster nuts are grown for their edible seeds and oil. Ccomposition of 100 g oyster nut kernel (dry weight) is; water 3.5 g, protein 27 g, and fat 66 g. Oyster nut oil contains 11.5% oleic acid, 32.5% linoleic acid, 5% linolenic acid, 24.5% palmitic acid and 18% stearic acid (Table 7). The seed has good keeping quality and may keep for up to eight years and still remain in excellent condition when husked (Ajayi et al., 2004; Okoli, 2007).

Table 7: Fatty acid composition of commercial oil seeds

		Oil seed		
Fatty acid	Formula	Oyster nut	Simsim	Soy bean
Oleic acid	C18.1	11.5%	38.22%	20.09%
Linoleic acid	C18.2ω6	32.5%	43.55%	56.79%
Linolenic acid	C18.3ω3	5.0%		11.24%
Palmitic acid	C16.0	24.5%	10.09%	9.79%
Stearic acid	C18.0	18.0%	5.15%	2.02%

Source: van der Vossen and Mkamilo (2007); Sultan, Dikshit and Vaidya (2015)

2.3.4 Health Benefits

Although few studies are available on the benefits of oyster nut to health, clinical data are too scanty to give basis of any nutritional benefit. Reports by Ajayi et al. (2004); Pareek and Sharma (2009) have suggested that oyster nuts have been successfully used to promote lactation in nursing mothers. Furthermore, the nuts are reported to contain high amounts of linoleic acid, an essential FA (Table 7). Linoleic acid improves serum profile of cholesterol and in essence reduces risk of cardio vascular disease. Consumption recommendations are limited.

2.3.5 Other Uses of Oyster Nut

Oyster nut oil is useful in cosmetics and in soap and candle making. The oil is used as medicine for stomach troubles and rheumatism in East Africa. The Wachagga of Tanzania use the seed as tonic after childbirth. After oil extraction, the press cake makes valuable feed for livestock, being rich in protein (van der Vossen and Mkamilo, 2007).

2.4 Fats, Oils and Fatty Acids

2.4.1. Fats and Oils

Fats and oils consist of a large number of organic compounds, including fatty acids (FA), monoacylglycerols, diacylglycerols and triacylglycerols, phospholipids, eicosanoids, resolvins, docosanoids, sterols, sterol esters, carotenoids, vitamins A and E, fatty alcohols, hydrocarbons and wax esters (FAO, 2010). They occur naturally in most plants, animals, microorganisms and are used as cell membrane components, energy storage molecules, insulation, and hormones. Fats and oils are esters of glycerol and three FA (Figure 3). Various FA exist and greatly influence the melting behaviour of lipids. When melting point is below ambient, the lipid is referred to as an oil, if above, then it is a fat (List, 2009).

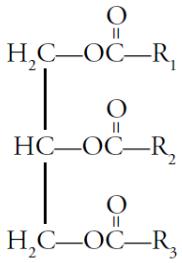

Figure 3: Typical structure of a triglyceride (List, 2009)

2.4.2. Fatty Acids

Fatty acids (FA) consist of a straight alkyl chain, terminating with a carboxyl group (CH_3-CH_2-CH_2-CH_2-COOH) (List, 2009), Figure 5). Fatty acids may be classified based on level of saturation. Saturated fatty acids (SFA) contain no double bonds. These are the most stable FA; whether free or in combination. Most are straight chains with an even number of carbon atoms. The most common SFA contain 12 to 22 carbon atoms and include lauric acid (C12.0), palmitic acid (C16.0), and stearic acid (C18.0) (Rustan and Drevon, 2005). Saturated FA are very resistant to oxidation and other forms of chemical attack. Unsaturated fatty acids (UFA) are

those that contain one or more carbon-carbon double bonds per molecule hence the name monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) (Figure 5). The most common MUFA have a chain length of 16 to 22 carbon atoms (Gunstone, 1996). Oleic acid (C18.1) and palmitoleic (C16.1) are the dominant MUFA in plants, animals and oil seeds. Oleic acid is not very susceptible to oxidation hence the high stability of olive and groundnut oil that contain this FA is high amounts (List, 2009). Polyunsaturated FA such as linoleic acid (C18.2) and alpha linolenic acid (C18.3), contain more than one carbon-carbon double bond (Figure 5).

Figure 4: Single and double bonds (Nettleton, 1995).

Double bonds are potential points of oxidation and other forms of chemical attack. Vulnerability to oxidation increases rapidly with increase in number of double bonds (List, 2009).

Fatty acids may be classified based length of the carbon chain; where short chain (<6 carbons); medium (6 to10 carbon atoms) and long medium (12 or more carbon atoms). Short and medium chain FA are found in palm oil, coconut oil and milk fat while long chain FA are found mainly in meat (Caponio, Gomes, and Bilancia, 2003; Haug, Høstmark, and Harstad, 2007; Orsavova et al., 2015). Location of the double bond; whereby unsaturated FA are identified by position of the double bond nearest the methyl end (CH_3) of the carbon chain (Figure 5).

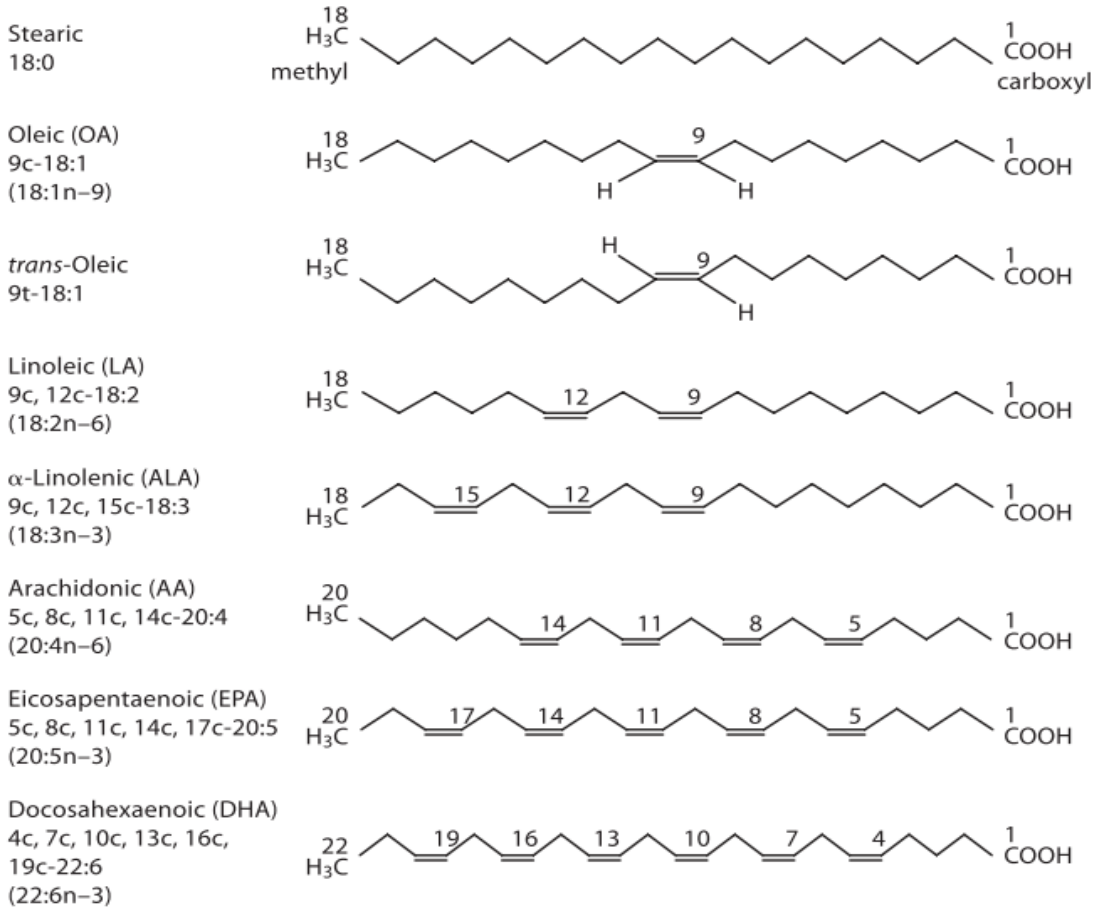

Figure 5: Structures of common fatty acids (Ratnayake and Galli, 2009)

This is described as an omega number (Ratnayake and Galli, 2009). If the unsaturated FA has the first bond 3 carbons from the methyl end, then it is an omega-3 while 6 carbons from the methyl end is omega-6. Linoleic acid (C18.2ω6) and arachidonic acid (C20.4ω6) belong to the omega (ω)-6 group of FA, since the first double bond, counting from the methyl end of the molecule, occurs at carbon number 6., and alpha linolenic acid (C18.3ω3) belongs to the ω3 group (Ratnayake and Galli, 2009). Common sources of ALA include soya bean oil, flax seed oil and rape seed oil (Dieffenbacher, Holm, and Beare-Rogers, 2001; Gates, Nykter, and Kymäläinen, 2006; Kassis, Gigliotti, Beamer, Tou, and Jaczynski, 2012). Other omega-3 FA include; eicosapentaenoic acid (EPA; C20:5 ω3), and docosahexaenoic acid (DHA; C22:6

ω3).these are common in fatty fish and fish oils. Omega-3 FA are associated with anti-inflammatory, anti-thrombic, anti-arrhythmic, hypolipidemic, vasodilatory properties, cognitive ability as well as growth and development (Johnson and Bradford, 2014).

Saturated FA are strongly correlated with coronary heart disease while PUFA associated with reduced incidence of coronary heart disease (Rustan and Drevon, 2005). The minimum requirement of SFA is ≤10% energy while 0.5% ALA and 2.5% LA is recommended to prevent nutritional deficiency (FAO/WHO, 2008).

2.4.3 Essential Fatty Acids

These are FA required for biological processes in humans and other animals but must be ingested because the bodies cannot synthesize them. The PUFA linoleic acid and linolenic acid (Figure 6) are essential fatty acids (Forbes and Parsons, 2012). Some FA are classified as "conditionally essential," meaning that they can become essential under some developmental or disease conditions; examples include docosahexaenoic acid (DHA) an omega-3 FA and gamma-linolenic acid (GLA),an omega-6 FA (Makrides, Neumann, Simmer, Gibson, and Pater, 1995).

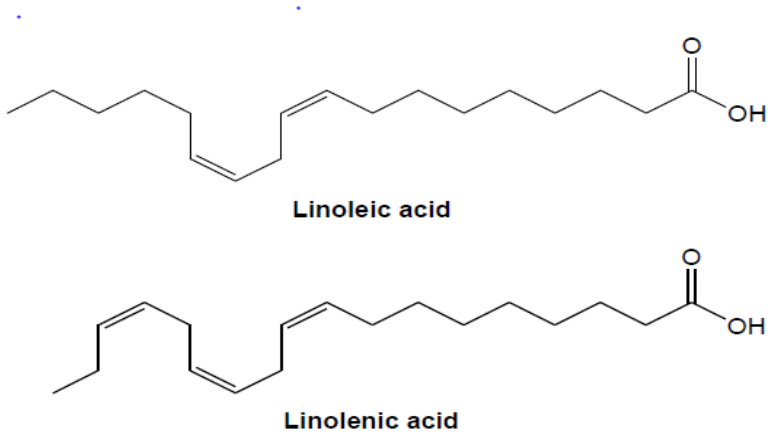

Figure 6: Structures of essential fatty acids

Essential FA are important for cardiovascular health, cognitive health, pregnancy, lactation and infancy to ensure proper development of the brain and nervous as well as vision system (Jumbe et al., 2016). Essential FA also regulate blood clotting; omega-6 FA encourage blood clot formation, whereas omega-3 FA reduce clotting. (Arbex et al., 2015).

Table 8: Common fatty Acids

No. of Carbons	Structure	Common name
C12.0	$CH_3(CH_2)_{10}COOH$	Lauric acid
C14.0	$CH_3(CH_2)_{12}COOH$	Myristic acid
C16.0	$CH_3(CH_2)_{14}COOH$	Palmitic acid
C18.0	$CH_3(CH_2)_{16}COOH$	Stearic acid
C20.0	$CH_3(CH_2)_{18}COOH$	Arachidic acid
C24.0	$CH_3(CH_2)_{22}COOH$	Lignoceric acid
C16.1ω9	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	Palmitoleic acid
C18.1ω9	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Oleic acid
C18.2ω6	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	Linoleic acid
C18.3ω3	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	Linolenic acid
C20.4ω6	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	Arachidonic acid

Source: Rustan and Drevon (2005)

Alpha linolenic acid (ALA, C18.3ω3) can be desaturated and elongated in the human body to its longer-chain derivatives but the efficiency of this conversion is reduced by high intake levels of LA, which competes more effectively than ALA for desaturation and elongation enzymes due to the high abundance of LA in today's diet. Linoleic acid (C18.2ω6) and ALA produce hormone like substances known as eicosanoids (Galli and Calder, 2009; Jandacek, 2017). Eicosanoids are oxygenated derivatives of C20 polyunsaturated fatty acids e.g. arachidonic acid (Simopoulos, 2008). These affect the cells where they are made and may have different effects to cells including causing muscles to contract or relax, regulation of blood pressure, blood clot formation, regulation of blood lipids and immune response. Eicosanoids include prostaglandins, thromboxanes and leukotrienes. Eicosanoids generated from ω3 FA are anti-inflammatory while those generated from ω6 FA are pro-inflammatory (Lunn and Theobald, 2006). Dietary guidelines recommend a ratio of 4:1 for ω6 to ω3 for good health (Simopoulos, 2002, 2004, 2016).

2.4.4 Cholesterol

Cholesterol is a component of membranes in body cells and is required for normal development of the brain and nervous tissue (Priya, Maurya, and Khan, 2013). It is the precursor to bile acids, steroid hormones, and 7-dehydrocholesterol in the skin, which in turn is the precursor to vitamin D (Gurr, 1999).The highest concentrations are found in liver and egg yolk, but red meats, poultry (especially the skin), whole milk, and cheese make significant contributions to the diet (Huff, 2003). Cholesterol is insoluble in the blood, so when it is released into the blood stream it forms complexes with lipoproteins (Sacks et al., 2017). A lipoprotein is an association of protein and lipids and it exists in combination with proteins in order to allow fats to move across the cell.

The protein component plays a part in emulsification of fat molecules (Hegele, 2009). Depending on the ratio of fat to protein content, lipoproteins can be classified as low density lipoprotein (LDL) which is known as bad cholesterol, high density lipoprotein (HDL) termed as good cholesterol and very low density lipoprotein (VLDL) which is similar to LDL and triglycerides (Priya et al., 2013). Low density lipoproteins (LDL) are lipoproteins which transport cholesterol from liver and intestine to the cells and tissues of the body through bloodstream. High density lipoprotein (HDL) on the other hand transfers cholesterol from the cells and tissues to the liver and thus it reduces cholesterol in the blood. (Priya et al., 2013). If HDL levels are low, then the blood level of cholesterol will increase. High levels of blood cholesterol are associated with plaque formation in the arteries, which can lead to heart disease and stroke. While most cholesterol in the body is synthesized in the liver, dietary cholesterol also adds to the total blood levels. Cholesterol intake from the diet enters the bloodstream in the LDL form (Grundy and Denke, 1990).

2.4.5 Effect of Fatty Acids Plasma Lipids

Individual SFA have different effect on concentration of plasma lipoprotein cholesterol fractions. Myristic, palmitic and lauric acid increase low density lipoprotein cholesterol (LDL-c) while stearic acid has a neutral effect (FAO/WHO, 2008; Mente et al., 2017). Stearic acid (C18.1) is converted to oleic acid (C18.1) and hence doesn't affect cholesterol concentration in the blood (Mente et al., 2017). Saturated FA enhance levels of total cholesterol and LDL-c and promote obesity and arteriosclerosis (Attia, Al-harthi, Korish, and Shiboob, 2017). Oleic acid (C18.1) decreases LDL-c while linoleic acid (C18.2ω6) decreases LDL-C, however, in very high concentrations it may decrease HDL-c levels. Omega-3 PUFA such as alpha linolenic acid

(ALA, C18.3ω3) decrease the level of very low density lipoprotein cholesterol VLDL-C and triglycerides in the body hence lowering the risk of cardiovascular diseases (Abbey, Clifton, Kestin, Belling, and Nestel, 1989).

2.4.6 Health Lipid Indices

Health lipid indices are important in evaluating nutritional quality of oil. These include; PUFA/SFA, Omega-3/omega-6 or omega-6/omega-3, atherogenic and thrombogenic indices, hypocholesterolemic and hypercholesterolemic index (Garaffo, Vassallo-Agius, Nengas, Lembo, Rando, Maisano, and Giuffrida, 2011; Ghaeni, Ghahfarokhi, and Zaheri, 2013). Proportions of polyunsaturated and saturated FA as well as omega-6 to omega-3 must be well balanced to benefit nutrition and health. Minimum requirement for PUFA/SFA is 0.45 while omega-6 to omega-3 should be in a ratio of 4:1 to promote health. A low ratio of omega-6/omega-3 FA is more desirable in reducing the risk of chronic diseases (Simopoulos, 2002; 2010). The reverse ratio omega-3/omega-6 ratio has proven to be good index for comparing relative nutritional value of lipids. A high ratio may reduce risk of non-communicable disease and maintain serum cholesterol at safe levels (Wertz, 2009). Today, however, the levels of omega-6 in diets supersedes the amount of omega-3 consumed hence creating an imbalance (Simopoulos, 2002; 2004)). More omega-3 should be consumed to prevent the negative effect that comes with excessive levels of omega-6 in the body (Simopoulos, 2008). Atherogenic and thrombogenic indices show the influence of individual FA on the possibility of causing atheroma and thrombosis (Garaffo et al., 2011). Thrombosis refers to the formation of clots in blood vessels while atheroma refers to the formation of plaque in blood vessels which is a factor in coronary diseases (Ulbricht, and Southgate, 1991). According to S˘imaT, Bogdanovic´,

Poljak, and Petricˇevic´(2015) low levels less than one (<1) are required for health benefit. Hypocholesterolemic/hypercholesterolemic ratio (h/H) is an index that takes into account the functional activity of FA in the metabolism of plasma cholesterol lipoproteins, whose types and quantities are associated with higher or lower risk of cardiovascular disease (Santos-Silva, Bessa, and Santos-Silva, 2002; Pamukova and Naydenova, 2018).

2.5 Lipid Oxidation

Lipid oxidation involves modification of fats in presence of oxygen, water, and pro-oxidant elements such as iron and copper (Berton-carabin, 2014). Different forms of lipid oxidation can occur; autoxidation, photo-oxidation, and enzymatic oxidation. In each of these processes, hydroperoxides are formed as primary oxidation products. (Gheysen, 2019) Oxidation of unsaturated lipids not only produces offensive odor and flavor but can also decrease the nutritional quality of long chain PUFA as well as their safety by the formation of secondary oxidation products in foods after cooking and processing (Frankel, 1980; Kamal-Eldin, 2006).

2.5.1 Auto-oxidation

Auto oxidation of lipids proceeds via a chain reaction involving initiation, propagation and termination steps (Figure 7). This reaction is triggered by the interaction of unsaturated lipids with oxygen (Fisk, 2007). Lipid hydroperoxides are the initial products; these either breakdown to form volatile products or polymerise to form large molecular weight products. Lipid oxidation occurs spontaneously and is often initiated by trace metals, light, heat, peroxides or hydroperoxides (Wasowicz et al., 2004). Metals can initiate fatty acid oxidation by reaction with oxygen. The anion thus produced can either lose an electron to give singlet oxygen or react with

a proton to form a peroxy radical, a good chain initiator (Choe and Min, 2006; Xia and Budge, 2018). A variety of volatile and non-volatile secondary products are formed from hydroperoxides when lipid oxidation is carried out to high conversion or at elevated temperatures (Choe and Min, 2006). The first step of the decomposition of an unsaturated hydroperoxide is the homolytic cleavage of the oxygen-oxygen bond to yield an alkoxy and hydroxy radical. This is followed by carbon-carbon cleavages which lead to formation of aldehyde esters and aldehydes (Madhujith and Sivakanthan, 2018). Unsaturated lipids, in particular those containing ω3 long chain PUFA, are highly susceptible to lipid oxidation due their chemical structure, which includes a large number of double bounds. Some intermediate and final products of lipid oxidation reactions are potentially toxic to consumers' health (Wasowicz et al., 2004; Summo, Caponio, and Bilancia, 2005)

2.5.2 Hydrolytic Rancidity

Hydrolytic rancidity of oil occurs when tri-, di- and mono aclyglycerides are degraded into free FA and glycerol (Das, Babylatha, Pavithra, and Khatoon, 2013, Chen et al., 2019;) . According to Goffman and Bergman (2003), this process is primarily related to lipase activity. The free FA are then oxygenated by lipoxygenase or via auto-oxidation to form hydroxyl-peroxides that are further broken down to form compounds with off odors and off flavors (Chen et al., 2019; Shahidi, 2005; Summo, Caponio, Paradiso, Pasqualone, and Gomes, 2010). Hydrolysis of FA with less than 12 carbon atoms results in off odors and off flavors due to their volatility (Nielsen, 2017). Hydrolytic degradation is enhanced by presence of moisture, light, air (Su, 2003). Lipases however, may be inactivated during high temperature short time processes hence inhibiting the hydrolytic processes (Summo et al., 2010; Nielsen, 2017). Acid value, a measure

of free FAs in oil, indicates the edibility of oil. Good quality oil should have low acid value less than 40 mg/kg (FAO/WHO, 2005).

Lipid oxidation strongly affects shelf life and sensory characteristics of oil and depends on many factors such as the concentrations of unsaturated fatty acids, enzymatic activity, mineral composition and the presence of natural antioxidants (Tenyang, Ponka, Tiencheu, Djikeng, Azmeera, Karuna, et al., 2017)

Peroxide value (PV) is used as an index for oxidative stability of oil (Ali, Islam, Othman, and Noor, 2017). Peroxide value determines the concentration of hydro-peroxides, the primary oxidation products (Gromadzka and Wardencki, 2011, Table 9). Hydroperoxides later breakdown to form free fatty acids, alcohols, aldehydes, and ketones, eventually leading to a rancid product (Zahir, Saeed, Hameed, and Yousuf, 2017). A general rule is that PV should not be above 10 to15 mEq/kg oil to avoid rancidity flavour (FAO/WHO, 2005).

2.5.3 Enhancing Oxidative Stability of Lipids

Oxidative stability is the resistance of lipids to oxidation during processing and storage Oxidative stability depends on level of unsaturation and presence of anti-oxidant components (Dubois, Breton, Linder, Fanni, Parmentier, 2007). High proportions of PUFA increase the susceptibility of oil to oxidation leading to production of hydro-peroxides and other oil degradation products (Hemery et al., 2015).

Table 9**:** Measurement of oxidation products

Products of lipid oxidation		Means of measurement
Primary products of lipid oxidation	Non- esterified hydroperoxyl fatty acids e.g. hydroperoxy linoleate 13 HPODE	Peroxide value, iodine value, conjugated dienes
	Non esterified hydroxy fatty acids e.g. hydroxyl linoleate -13-HODE	Conjugated dienes
	Non esterified epoxy fatty acids e.g. 9,10-epoxy linoleate	Gas chromatography and mass spectrometry (GC-MS)
	Oxidized triacylglycerol monomers and dimers	Polar compounds
	cyclic triacylglycerol monomers and dimers	Polar compounds
Secondary products of lipid oxidation	,alcohols, aldehydes, ketones,hydrocarbons	Anisidine value, acid value, thiobarbututic acid reactive substances (TBARS)

Source: Jackson and Penumetcha (2019)

Blending of oils with high proportions of PUFA with oleic acid rich oils could improve oxidative stability of oil (Hashempour-Baltork, Torbati, Azadmard-Damirchi, and Savage, 2018). In order to retard or inhibit oxidation, anti-oxidation may be achieved by adding low concentrations of chain-breaking antioxidants that interfere with either chain propagation or initiation.

Synthetic chain-breaking antioxidants include phenol and aromatic amino compounds hindered with bulky alkyl substituents. Common chain-breaking antioxidants used in food lipids include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), butylated hydroquinone (TBHQ), and propyl gallate (Patil, 2014). These compounds generally lose their efficiency at elevated temperatures because of homolytic decomposition of hydroperoxides formed by reaction and because of a reaction of the antioxidants with oxygen (Wasowicz et al., 2004). Naturally occurring anti-oxidants include vitamin A, tocopherols, vitamin C and beta carotene (Kalogeropoulos, Mylona, Chiou, Ioannou, and Andrikopoulos, 2007). Polyphenols and flavonoids have been cited to have efficient anti-oxidant properties (Kalogeropoulos et al., 2007; Mahatma, Thawait, Bishi, Khatediya, and Rathnakumar, 2016). These work in synergy to inhibit or reduce the extent of oxidation. Synergism is known for the reinforcing effect of multi-component stabilizer systems exhibiting a greater combined effect than the sum of their individual effects (Elmadfa, 2001; Ratnayake and Galli, 2009; Patil, 2014). Significant synergism is generally observed when chain-breaking and preventive antioxidants are used together because they suppress both initiation and propagation (Frankel, 1980; Patil, 2014).

Presence of anti-oxidants can delay progress or prevent oxidation reactions in oil (Dauqan, Abdullah Sani, and Abdullah, 2011; Pignitter and Somoza, 2017; Weber, Eggersdorfer, Blumberg, Birringer, and Frank, 2019). Unsaturated FAs are particularly unstable when exposed to light, heat, metallic ions and oxygen (Yanishlieva and Marinova, 2001; Madhujith and Sivakanthan, 2018). Tocopherols and carotenoids are naturally occurring antioxidants in unrefined oil. Beta-carotene acts by light filtering, quenching singlet oxygen, inactivating photo sensitizers and free radical scavenging (Madhujith and Sivakanthan, 2018). Beta-carotene together with other natural anti-oxidants was shown to contribute to oxidative stability of oil

during frying and storage (Sunil, Reddy, and Krishna, 2015). Similarly, α-tocopherol is reported to have high anti-oxidant properties together with γ-tocopherol. Alpha-tocopherol, however, has higher biological activity in the body than other tocopherols (Kamal-Eldin, 2006). Alpha-tocopherol interrupts oxidation reactions by combining with reactive species such as peroxyl radicals in oil. These anti-oxidants, however, are unstable at high temperatures (Yanishlieva and Marinova, 2001, Kamal-eldin, 2006).

2.6 Anti-oxidant Vitamins

2.6.1 Beta-carotene

Carotenoids are polyisoprenoids with differing degrees of conjugated double bonds. They are abundant in most colourful plant foods (Bolling et al., 2010). Beta-carotene (Figure 7) is a source of pro-vitamin A, a potent anti-oxidant and natural colourant in many natural and processed foods (Mathews-Roth, 1986). As an anti-oxidant, beta carotene destroys free radicles produced during digestion and in foods, those formed through enzymatic and various catalysts such as light and trace elements. Beta-carotene is a scavenger of singlet molecular oxygen and peroxyl radicals, at low partial pressures of oxygen, such as those found in most tissues under physiological conditions (Eldahshan and Singab, 2013). It protects cellular membranes and lipoproteins against oxidative damage (due to lipophilicity and specific property to scavenge peroxyl radicals). Cooperative effects with other antioxidants (i.e., vitamin C regenerates vitamin E in lipid systems, β- carotene tocopherol from the tocopheroxyl radical; vitamins E and C and β-carotene exhibit cooperative synergistic effects scavenging reactive nitrogen species)(Ciccone et.al., 2013).

Besides their food functions carotenoids are considered as anti-carcinogens (Gunstone, 2002). Beta-carotene co-exists with other carotenoids such as lutein, lycopene, alpha–carotene and beta-cryptoxanthin (Colditz et al., 2007). Most vegetable oils contain low sources of carotenoids except palm oil and the orange-red colour is useful as a natural pigment in food preparations (May and Nesaretnam, 2014). The maximum amount of beta-carotene in groundnut oil is 60 µg /litre of oil and this concentration decreases with maturity (Nautiyal, 2002). There is no documented RDA for beta carotene and this may be attributed to its multi-functional roles in the body.

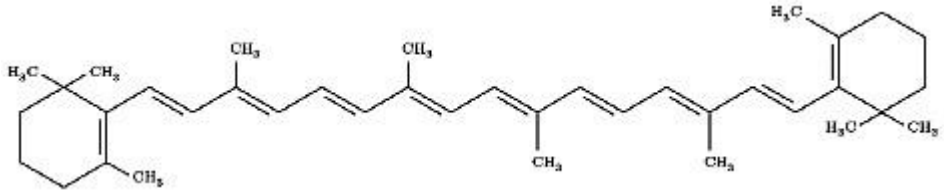

Figure 7. Chemical structure of beta-carotene

2.6.2 Vitamin A

Vitamin A (Figure 8) is a group of related compounds, called retinoids, with retinol the most common form (Olson and Mello, 2010). It's a fat-soluble vitamin that the body stores in the liver, ready to use when needed. Vitamin A supports the immune system and bones and is part of a compound called rhodopsin that absorbs light in your retina to facilitate vision (Kong and Du, 2018). It is also crucial during development because it is used to produce new cells. Vitamin A designates a group of compounds essential for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system. The body's need for vitamin A can be met by dietary intake of preformed retinoids with vitamin A activity (usually in animal products) or by consumption of carotenoid precursors of vitamin A such as β-carotene, α-

carotene, and cryptoxanthin formed by plants and present in some animal fats. In spite of being fat soluble, most vegetable oils with the exception of palm oil are poor sources of vitamin A (Thurnham and Northrop-clewes, 2018).

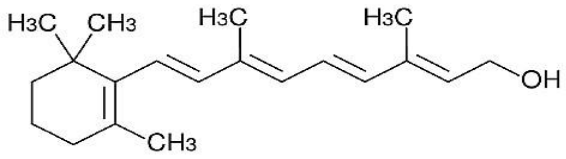

Figure 8. Chemical structure of vitamin A

Dietary needs of vitamin A can be met by preformed vitamin A or by beta carotene (Tang, 2010). Preformed vitamin A (retinol) is found in animal products (Leitner et al., 2018). In the small intestine, retinyl esters are hydrolyzed; the products are associated first with lipid globules and then with bile salt-containing mixed micelles in the upper part of the small intestine. These mixed micelles contain carotenoids as well as retinol. However, absorption of retinol and carotenoids, especially β-carotene, differs in several ways. Absorption of beta-carotene is about 30% efficient as compared to 50% absorption efficiency for retinol (Tang, 2010).

Absorbed retinol is largely esterified in intestinal mucosal cells and incorporated into chylomicrons, as is the portion of absorbed β-carotene and other biologically active carotenoids that is not cleaved in intestinal cells. Most absorbed β-carotene normally is converted to retinol and then to retinyl esters in mucosal cells. The relationship between retinol and beta-carotene is shown by the following conversion factors;

6 µg of beta carotene are equivalent to 1 µg of retinol

While 3 µg of retinol are equivalent to 1 µg = RE of vitamin A activity. 1 RE is defined as 1 µg of all-trans retinol, 6 µg of all-trans β-carotene, or 12 µg of other provitamin A carotenoids

(Tang, 2010, 2012). On the basis of all these considerations, the assumed relationship between the biological effectiveness of β-carotene and retinol was changed, so that 6 µg of dietary β-carotene was assumed to be nutritionally equivalent to 1 µg of retinol (FAO/WHO, 1967; NRC, 1980). Vitamin A activity in foods is thus currently expressed as retinol equivalents (RE) (Pee, West, Permaesih, Martuti, and Hautvast, 1998).

Table 10: Recommended Daily Allowances of vitamin A

Group	Mean requirement µg RE/day	Mean requirement µg Retinol/day
0-6 months	180	60.0
7-12 months	190	63.3
1-3 years	200	66.7
4-6 years	200	66.7
7-9 years	250	83.3
10-18 years Female	330-400	110-133.3
19-65	270	90.0
65+	300	100.0
19-65 years Male	300	100.0
65+	300	100.0
Pregnant	370	123.3
Lactation	450	150.0

Source :FAO/WHO (2004)

Groundnuts have little or no vitamin A activity. They contain carotenes (pro-vitamin A) but the level is too small to be of significance. According to Cobb and Johnson (1973), carotenes

characterized in oil from immature groundnuts include alpha (trace), beta (60 µg/l), zeta (0.088 µg/l) and an unknown (12 µg/l).

2.6.3 Vitamin E

This includes a broad class of chemically related compounds called tocopherols. Tocopherols function as weak antioxidants in expressed groundnut oil and may also serve a similar *in vivo* function in the metabolizing seed (Eitenmiller and Lee, 2004). Several chemical forms exist but α, γ and δ-tocopherol have been demonstrated in groundnuts. Groundnut oil exhibits a total tocopherol range of 26.3 to 59.4 mg/100 g of oil after chromatographic purification of saponified oil fractions. USDA (2011), however reported that groundnuts have vitamin E amounting to about 6.93 mg/100 g. High storage temperatures are destructive to tocopherol content of nuts. Higher temperatures are responsible for a loss of about 25% of the alpha form over four-month storage. Vitamin E appears to be associated with the physiology of muscular and vascular tissue and may be involved in electron transport (Cobb and Johnson, 1973). The recommended daily intake of vitamin E for adults is 15 mg (FAO/WHO, 2005). This makes groundnuts and groundnut oil efficient sources for supplementing vitamin E to the body.

Vitamin E is a group of compounds consisting of tocopherols (α-, β-, γ-, and δ-) and tocotrienols (α-, β-, γ-, and δ-) that have similar biological activities (Eitenmiller and Lee, 2004; Zielinska and Nowak-Adam Mickiewicz, 2014, Table 11). Tocopherols, are characterized by a ring system and a long, saturated side chain (Figure 9). Tocopherols only differ in the number and position of methyl groups on the ring. Tocotrienols on the other hand differ from the tocopherols by having an unsaturated side chain (Colombo, 2010). They possess powerful neuroprotective,

anticancer, and cholesterol-lowering properties that are often not exhibited by tocopherols (Colombo, 2010). Although the most biologically active form of vitamin E is alpha-tocopherol, β-, γ-, and δ-tocopherols also have essential roles in the body. Alpha-tocopherol is the primary lipid-soluble antioxidant in mammalian and plant cells located in the cell membranes and available to protect lipoproteins. It functions as a primary, chain-breaking antioxidant, scavenging peroxy free radicals (Traber and Packer, 1995). Protection of polyunsaturated fatty acids (PUFAs) is facilitated by the greater affinity of lipid-generated free radicals for reaction with α-tocopherol than with PUFA located in membrane phospholipids (Eitenmiller and Lee, 2004). In that way it prevents the body from non-communicable diseases (cancers, cardiovascular disease). Additionally, it strengthens blood vessels and improves their elasticity and lowers blood clotting (Zielinska and Nowak, 2014).The plasma concentration of α-tocopherol level is normally 5 to 20 μg/ml. Inadequate Vitamin E levels in the body cause degeneration of the axons of neurons (nerve cells) resulting in neurologic deficits, and fragility of red blood cells. Vitamin E is not synthesized in human body, therefore tocopherols supplied with food are mainly stored in fat tissue. The absorption of vitamin E in intestines is determined by the content of fats in food (Zielinska and Nowak, 2014). Groundnuts, sunflowers seeds, vegetable oils, wheat germ and green leafy vegetables are good sources of vitamin E.

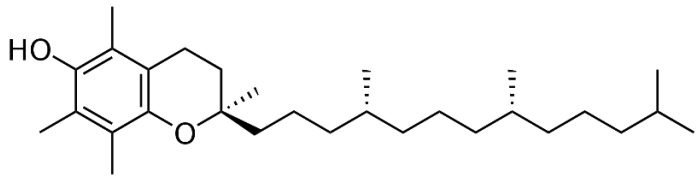

Figure 9. Structure of tocopherol

Table 11: Tocopherols classification

Trivial name	Chemical name	Abbreviation	Ring position		
			R_1	R_2	R_3
α-tocopherol	5,7,8-trimethyltocol	α-T	CH_3	CH_3	CH_3
β-tocopherol	5,8-dimethyltocol	β-T	CH_3	H	CH_3
γ-tocopherol	7,8-dimethyltocol	γ-T	H	CH_3	CH_3
δ-tocoherol	8-methlytocol	δ-T	H	H	CH_3

Source: Zielinska and Nowak – Adam Mickiewicz (2014).

2.6.3.1 Tocopherols as Anti-oxidants

Oxidation of lipids processed by a chain reaction assisted by free radicals in which the lipid peroxyl serves as a chain carrier. The reaction occurs in three stages initiation, propagation and termination.

Initiation $RH+O_2 \rightarrow R\cdot+HOO\cdot$

$RH \rightarrow R\cdot+H\cdot$

Propagation $R\cdot+ O_2 \rightarrow ROO\cdot$

$ROO\cdot+RH \rightarrow ROOH+ R\cdot$

Termination $R\cdot+ R\cdot \rightarrow RR$

$ROO\cdot+ R\cdot \rightarrow ROOH$

Where RH is the lipid and R·, HOO·, ROO· and H· are free radicals, ROOH is the lipid hydro-peroxide. The role of alpha-tocopherol is to neutralise the free radicals before they react with other lipids to continue the chain reaction.

$ROO\cdot+ TOH \rightarrow ROOH+ TOO\cdot$

Where TOH is the tocopherol and TOO is the tocopheroxyl radical.

Table 12: Recommended Daily Intake for Vitamin E

Category	Age (years)	Alpha tocopherol (mg/day)
Children	1 to 3	6
	4 to 8	7
Boys	9 to 13	11
	14 to 18	15
Girls	9 to 13	11
	14 to 18	15
Adult men	>18	15
Adult women	>18	15
Pregnant		15
Lactating		19

Source: FAO/WHO (2004)

2.7 Tannins

Tannins are polyphenolic secondary metabolites of higher plants, and are either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol, catechin and triterpenoid cores (gallo- tannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins) (Khanbabaee and Ree, 2001). Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and other macromolecules (Ashok and Upadhyaya, 2012; Mangan, 1988). Molecular weights of tannins range from 500 to >3000 (gallic acid esters) and up to 20,000 (proanthocyanidins)

(Khanbabaee and Ree, 2001). The name ‘tannin’ is derived from the French word ‘tannin’ (tanning substance) and is used for a range of natural polyphenols.

While soluble, astringent materials are found in some plants like tea and coffee, tannins are supplemented to various processed foods, including ice-cream and caramel. They are also used as refining materials to precipitate proteins in wines and beer. As tannins often lower the absorption of some materials into the body, tannins are also often known as anti-nutrients. For example, tannins are found in tea and coffee and consuming too much of these beverages without milk may lead to calcium and iron deficiency in the body and often lead to osteoporosis and anemia. Tannins may be classified as either hydrolysable or condensed (Kumari and Jain, 2015; McMahon, McAllister, Berg, Majak, Acharya, Popp, and Cheng, 2000, Figure 10).

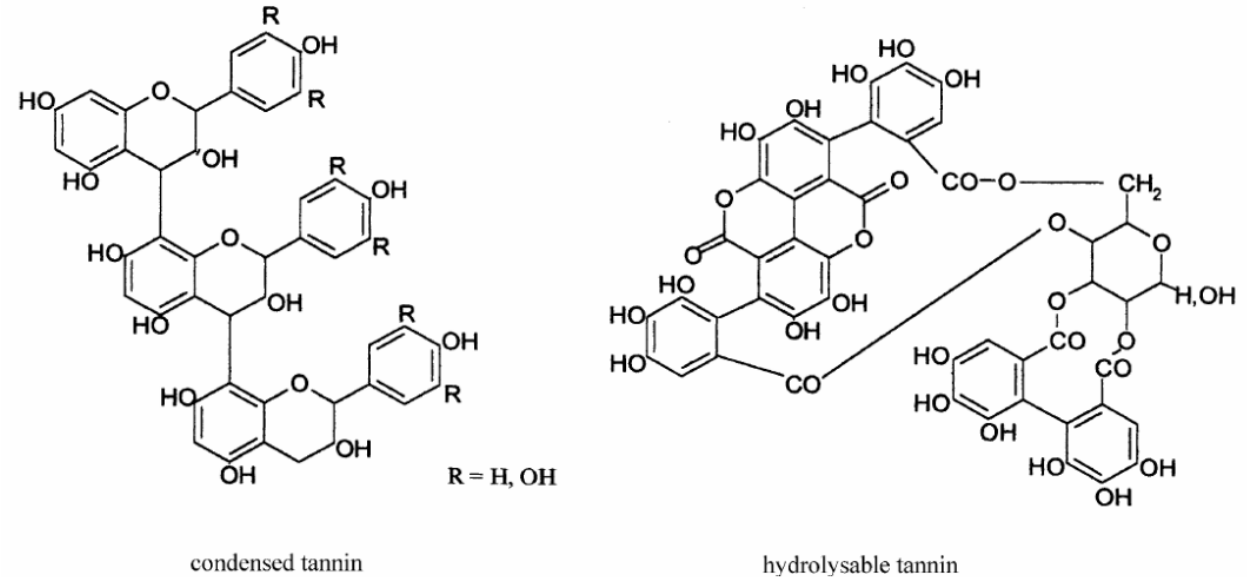

Figure 10. Classification of Tannins (Hassanpour, Maheri-sis, Eshratkhah, and Mehmandar, 2011)

2.7.1 Hydrolysable Tannins

Hydrolysable tannin molecules contain a carbohydrate (usually D-glucose) at the centre. Hydroxyl groups of the carbohydrate are partially or totally esterified with phenolic groups such as gallic acid (in gallotannins) or ellagic acid (in ellagitannins). Hydrolyzable tannins are hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids.

2.7.2 Condensed Tannins

These are also known as proanthocyanidins, are polymers of 2 to 50 (or more) flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis. They occur mostly in plant sources (Kumari and Jain, 2015). While hydrolysable tannins and most condensed tannins are water soluble but few very large condensed types of tannin are insoluble. Tannins (mainly condensed tannins) are also found in wine, particularly red wine. Tannins in wine can come from many sources and the tactile properties differ depending on the source. Wine tannins come from grape skins, stems and seeds, and their extraction is heavily dependent on the particular winemaking process involved. Some tannin also comes from barrels, particularly new ones, where these are used to age wine. The complicating factor here is that the chemical make-up of the tannins is actually changed during the winemaking process (Ashok and Upadhyaya, 2012). Tannins are phenolic compounds that interfere with iron absorption through a complex formation with iron when it is in the gastro-intestinal lumen which decreases the bioavailability of iron. There is an importance difference between the way in which the phenolic compound interact different hydroxylation patterns (gallic acid, catechin, chlorogenic acid) and the effect on iron absorption. Content of the iron binding galloyl groups may be the major determinant of the inhibitory effect of phenolic

compounds (Ashok and Upadhyaya, 2012). Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhoea, dysentery, haemorrhaging, fatigue, skin ulcers. Tannins can cause regression of tumours that are already present in tissue, but if used excessively over time, they can cause tumours in healthy tissue (Ashok and Upadhyaya, 2012).

Phenolic tannins have both positive and negative effects. They possess anti-nutritive properties, but are also beneficial for health due to their role as antioxidants and their ability to stimulate the immune system and various effectors (Chung et al., 1998; Makkar et al., 2007; Quideau et al., 2011). Tannic compound has also been reported to have antimicrobial activity against fish pathogens (Schrader, 2008).

Tannins are heat stable and they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (Zhu et al., 2016) Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. If tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed (Zhu et al., 2016). Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the anti-nutritional effects of tannin containing foods (Muhammad Aslam et al., 2009). This is because the heart contains saponins that impart a bitter flavor, and skin contains catechol tannins and related compounds, which give finished products an un-desirable color (Woodroof 1983).

Satish Ingale andShrivastava (2011), reported 0.412 g/100 g of tannin in groundnut variety JL-24 in India. The red skins constitute 2.0 to 3.5% of groundnut kernels and contain tannin and related pigments.

CHAPTER THREE

3 Materials and Methods

3.1 Sample Collection and Preparation

Groundnuts were collected between 2016 and 2018 using the purposive sampling method. Total population of Improved cultivars (Serenut 1 to 14) was taken and six traditional (Acholi white, *Igola*, *Egoromoit*, *Rudu* white, *Rudu* red and Red beauty) groundnut cultivars were selected due to availability and popularity in the region. Serenut 1 to 14 were collected from National Arid and Semi-Arid Research Resources Institute (NaSARRI) located in Serere District. Traditional cultivars were purchased from Soroti, Arapai, and Achorimongin markets in Soroti and Katakwi Districts of Teso sub-region, Eastern Uganda (Figure 11). Groundnuts were shelled, sorted, hulled and finely crushed to obtain groundnut flour that was stored at 4^{o}C in a refrigerator until use.

Oyster nuts were obtained from Kamuli District in Eastern, Dokolo District in Northern, and Luwero District in Central Uganda based on recommendation from key informants about their availability in the country. Dried oyster nuts were transported to Chemiphar and Uganda Industrial Research Institute laboratories. Nuts were cleaned, and sorted according sex. The flat nuts were classified as female and the creased nuts as male. A total of 18 samples comprising of 9 males and 9 females were peeled to remove the fibrous shell. The inner shell was split using a knife to release the oil-bearing cotyledon. The cotyledons were pounded using a mortar and pestle to obtain a paste that was stored at 4^{o}C in a refrigerator until analysis.

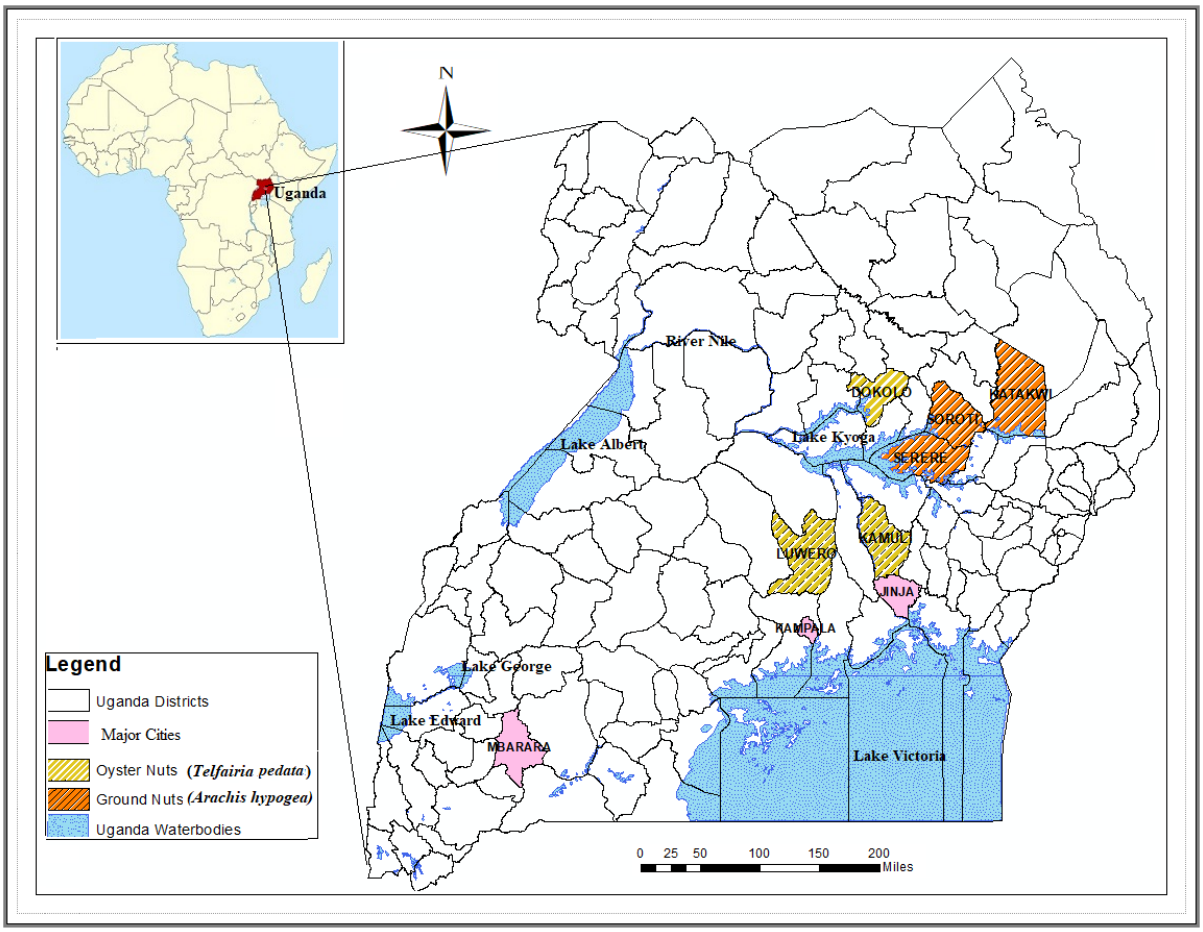

Figure 11: Districts where *Arachis hypogea* and *Telfairia pedata* used in the study were collected. (C) 2020

3.2 Oil Extraction

Oil extraction was performed according to the method of Bligh and Dyer (1959) with slight modifications. Groundnut flour (10 g) and oyster nut paste (10 g) were separately weighed into a 250 ml flat bottomed flask. Then, 100 ml chloroform and 100 ml of methanol was added to each flask. Fifty microliters (50µl) of 0.5% (w/v) butylated hydroxyanisole was added to prevent oxidation. Flask contents were mixed using an ultraturax homogenizer (IKA T18, Bergkirchen, Germany) for 2 min. The mixture was transferred into a 40 ml dionex vial and centrifuged at

2000 rpm for 5 min. The mixture was filtered through a Macherey-nagel filter paper (MN No. 616, 125 mm, Germany), containing anhydrous sodium sulphate. The filtrate was concentrated using Techne Dri-Block (DB-3D, USA) under a stream of nitrogen at 40 ºC. Oil yield was expressed as percentage of the original weight of the sample extracted.

3.3 Determination of Stability

3.3.1 Acid Value

Acid value was determined by titration according to AOAC (2000) method number 920.212. Oil (2 g) from traditional and improved groundnuts and oyster nuts was separately weighed into a flask. Fifty millilitres of ethanol (99.9%) containing 0.5 ml of phenolphthalein indicator were heated in a second flask. The contents of the first flask were neutralized with a solution of 0.1 M potassium hydroxide until a pink colour developed and persisted for at least 15 s. The neutralized ethanol was added to the sample in the first flask and mixed thoroughly. The contents were brought to boil and titrated with 0.5M sodium hydroxide until the mixture turned colourless. Acid value was calculated according to the following expression;

$$\text{Acid Value} = \frac{\text{VxCx56.1}}{\text{M}}$$

Where;

V: Volume of standard sodium hydroxide solution

C: Concentration of sodium hydroxide solution

M: Mass of test sample

56.1: Molar mass of sodium hydroxide

3.3.2 Peroxide Value

Peroxide value was determined using the titration method as described by AOCS (2011) method number Cd 8b-90. Oil (1 g) was dissolved in 15 ml of glacial acetic acid: chloroform (3:2, v/v) into a dry conical flask to dissolve the fat while swirling. Fresh saturated potassium iodide solution (1 ml) was added, the flask was stoppered and shaken for 1 min. Water (75 ml) was then added to the mixture which was then titrated with 0.1M sodium thiosulphate solution using starch indicator (1%) until the solution turned colourless. Peroxide value was calculated using the expression below;

$$\text{Peroxide value} = \frac{(V_1 - V_0)T}{\text{Meq}}$$

Where;

T: Morality of thiosulphate solution

M: Mass of the sample

V_1: Volume of sodium thiosulphate solution

V_0: Volume of the blank

3.3.3 Iodine Value

Iodine value was calculated from the fatty acid composition using the method of Hashim et al. (1993).

IV = (% Oleic acid x 0.8601) + (% Linoleic acid x 1.7321) + (% Eicosanopentanoic acid x 0.7854)

Where; 0.8601, 1.7321 and 0.7854 are conversion factors representing the potential number of iodine atoms added to each fatty acid in the formula for groundnut oil (Nielsen, 2017).

3.4 Determination of Fatty Acid Composition

3.4.1 Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) were prepared according to AOAC (2000) method number 969.33. Oil (0.5 g) was weighed into a 40 ml glass vial and 2 ml diethyl ether added. Mixture was vortexed until oil was dissolved. Methanolic potassium hydroxide (0.5 ml; 5%) was added and the mixture allowed to react for 15 min. during which the solution became cloudy due to soap formation.

3.4.2 Extraction of Fatty Acids from the Soap Solution

Distilled water (2 ml) was added to the FAME solution followed by 10 ml of hexane. Mixture was vortexed for 1 min. to allow phase separation. Organic layer (~5 ml) was transferred to a clean test tube then 2 ml distilled water was added. Mixture was allowed to stand for 2 min. to allow phase separation. This was repeated until the water used to wash the organic layer showed no color change with phenolphthalein. Organic layer (1 ml) was transferred to a 1.5 ml GC vial and 1 µl injected onto a 30 m x 0.32 mm x 0.5 µm Sol Gel-Wax column (Agilent Technologies; USA) with polyethylene-glycol as the stationary phase and helium gas at 20 psi as the mobile phase. The column was mounted in a GC/FID (Varian chrompack CP-3800, USA). Injector temperature was 260°C. The temperature of the column was kept at 50°C for 5 min. after injection and thereafter increased to 180°C at a rate of 20°C/min., followed by an increase of 2°C/min. to 200°C, held for 11 min. and then finally ramped to 250°C at 2°C/min. and held for 2.5 min. Fatty acids were identified by analyzing a reference standard mixture (Supelco 47885-U, Sigma Aldrich, Belgium) under the same conditions as the test portion. The retention times of the standard were compared with those of the test portion. Calculation of the peak area was

by normalization which assumes all components of the test portion are represented on the chromatograms so that the sum of all the peals represents 100% of the constituents.

3.4.3 Effect of Heat Processing on Fatty Acid Composition

Heat treatment of nuts was done by the method of Ndidi, Ndidi, Aimola, Bassa, Mankilik, and Adamu (2014) with slight modifications in terms of heat source and duration of heating . While Ndidi et al used firewood to roast the nuts while a hot plate was used in this study. Furthermore Ndidi et al. (2014) roasted for 1 hr and cooled and pulverized roasted nuts with a mill while in this study, nuts were crushed using a domestic blender. Ndidi et al. (2014) crushed the nuts after boiling while in this study nuts were crushed before stewing. Samples each of traditional and improved groundnut cultivars were subjected to similar heat treatments. Traditional cultivars included *Egoromoit*, *Rudu* red and *Rudu* white while the improved comprised of Serenut 2, 4Tan and 5Red. Selected nuts are some of the most popularly consumed cultivars in Teso sub-region. Oyster nuts comprising male and female from Dokolo, Kamuli and Luwero were used in this study. Oyster nuts from each district represented one of the three replicates. Nuts were mannually shelled to release the seeds. Nuts were portioned into lots which were subjected to different treatments. The first lot (100 g) was set aside as control (raw). The control (raw nuts) were sorted, cleaned and crushed into flour and these were stored in a refrigerator at 4^{o}C until analysis. The second lot (100 g) was blended into a fine flour using a domestic blender (Magic Butler MB1001, Austria-4063 Horsching). Groundnut flour/oyster nut paste (100 g) was mixed with 100 ml of hot water (just boiled) and left to stand for 5 min. prior to stewing at 92^{o}C for 15 min. on a hotplate (Neo-Tech SA, 404, Belgium). Groundnut/oyster nut stew was allowed to cool for 5 min. and then packed in a plastic sample bottle. The third lot (100 g) was subjected

to dry roasting on an aluminium pan at 179.3ºC for 15 min. after which it was ground, packed in a plastic bag and stored in a refrigerator until use. Oil was extracted from the heat treated samples according to the procedure described in section 3.2 and the stability indices (Peroxide value and iodine value) of the oil were established according to the procedures in sections 3.3.2 and 3.3.3.

3.5 Health Lipid Indices

Health lipid indices were obtained empirically from the fatty acid composition data using the methods below.

3.5.1 Atherogenic and Thrombogenic Index

Atherogenic index shows the relationship between the sum of the main SFA and that of the main classes of unsaturated, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified FA, cholesterol, and phospholipids, there- by preventing the appearance of micro- and macro- coronary diseases). Thrombogenic index indicates the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MUFA, PUFA–ω6 and PUFA– ω3). Atherogenic index (AI) and thrombogenic index (TI) were calculated according to the following equations by Ulbricht and Southgate (1991);

$$\text{Atherogenic index} = \frac{(\text{C14.0x4} + \text{C16.0} + \text{C18.0})}{(\sum\text{MUFA} + \sum\omega6\text{PUFA} + \sum\omega3\text{PUFA})}$$

$$\text{Thrombogenic index} = \frac{(\text{C14.0} + \text{C16.0} + \text{C18.0})}{(0.5\text{xMUFA} + 0.5\text{x}\omega6\text{PUFA} + 3\text{x}\omega3\text{PUFA}) + \frac{\omega3}{\omega6}}$$

3.5.2 Hypocholesterolemic/hypercholesterolemic index (h/H)

Hypocholesterolemic/hypercholesterolemic index (h/H) indicates potential of a lipid to balance between the good and bad cholesterol. The index was calculated from the following equation adopted from Santos-Silva, Bessa, and Santos-Silva (2002).

$$\frac{\text{h}}{\text{H}} = \frac{(\text{C18.1}\omega9 + \text{C18.2}\omega6 + \text{C20.4}\omega6 + \text{C18.3}\omega3 + \text{C20.5}\omega3 + \text{C22.6}\omega3)}{(\text{C14} + \text{C16})}$$

h: Hypocholesterolemic; H: Hypercholesterolemic, C14.0: Myristic acid, C16: Palmitic acid; C18.1 : Oleic acid; C18.2 : Linoleic acid; C20.4:, C18.3 : Alpha linolenic acid; C20.5: Eicosanopentaoic acid, C22.6: docosahexaenoic acid ;ω: omega.

3.6 Anti-oxidant Vitamins

3.6.1 Determination of Vitamin A Content

Vitamin A content of the oil was analysed using high-performance liquid chromatography (HPLC) 1290 series (Agilent technologies, USA) equipped with a photometric detector and a reverse-phase column (C18, 10 µm; 4.6 x 250 mm) for the measurement of trans- and cis-isomers of retinol according to AACC (2000). Oil (3 g) was dissolved in a mixture of pure tetrahydrofuran and absolute ethanol (50:50, v/v) and the absorbance assayed at 328 nm. The mobile phase was prepared by mixing 860 ml of HPLC grade methanol and 140 ml deionised water. A vitamin A working standard was prepared by dissolving 50 mg of USP-vitamin A acetate concentrate (Sigma) in 100 ml absolute ethanol. The vitamin A working standard was

injected into the HPLC system (Hewlett Packard HP1100, Germany) with a mobile phase resolution of 1.5 for trans- and cis-isomers of retinol. Vitamin A was quantified by comparison of chromatographic peak areas for the standard and that corresponding to retinol in the sample extract (Appendix 2)

3.6.2 Determination of Beta-carotene

Beta-carotene was determined according to AOAC (1980). Oil (5 g) was mixed with 50 ml of 95% ethanol after which it was placed in a water bath (GP-200, China) at 75^{o}C for 20 min. with periodic shaking. Supernatant was decanted, cooled and swirled gently to obtain a homogeneous mixture. Mixture was left to stand until it separated into two layers. Volume of the supernatant was measured and recorded as initial volume. Concentration of ethanol was brought to 85% by adding 15 ml of water and further cooled in a container of ice water for 5 min. Mixture was then put into a separating funnel and 25 ml petroleum ether added followed by extraction by swirling. Ethanol (3 drops) was administered over the mixture, the funnel swirled gently to obtain a homogeneous mixture. Mixture was left to stand until it separated into two phases. Bottom phase was run off into a 100 ml beaker while the top phase was collected into a 50 ml conical flask. Bottom layer was transferred into the funnel and re-extracted with 10 ml of petroleum ether for 5 to 6 times until the extract became fairly yellow. Petroleum ether was collected into a 250 ml conical flask and transferred into a separating funnel for re-extraction with 50 ml of 80% ethanol. Final extract was put in a sample bottle for further analysis. Absorbance was measured on a UV/VIS spectrophotometer (Perkin Elmer Lambda 35, USA) at 436 nm. Instrument was calibrated to the zero-point using a cuvette with petroleum ether (blank). Samples of all the 20 groundnut oil extracts were put in different cuvettes to read their corresponding absorbances.

Concentration of beta-carotene was calculated using the Beer Lambert's law; A=ECL Where: C= concentration of carotene; A= absorbance; E=extinction coefficient; L= thickness of cuvettes (path length) =1 cm; E of β-carotene =1.25x104 µg/l

3.6.3 Determination of Vitamin E (Alpha-tocopherol)

Alpha-tocopherol was determined by high performance liquid chromatography (HPLC), (Perkin Elmer, 200 series, USA) using the method described by AACC (1989). A calibration curve (Appendix 3) was prepared using standard alpha-tocopherol (CAS 10191-41-0; Sigma Aldrich, Belgium). A vitamin E working standard was prepared by weighing 55 g of tocopherol palmitate into a 100 ml volumetric flask and a pea sized piece (~50 mg) of pyrogallic acid added. Solution was made up to the mark with hexane. Five millilitres (5 ml) of solution was transferred into a 250 ml flask, diluted to volume with 95% ethanol and then stored in the dark. Vitamin E working standard (5 ml) was transferred into a 125 ml Erlenmeyer flask and 25 ml of 95% ethanol added followed by ~50 mg pyrogallic acid. Oil (2 g) was weighed into a 125 ml Erlemmeyer flask and 40 ml of 95% ethanol added. Pyrogallic acid (50 mg) was added to each sample and a glass bead added to promote boiling. Flasks were swirled to ensure thorough dispersion of samples in the solution. Potassium hydroxide (50%, 10 ml) was transferred into each flask and placed on the hot plate under a reflux condenser. Refluxing took 45 min. with swirling every 10 min. After refluxing, flasks were left to cool to room temperature (25^{o}C), after which 10 ml of 1% glacial acetic acid was transferred into each flask to neutralize the potassium hydroxide. Flasks were left to cool to room temperature. Each of the solutions was transferred to a 100 ml volumetric flask using a 50:50, v/v, tetrahydrofuran (pure): ethanol (99.9%) solution and solution made to the mark using distilled water. Samples were centrifuged for 15 min. at 2000 rpm allowing FA

salts formed during saponification to precipitate. The extract (50 µl) was injected into the HPLC system. Samples were separated in a C8, 250 mm x 4.00 mm column (Perkin Elmer, 200 series, USA) by employing methanol-water (90:10 v/v) mobile phase with a flow rate of 1 ml/min. Value of α-tocopherol in the standard was detected by the diode array detector (Perkin Elmer 200 series, USA) set at 290 nm. The α-tocopherol in the sample was identified by comparing the retention times with those of α-tocopherol standard.

3.7 Determination of Minerals

Concentrations of calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) were determined by flame atomic absorption spectrophotometry (AAS) according to AOAC (2012) method number 927.02. Groundnut and oyster nut oil (10 g) was separately weighed into crucibles and placed into a muffle furnace (L15/12/B180, Nabertherm GmbH, Germany). Sample was heated at 550^{o}C for 360 min. to char any organic matter present. Resulting ash was digested in 5 ml of 20% hydrochloric acid, and filtered using Whatman No 1 filter paper. Procedure blank was also prepared. Standard solutions of Ca, Mg, Fe and Zn (Scharlau, Germany), were prepared by successive dilution of the stock standard solutions with distilled water. Mixtures were transferred into a 100 ml volumetric flask and filled to the mark with distilled water. Standard solutions were prepared in the concentrations of 0.1, 0.2 0.3, 0.4, 0.5 ppm and were used in calibration. Standard solutions (1 ml) were transferred into a 100 ml volumetric flask and diluted to the mark with 1% nitric acid. Standards for each of Ca, Mg, Fe and Zn were aspirated into the AAS (Perkin Elmer, Aanalyst 400 Shelton CT 06484-4794, USA) and the absorbances were recorded. Calcium, Mg, Fe and Zn of the sample were analysed using AAS by aspirating the liquid sample containing the metals into an air acetylene oxide lean, blue flame to allow

atomization of metal atoms. These were then excited by multi-element lamps corresponding to Ca, Mg, Fe or Zn. Absorbance was measured with a conventional UV-visible dispersive spectrometer. Respective wavelengths for detection of Ca, Mg, Fe and Zn were 442.7, 285.2, 248.3 and 213.9 nm.

3.8 Determination of Tannins

Amount of tannins was determined according to AOAC (2000). Groundnut and oyster nut oil was prepared by adding 5.0 ml of 25%:75% water/methanol to 5 g of sample in a 20 ml centrifuge tube and vortexed for about 10 min. Mixture was then centrifuged (3000 rpm) for 15 min. to allow proper separation. Extract (0.5 ml) was transferred into a 10 ml test tube containing 7.5 ml of distilled water. Folin-Ciocalteuphenol reagent (0.5 ml) was added followed by 1 ml of 35% sodium carbonate solution. Mixture was topped up to 10 ml with distilled water. Mixture was shaken and kept at room temperature for 30 min. after which it was read at 760 nm on a spectrophotometer (Agilent Cary 60 UV-Vis). Standard solution (1000 µg/ml) was prepared by weighing 10 mg of tannin reference standard in a 10 ml volumetric flask and thereafter made up to 10 ml with distilled water. Portions of 20, 50, 100, 200 and 400 µg were taken in different test tubes. Folin-Denis reagent (0.5 ml) and sodium carbonate solution (1 ml) were added to each tube and made up to 10 ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 min. and their absorbances were read in a spectrophotometer at 760 nm against the reagent blank. The concentration of tannins measured in µg/g of tannic acid.

3.9 Statistical Analysis

Samples were analysed in triplicate and data were computed for means ± standard deviations of the means using SPSS version 23.0 (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) with a Tukey's least significant difference (LSD) test was used to evaluate the differences in FA, anti-oxidant vitamins, minerals and tannins composition. Differences were considered statistically significant at $p<0.05$.

CHAPTER FOUR

4 Results and Discussion

4.1 Yield and Stability of Groundnut and Oyster Nut Oil

4.1.1 Groundnuts Oil Yield

Oil content in groundnuts ranged from 26.63 to 54.60% (Table 13) with a mean yield of 40.62%. In agreement with the hypothesis, all groundnut cultivars had high amount of oil. Among improved cultivars, Serenut 11Tan had the highest yield of 54.60% while Serenut 3Red had the lowest (26.65%). All traditional cultivars had oil yield above 40% except *Egoromoit* which had the lowest yield of 38.74%. Findings agree with Özcan and Seven (2003); Turner, (2010); Gunstone (2011); Shad et al. (2012); and Wang et al. (2013), who reported a range of 40 to 50% oil yield in groundnuts. In spite of differences ($p<0.05$) within the cultivars, results confirm that groundnuts have high oil content. Compared to traditional cultivars, there was wide variation in the oil content of the improved groundnut cultivars, an effect pertaining to cultivar and genetic background (Wilson et al., 2013). Isleib, Pattee and Giesbrecht (2004) and, (Gunstone, 2011) explained that oil yield is affected by genotype, environmental conditions during growth, maturity level and interaction amongst these factors. Other factors that influence oil yield include; pH, temperature, moisture availability and use of fertiliser (Vossen (2007); Ahmed, Pickova, Liaquat, and Jahangir, 2016). Bera et al. (2018) found high variation in oil yield in improved groundnuts in India during four different growing seasons. Studies by Adeeko and Ajibala (1990) suggest that conditions of extraction can also affect oil yield. In this study, all cultivars of groundnuts were subjected to similar treatments prior to oil extraction. Adeeko and Ajibala (1990) further noted that variation in temperatures and particle size of mass before oil extraction may affect oil yield. In their trial, it was observed that the coarse mass yields more

oil than the finely crushed mass. Vossen (2007) reported variations in oil yield in various cultivars of olives grown in different countries. This suggests dependency of oil yield on environmental conditions and cultivar.

Table 13: Groundnut oil yield

Cultivar	Oil yield	Cultivar	Oil yield
Improved Cultivars		S12R	34.54±0.01^{n}
S1R	49.16±0.01^{c}	S13T	35.50±0.17^{m}
S2	33.90±0.10^{o}	S14R	45.10±0.02^{f}
S3R	26.65±0.01^{p}	Traditional cultivars	
S4T	38.63±0.15^{l}	AW	43.61±0.01^{h}
S5R	39.85±0.01^{k}	IGO	47.19±0.01^{d}
S6T	46.50±0.17^{e}	RW	44.27±0.01^{g}
S7T	47.17±0.15^{d}	RR	42.28±0.01^{i}
S8R	40.32±0.02^{j}	EGT	38.74±0.01^{l}
S9T	46.63±0.25^{e}	RB	44.21±0.01^{g}
S10R	50.75±0.03^{b}		
S11T	54.60±0.17^{a}		

Values are means of three independent replicates ±standard deviations of the means. Values in columns with similar superscript letters are not significantly different ($p>0.05$).

Findings from this study suggest that *Egoromoit*, Serenut 2, 3Red, 4Tan, 5Red, 12Red and 13Tan could be best used for confectionary purposes while the rest of varieties could be exploited for commercial oil production. Genotypes with low oil content are best used in

confectionery products whereas high oil genotypes can be exploited for commercial oil extraction (Asibuo, 2008; Bishi et al., 2013).

4.1.2 Stability of groundnut oil

To investigate stability, the acid, peroxide and iodine values of groundnut and oyster nut oil were determined. Acid values (AV) of oil varied from 0.42 to 2.38 mg/100 g and peroxide values (PV) ranged between 0.00 and 0.7 mEq/kg (Table 14). Values higher than those specified in Table 14 are indicative of oxidative or hydrolytic degradation. Peroxide value in this study was low while the AV was high compared to that reported by Özcan and Seven (2003) for groundnut oil from Turkey. The higher PV could be attributed to the use of the oxidative Soxhlet method for oil extraction done at 40 to 60^{o}C while in this study the less oxidative cold extraction method of Bligh and Dyer (1959) was used. Results imply that oil from Serenut and traditional cultivars were stable to oxidative and hydrolytic degradation given the low AV and PV.

Iodine values (IV) of groundnut oil were within the recommended range of 86 to 106 (FAO/WHO, 2005) except *Egoromoit*, a traditional cultivar that had the lowest IV of 83.11. Highest IV observed were 98.95 and 96.20 corresponding to oil from Serenut 11Tan and 13Tan, respectively. Among the traditional cultivars, Acholi white and *Rudu* white had the highest IV of 95.92 and 95.40, respectively. The IV of groundnut oil differed significantly ($p<0.05$). Iodine values for groundnut oil in this study were similar to that reported by Singkham et al. (2010) for low and intermediate oleic acid groundnut groups. Singkham et al. (2010) found out that different seasons and differences in oleic acid contents affect IV.

Table 14: Oxidative and hydrolytic stability of groundnut oil

Cultivar	Acid value (mg/100 g)	Peroxide value (mEq/kg)	Iodine value (g/100 g)
Improved Cultivars			
Serenut 1Red	2.38±0.02	0.7±0.01	88.42±0.04^{hi}
Serenut 2	1.47±0.01	0.2±0.00	88.49±0.09^{hi}
Serenut 3Red	2.22±0.00	0.1±0.00	84.73±0.12^{k}
Serenut 4Tan	1.81±0.01	0.02±0.00	85.38±0.08^{k}
Serenut 5Red	2.21±0.00	0.5±0.00	88.98±0.04^{h}
Serenut 6Tan	2.22±0.01	<0.01	87.91±0.05^{ij}
Serenut 7Tan	1.11±0.01	0.02±0.00	93.18±0.08^{ef}
Serenut 8Red	2.23±0.00	0.20±0.00	92.54±0.04^{fg}
Serenut 9Tan	2.11±0.04	0.20±0.00	94.86±0.06^{d}
Serenut 10Red	2.23±0.00	0.00±0.00	87.35±0.07^{j}
Serenut 11Tan	2.32±0.01	0.10±0.00	98.95±0.11^{a}
Serenut 12Red	2.22±0.00	0.50±0.00	92.56±0.09^{fg}
Serenut 13Tan	1.09±0.05	0.10±0.00	96.20±0.07^{b}
Serenut 14Red	2.23±0.00	0.60 ±0.02	93.48±0.00^{e}
Traditional cultivars			
Acholi white	1.84±0.00	0.20±0.01	95.92±0.05^{bc}
Igola	0.56±0.04	1.25±0.01	93.76±0.04^{e}
Rudu white	0.42±0.00	3.00±0.00	91.97±0.03^{g}
Rudu red	0.53±0.00	1.80±0.06	95.40±0.03^{cd}
Egoromoit	0.59±0.04	1.30±0.01	83.11±0.06^{l}
Red beauty	0.53±0.02	2.70±0.04	93.69±0.08^{e}
Recommended level*	4	15	86-107

Values are means of three independent replicates ±standard deviations of the means. Values in columns for iodine value with similar superscript letters are not significantly different ($p>0.05$).
* (FAO/WHO, 2005)

Their study suggested that when oleic acid content is low, IV is high. This study however, did not consider effect of growth seasons or oleic acid levels while testing for stability.

Iodine value is a measure of unsaturation (Mandloi, Radadia, Visavadia, and Vaghela, 2014). Lower level of unsaturation implies higher shelf stability in oil while the converse is true for high unsaturation (Kratz, Cullen, and Wahrburg, 2002; Davis, Price, Dean, Sweigart, Cottonaro, and Sanders, 2016). This suggests that oils from Serenut 11Tan, 13 Tan, *Rudu* white and *Acholi* white are more prone to oxidation than that from *Egoromoit*.

4.1.3 Oyster Nut Oil yield

The oil yield of oyster nut ranged from 45.32 to 56.14% in male nuts from Kamuli and Luwero, respectively, and 47.99 to 51.57% in female nuts from Kamuli and Dokolo, respectively (Table15). Significant differences ($p<0.050$ were observed.

Table 15: Oyster nut oil yield

Geographical source	Oil yield	
	Male oyster nut	Female oyster nut
Dokolo district	51.22 ± 0.04^{b}	51.57 ± 0.03^{b}
Kamuli	45.32 ± 0.03^{c}	47.99 ± 0.05^{c}
Luwero	56.14 ± 0.02^{a}	50.49 ± 0.01^{a}

Values are means of three independent replicates ±standard deviations of the means. Values in columns with different superscript letters are significantly different ($p<0.05$).

Findings of this study are close to a report by Ajayi, Dulloo, Vodouhe, Berjak, and Kioko (2004) who asserted that oyster nut contains 55 to 60% extractable oil. Oil yield of oyster nuts in this study, however, was higher than the 38.3% oil reported by Hopkins and Chisholm(1964) but was lower than that of Nyagah (2016) for oyster nuts grown in South Africa and Kenya,

respectively. Differences could be attributed to origin and maturity of oyster nuts, growing conditions, post-harvest conditions and experimental conditions. A three year long study by Król and Paszko (2017) on effect of maturity stage on oil yeild of *Calendula officinalis* cultivars revealed that more oil yeild was obtained at 65% compared to 50% and 80% maturity. In addition, Wen, Tang, Sun, Zhu, and Wei (2012) reported that climatic factors such as temperature and sunshine as well as soil type significantly affected oil content of *Jatropha curcas.* As a major variation, this study detected twice as much oil as that repoted by Minzangi et al. (2015) in oyster nuts obtained from D.R. Congo. Oyster nuts in this study were obtained from three different districts; Dokolo, Kamuli and Luwero, hence geagraphical source of nuts could have influenced oil yeid. In another study, Wang et al. (2012) asserted that seed texture and the possibility that more oil remained in the cake after extraction could affect the oil yield. They further cited that moisture content and maturity as other factors that may be negatively correlated with oil content. Further still, there were differences in oil content in oyster nuts of different sexual orientation. This implies that sex differences may affect oil yield. Based on oil content, oyster nuts have great potential pending exploitation.

4.1.4 Stability of oyster nut oil

The AV and PV of oyster nut oil varied from 0.85 to 2.30 mg/100 g and 0.33 to 2.30 mg/100 g, respectively, for oil from male and female nuts (Table 16). The AV and PV of the oil were not significantly different ($p>0.05$) and were within the acceptable limits of 4 mg/g and 15 mEq/kg established by FAO/WHO (2005). Results suggest that oil from oyster nuts was stable to oxidative and hydrolytic degradation given the low values of AV and PV. The IV of oyster nut oil ranged from 77.13 to 84.88 g/100 g (Table 16). Significant differences ($p<0.05$) were

observed in iodine values among different districts. The IV of oyster nut oil in this study was close to the 83.2 reported by Hopkins and Chisholm (1964), for oyster nut oil but lower than the 109 obtained by Nyagah (2016) for oyster nut oil. Ajayi, Dulloo, Vodouhe, Berjak, and Kioko (2004) described oyster nut oil as a non-drying oil with typical IV <100. Although the IV of oyster nut oil was not included in the codex standard for oil, it was close to that of safflower and high oleic sun flower oil which range from 80 to 100 and 78 to 90, respectively (FAO/WHO, 2005).

Table 16: Stability of oil from oyster nuts

Location	Gender	Acid value (AV) (mg/100 g)	Peroxide value (PV) (mEq/kg)	Iodine value (IV) (g/100 g)
Dokolo	Male	0.85±0.08	1.00±0.01	82.76±0.06
	Female	0.96±0.02	0.87±0.01	87.59±0.07
Kamuli	Male	1.51±0.04	0.37±0.00	83.28±0.04
	Female	1.63±0.20	0.33±0.00	84.45±0.10
Luwero	Male	2.18±0.01	2.23±0.00	85.43±0.02
	Female	2.30±0.03	2.23±0.01	86.91±0.07
Recommended *		4	15	NA

Values are means of three independent replicates ±standard deviations of the means. Values in columns were not significantly different ($p>0.05$). * (FAO/WHO, 2005); NA: Not available.

4.2 Fatty Acid Composition of Groundnut and Oyster Nut Oil

4.2.1. Fatty Acid Composition of Groundnut Oil

Dominant fatty acids (FA) in groundnut oil were oleic (C18.1), linoleic (C18.2ω6) and palmitic (C16.0) with amounts more than 10% each (Table 17). Levels of the above mentioned FA varied significantly ($p<0.05$) indicating compositional differences among the cultivars. Oleic acid (C18.1) amounts in the oil varied from 39.83 to 55.89%. The highest amounts of oleic acid (C18.1) did not differ ($p<0.05$) in traditional and improved groundnut cultivars. However, the lowest value, 39.83%, was observed in Acholi white, a traditional cultivar. These findings are similar to data by Khetarpaul, Jood, and Goyal (2007), Özcan (2010) and Dorni et al. (2018) who reported 48.90%, 48.4 to 57.3% and 53.77% oleic acid (C18.1), respectively, in groundnut oil consumed in India and Turkey.

Linoleic acid (C18.2ω6) ranged from 20.23 to 35.59% in oil from *Egoromoit* and Acholi white, respectively. Among improved cultivars, oil from Serenut 11Tan showed the highest linoleic acid level (34.05%). Findings were in agreement with the findings of Achola et al. (2017) who reported levels ranging from 26.79 to 33.44% for groundnuts in Uganda. Similarly, Dorni et al. (2018) reported 26.96% linoleic acid (C18.2ω6) while Gulluoglu et al. (2016) reported a range of 2 to 43% linoleic acid (C18.2ω6) in groundnut oil from India and Poland, respectively. Linoleic acid (C18:2ω6) levels corroborated with the range 27.3 to 38.3% reported by Özcan (2010) for oil from groundnuts grown in Turkey. Similar results; 31.28 to 36.15% linoleic acid were detected by Singkham et al. (2010) for oil from low-oleic groundnuts in India. According to Hashim et al. (1993), exposure of groundnuts to drought during maturation may lead to enhancement of C16.0 and C18.2ω6 while reducing C18.1, C18.0 and C20.1. On the other hand,

high temperature 4 weeks before harvest causes synthesis of more C18.1 than C18.2ω6. Singkham et al., 2010) argued that a dry season favoured production of C18.2ω6 than C18.1 in groundnuts and further noted that genotype and environment affect FA composition.

Other unsaturated FA such as gamma linolenic (C18.3ω6), alpha linolenic (C18.3ω3), eicosenoic (C20.1) and eicosadienoic (C20.2) acids occurred in amounts less than 2% in all cultivar oil. Levels of these FA were more pronounced in oil from traditional than improved cultivars. Alpha linolenic acid (C18.3ω3) amounts were less than 2% in all cultivars. Wang, Raymer, Chinnan, and Pittman (2012) reported 0.01 to 0.1% alpha linolenic acid (C18.3ω3) in oil from raw groundnuts. Levels of C18.3ω3 in this study were consistent with the Codex specifications (nd to 0.3%) in groundnut oil (FAO/WHO, 2005). Results imply that groundnut oil is not a good source of alpha linolenic acid. Hence other rich sources of C18.3ω3 such as fish oil, flaxseed and soybean oil should be included in the diet. Cis 11-eicosenoic acid (C20.1), cis 11, 14 eicosadienoic acid (C20.2) and cis 11, 14, 17 eicosatrienoic acid (C20.3ω3) were only detected in oil from improved cultivars and not in the traditional ones. Achola et al. (2017) examined the FA composition of Serenut 1 to 10 but did not report the presence of 11, 14, 17 eicosatrienoic acid (C20.3ω3). The presence of these FA in oil from the Serenut cultivars implies that there may be other factors that influence levels of the above mentioned FA such as extraction method, genotype, growth season or source of groundnuts besides breeding (Singkham et al., 2010; Rodrigues et al., 2011; Hassan and Ahmed, 2012).

Palmitic acid (C16.0) was the major saturated FA and varied from 11.90 to 17.17%. Findings were close to data by Achola et al. (2017) who reported a range of 14.61 to 18.6% palmitic acid

(C16.0) in Serenut 5 to 10 oil. Levels of palmitic (C16.0) were higher in some traditional cultivars than the improved cultivars (Table 17). Oil from Acholi white had higher palmitic acid (17.17%) than all other groundnut oil (Table 17). Oil from Serenut 1Red showed highest level palmitic acid 15.73% among the improved cultivars. Shahidi (2005) and Özcan (2010) presented 8 to14% and 7.63 to 11.41% palmitic acid (C16.0), respectively, in groundnut oil. The palmitic (C16.0) levels were consistent with those reported by Achola et al. (2017), however, they were higher than those reported by Singkham et al. (2010) for low oleic groundnuts. Differences may pertain to genotype (Rodrigues et al., 2011). Palmitic acid (C16.0) is regarded as a catastrophic SFA with regard to cardiovascular disease (Carta, Murru, Banni, and Manca, 2017).

Levels of stearic acid (C18.0) were higher in oil from traditional than the improved cultivars. Oil from *Egoromoit* and Acholi white oil had the highest amounts; 4.93% and 4.88% stearic acid (C18.0), respectively. With exception of Serenut 4Tan, the content of stearic acid (C18.0) in oil from improved cultivars was generally low with amounts less than 4%. Stearic acid (C18.0) ranged from 2.03 to 4.93%. Berry (1982); and Achola et al. (2017) reported 3.17 to 3.68% and 2.19 to 3.46% stearic acid (C18.0), respectively, in groundnut oil. Stearic acid levels in this study were in accordance with those of Özcan (2010) who detected 2.14 to 4.13% and within the 2.37 to 6.02% reported by Singkham et al. (2010)for oil from low oleic groundnuts. Stearic acid is considered neutral to cholesterol metabolism, Gurr (1999; Valenzuela, Delplanque, and Tavella,(2011).

Arachidic acid (C20.0), the characteristic FA in groundnuts and their products tanged from 0.68 to 1.36% in oil from Serenut 13Tan and Serenut 10Red, respectively. Among traditional

cultivars, the lowest amount (0.82%) was detected in oil from *Igola* while the highest level (2.80%) was found in that from *Rudu* red. Dorni et al. (2018) and Özcan (2010) reported respective proportions of 1.44 to 2.36% and 1.42% arachidic acid (C20.0) in groundnut oil. Berry (1982) and FAO/WHO (2001; 2005) cautioned that arachidic acid (C20.0) levels in excess of 4.8% in groundnut oil may cause atherogenicity. Levels of arachidic acid (C20.0) in this study were within the recommended limits. Behenic acid (C22.0) in oil from all groundnut cultivars was less than 2% with the exception of *Rudu* red oil which had 3.21%. Behenic acid (C22.0) was higher in the traditional cultivar oil compared to improved counterparts.

4.2.2 Total Fatty Acids in Groundnut Oil

Approximately 80% of FA in oil from all groundnut cultivars were unsaturated (Table 18). Oil from traditional groundnut cultivars generally had higher amounts of SFA compared to the improved. The highest level of SFA was observed in Acholi white at 23.52% while the lowest was observed in Serenut 2 at 17.89%. Saturated FA in groundnuts were similar to the reports of Dorni et al. (2018); Kostik et al. (2013) for groundnut oil. In contrast, this study found higher SFA than that reported by Orsavova, Misurcova, Vavra Ambrozova, Vicha, and Mlcek (2015) in groundnut oil over two separate seasons. Al-bachir (2015) too reported lower levels of SFA in gamma irradiated groundnut oil.

Highest MUFA among Serenut was detected in Serenut 3Red at 56.01% while *Egoromoit* had the highest MUFA at 57.01% among traditional cultivars. The rest of the cultivars had a mean value of about 50% MUFA except Acholi white which had the lowest level at 39.92%. As expected, the levels of MUFA particularly C18.1 were high compared to other FA in this study.

Oleic acid (C18.1) is reported to enhance shelf life due to its resistance to oxidative degradation (Su, 2003; Yun and Surh, 2012). The MUFA levels in this study were lower than the 60.73 to 85.93% and 58.5% reported by Shad, Pervez, Zafar, Nawaz, and Khan (2012),and Kostik et al., (2013), respectively, for groundnut oil. Orsavova et al. (2015) reported a MUFA content of 71.1% in groundnut oil. Differences in MUFA levels may be ascribed to cultivar.

PUFA in traditional groundnut oil ranged from 22.01% in *Egoromoit* to 36.22% in Acholi white. Among improved cultivars, Serenut 1Red had the highest PUFA content (35.32%), whereas Serenut 3Red contained the lowest (24.27%). Levels of PUFA in groundnut oil were similar to amounts (26.96%) reported by Dorni et al. (2018) but higher than the 18.2% PUFA detected by Orsavova et al., (2015) in groundnut oil.

Total FA in oil from groundnuts varied significantly ($p<0.05$) among traditional and improved cultivars. Acholi white showed a unique FA composition with low levels of MUFA, mainly oleic acid, while the PUFA levels, mainly linoleic acid were significantly higher than those of other cultivars. This could be attributed to genotype as explained by Wang et al. (2012).
Various studies have shown that oleic and linoleic acid have been reported to lower total serum cholesterol (Grundy, 1997; Gurr, 1999; Alabdulkarim, Bakeet, and Arzoo, 2012). A similar assertion was made by Kris-Etherton (1999); and Sacks et al. (2017) who examined the role of dietary fats on cardiovascular disease.

Table 17: Fatty acid composition (%) of groundnut oil

GNC	C16.0	C18.0	C18.1	C18.2	C18.3ω3	C18.3ω6	C20.0	C20.1	C20.2	C20.3ω3	C22.0
Improved Cultivars											
S1R	15.73±0.03^{c}	2.13±0.03	50.29±0.04^{f}	26.08±0.02^{i}	1.09±0.07^{a}	0.34±0.03^{de}	1.02±0.07^{gh}	0.74±0.01^{de}	0.94±0.01^{b}	0.30±0.03^{c}	1.09±0.02^{f}
S2	14.85±0.04f	2.02±0.03	52.27±0.02^{c}	25.13±0.06^{j}	1.05±0.04^{a}	0.36±0.02^{de}	1.24±0.02^{def}	0.55±0.03^{f}	0.93±0.04^{b}	0.34±0.02^{c}	1.05±0.01^{fgh}
S3R	14.51±0.01^{h}	2.88±0.02	54.86±0.04^{b}	21.68±0.05^{l}	0.08±0.01^{gh}	0.28±0.00^{def}	1.10±0.03^{fg}	0.74±0.00^{de}	1.36±0.02^{a}	0.87±0.02^{a}	1.41±0.01^{cd}
S4T	13.63±0.02^{j}	4.15±0.04	55.07±0.05^{b}	21.95±0.02^{l}	0.08±0.01^{gh}	0.34±0.02^{de}	0.68±0.03^{jk}	0.81±0.01^{ab}	1.41±0.01^{a}	0.88±0.07^{a}	0.84±0.04^{jk}
S5R	14.88±0.03^{e}	2.76±0.03	48.72±0.03^{h}	27.18±0.02^{h}	0.05±0.01^{hi}	0.57±0.05^{c}	1.32±0.01^{de}	0.55±0.02^{f}	1.16±0.07^{ab}	0.91±0.07^{a}	1.59±0.01^{b}
S6T	15.87±0.02^{b}	3.43±0.03	50.30±0.03^{f}	25.77±0.03^{i}	0.07±0.01^{ghi}	0.51±0.02^{c}	0.70±0.01^{jk}	0.56±0.01^{f}	0.92±0.07^{b}	0.58±0.08^{b}	1.07±0.02^{fg}
S7T	14.65±0.04^{i}	3.17±0.01	46.32±0.02^{j}	30.79±0.04^{e}	0.06±0.02^{ghi}	0.02±0.00^{g}	1.20±0.01^{ef}	0.77±0.00^{cd}	0.95±0.05^{b}	0.36±0.03^{c}	1.46±0.03^{c}
S8R	13.75±0.05^{g}	3.14±0.05	48.63±0.01^{h}	29.27±0.03^{fg}	0.23±0.01^{e}	0.24±0.03^{def}	1.15±0.07^{fg}	0.85±0.02^{a}	1.03±0.06^{b}	0.13±0.01^{d}	1.33±0.04^{d}
S9T	14.59±0.02^{h}	2.08±0.06	45.96±0.03^{k}	31.94±0.03^{d}	0.27±0.01^{e}	0.28±0.01^{def}	1.33±0.04^{de}	0.80±0.02^{bc}	0.43±0.03^{c}	0.14±0.02^{d}	1.13±0.02^{ef}
S10R	15.09±0.05^{d}	2.46±0.03	51.87±0.03^{d}	24.67±0.05^{k}	nd	0.22±0.01^{ef}	1.36±0.03d	0.72±0.04e	1.20±0.01^{ab}	0.89±0.06^{a}	1.36±0.02^{d}
S11T	13.79±0.01^{i}	2.36±0.03	46.47±0.07^{j}	34.05±0.05^{b}	0.97±0.05^{b}	0.19±0.01^{f}	0.92±0.07^{hi}	nd	nd	nd	0.95±0.07^{hi}
S12R	15.41±0.03^{c}	2.56±0.01	52.10±0.02^{c}	27.57±0.03^{h}	nd	0.31±0.01^{def}	1.02±0.04^{gh}	nd	nd	nd	0.98±0.06^{gh}
S13T	14.65±0.04^{g}	2.36±0.00	50.20±0.03^{f}	30.61±0.01^{e}	nd	0.37±0.03^{d}	0.70±0.02^{jk}	nd	nd	nd	1.08±0.02^{fg}
S14R	14.86±0.05^{f}	2.65±0.04	49.72±0.03^{g}	28.87±0.05^{f}	nd	0.97±0.07^{b}	1.03±0.06^{gh}	nd	nd	nd	1.14±0.03^{ef}
Traditional Cultivars											
AW	17.17±0.02^{a}	4.88±0.03	39.83±0.02^{n}	35.59±0.01^{a}	nd	0.53±0.02^{c}	1.60±0.02^{d}	nd	nd	nd	0.86±0.03^{ij}
IGO	14.83±0.02^{f}	2.63±0.01	50.86±0.01^{e}	28.87±0.02^{g}	nd	1.40±0.05^{a}	0.82±0.02^{ij}	nd	nd	nd	0.74±0.04^{k}
RR	12.80±0.04^{k}	4.22±0.02	44.77±0.02^{l}	30.86±0.03^{e}	0.14±0.01^{fg}	nd	2.80±0.06^{a}	nd	nd	nd	3.21±0.05^{a}
RW	12.71±0.02^{k}	4.81±0.02	44.29±0.02^{m}	33.09±0.02^{c}	0.21±0.02^{ef}	nd	2.12±0.03^{c}	nd	nd	nd	1.14±0.02^{ef}
EGT	13.76±0.04^{i}	4.93±0.03	55.89±0.02^{a}	20.23±0.03^{m}	0.67±0.02^{c}	nd	2.22±0.04^{c}	nd	nd	nd	1.21±0.01^{e}
RB	11.90±0.04^{l}	4.75±0.01	46.98±0.01^{i}	30.76±0.0^{e}	0.59±0.02^{d}	nd	2.44±0.05^{b}	nd	nd	nd	1.32±0.03^{d}

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values followed by the same superscript letter within each column are not significantly different ($p>0.05$). nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: red; T: Tan; AW: Acholi white; IGO: *Igola*; RR:*Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; RB: Red beauty.

Table 18*:* Total Fatty Acids (%) of Groundnut Oil

GNC	SFA	MUFA	PUFA	TUFA
Improved Cultivars				
S1R	20.10±0.09	51.15±0.04^{f}	28.74±0.04^{h}	79.89±0.01^{fg}
S2	19.31±0.04	53.57±0.01^{c}	27.81±0.02^{i}	81.39±0.03^{abc}
S3R	20.03±0.04	56.01±0.07^{b}	24.27±0.08^{k}	80.33±0.00^{ef}
S4T	19.35±0.02	55.85±0.05^{b}	24.65±0.08^{k}	80.50±0.04^{def}
S5R	20.75±0.05	50.15±0.06^{g}	29.89±0.05^{g}	80.02±0.02^{fg}
S6T	21.18±0.06	51.10±0.04^{f}	27.86±0.04^{i}	78.96±0.06^{h}
S7T	20.62±0.00	47.20±0.03^{k}	32.18±0.03^{de}	79.38±0.00^{gh}
S8R	19.51±0.01	49.59±0.01^{h}	30.90±0.01^{f}	80.49±0.11^{def}
S9T	19.25±0.06	47.65±0.07^{g}	33.92±0.06^{c}	81.58±0.11^{ab}
S10R	20.42±0.02	52.59±0.06^{d}	26.99±0.06^{j}	79.58±0.02^{gh}
S11T	17.09±0.01^{b}	46.58±0.07^{l}	35.32±0.02^{b}	81.90±0.03^{a}
S12R	19.98±0.06	52.10±0.07^{e}	27.87±0.01^{i}	79.97±0.02^{fg}
S13T	19.01±0.04^{ab}	50.20±0.02^{g}	30.98±0.03^{f}	81.18±0.02^{bc}
S14R	20.14±0.02	49.72±0.03^{h}	30.25±0.04^{g}	79.97±0.05^{fg}
Traditional Cultivars				
IGO	19.22±0.02	50.86±0.01^{f}	30.28±0.06^{g}	81.14±0.08^{bcd}
AW	23.52±0.05	39.92±0.03^{n}	36.22±0.01^{a}	76.15±0.05
RR	23.14±0.12	45.81±0.01^{m}	32.03±0.05^{e}	77.84±0.05^{i}
RW	20.78±0.04	45.91±0.02^{m}	34.92±0.04^{b}	80.83±0.03^{cde}
EGT	22.12±0.05	57.01±0.04^{a}	22.01±0.06^{l}	79.03±0.01^{h}
RB	20.40±0.09	48.27±0.04^{i}	32.64±0.01^{d}	80.91±0.04^{bcde}

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values followed by the same superscript letter within each column are not significantly different ($p>0.05$) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: Red; T: Tan; AW:Acholi white; IGO: *Igola*; RR:*Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; RB: Red beauty. S: saturated fatty acid; M: monounsaturated fatty acid; P: polyunsaturated fatty acid; TUFA: total unsaturated fatty acid.

4.2.3 Fatty Acid Composition of Oyster Nut Oil

Oyster nut oil was dominated by linoleic (C18.2ω6) as the major unsaturated FA and palmitic (C16.0) as the major SFA. Linoleic acid (C18.2) levels ranged from 41.50 to 44.65%. This result was in agreement with that obtained by Hopkins and Chisholm (1964) at 44% but lower than that of Nyagah (2016) who detected 53.66% linoleic acid in oyster nut oil. Levels of linoleic

acid (C18.2ω6) in this study doubled the 22.03% obtained by Minzangi et al. (2015). Linoleic acid (C18.2ω6) level reported by Jumbe et al. (2016) was significantly ($p<0.05$) lower than the findings of all the above stated scholars. Differences could be associated with geographical source of nuts. Linoleic acid (C18.2ω6) lowers total serum cholesterol, a positive attribute in reducing cardiovascular risk (Ristić-Medić et al., 2013).

Oleic acid (C18.1) ranged from 5.69 to 8.78% (Table 18). Similarly, Ajayi et al. (2004); Hopkins and Chisholm, (1964) and Nyagah (2016) reported low levels of oleic acid (<10%) in oyster nut oil. Contrary to the above reports, Minzangi et al. (2015) detected 41.77% oleic acid in oyster nuts suggesting that these nuts are a good source of this FA. Oleic acid (C18.1) levels of in this study were was four times lower. On the other hand, Jumbe et al. (2016) reported that oyster nut oil contained only 2.05% oleic acid. Given the wide variation of oleic acid and lack of information regarding their study, it was impossible presume the causes of such variation. Oyster nut oil contained very low levels of alpha linolenic acid (ALA, C18.3ω3) amounting to <1%. Literature sources, (Hopkins and Chisholm, 1964; Jumbe et al., 2016) indicated that ALA levels in oyster nuts are either too low or undetected. Small quantities (<1.0%) of C18.3ω6, C20.2 were also detected in oyster nut oil.

Palmitic acid (C16.0) which ranged from 33.58 to 38.75% (Table 18), concurred with that reported by Hopkins and Chisholm (1964), and Nyagah (2016) who, respectively, reported 35% and 32.03% palmitic acid (C16.0) in oyster nut oil. Palmitic acid (C16.0) amounts as low as 14.06% were reported for oyster nut oil (Minzangi et al., 2015). Palmitic acid (C16.0) is linked to increase in serum cholesterol and hence cardiovascular risk (Galli and Calder, 2009; Fattore

and Fanelli, 2013; Mancini et al., 2015 and Carta et al., 2017). The high amount of palmitic acid (C16.0) in this study, therefore, suggests that oyster nut oil may not favour cardiovascular health. In addition, high levels of palmitic acid (C16.0) may enhance metabolic complications such as insulin resistance and decreased oxidation of FA and glucose in muscles hence their accumulation in tissues (Iggman and Risérus, 2017). However, moderate consumption of oil rich in palmitic acid (C16.0) could provide health benefits as it forms a significant part of cell membrane phospholipids (Calder, 2015).

Stearic acid (C18.0) in oyster nut oil ranged from 9.48 to 13.65%. these findings are comparable with the 14%, 10.31% and 9.3% stearic acid reported by Hopkins and Chisholm (1964), Nyagah (2016) and Minzangi et al. (2015, respectively. Stearic acid (C18.0) is converted to oleic acid (C18.1) and this may help to lower plasma cholesterol in the body (Bonanome and Grundy, 1988; Strayer et al., 2006; Mente et al., 2017). It is, therefore, implied that diets containing more stearic than palmitic acid may cause improvement in the plasma lipid profile.

Despite the small quantities, higher levels of C18.3ω3, C18.3ω6, C20.2 and C23.0 were observed in oil from female nuts than that from the male ones. This implies that sex of nut affects FA composition. Significant differences ($p<0.05$) were observed among nuts for similar and different locations. A study by Lutz, Álvarez, and Loewe (2017) on the chemical composition of pine nut sourced from three geographical macro zones in Chile suggested that FA composition may be affected by source of nut. Similarly, this study showed significant differences ($p<0.05$) in the FA composition of oyster nuts from three different districts in Uganda. Method of extraction could be another reason for variation in FA composition by

different scholars. Hopkins and Chisholm (1964) employed the Soxhlet method of extraction followed by air drying while Minzangi et al. (2015) used the Soxhlet method coupled with rota-evaporation to remove excess solvent. According to Bligh and Dyer (1959), the Soxhlet method is oxidative and may modify the FA composition of oil. In this study, the rapid extraction method of Bligh and Dyer (1959) was applied.

4.2.4 Total Fatty Acids in Oyster Nut Oil

Saturated fatty acids (SFA) in oyster nut oil ranged from 42.98 to 51.82%, palmitic acid (C16.0) being the predominant SFA (Table 19). Unsaturated fatty acids (UFA) ranged from 47.51 to 53.86% and mainly comprised of poly unsaturated fatty acids (PUFA) which ranged from 41.57 to 44.90%, the rest were monounsaturated fatty acids (MUFA). Significant differences ($p<0.05$) were observed for SFA, MUFA and PUFA amongst the different sex orientations and districts of origin. Results obtained in this study were comparable with those of Hopkins and Chisholm (1964) who reported 49% SFA, 7% MUFA and 44% PUFA. Oil from the female nuts appeared to contain more PUFA than the male while the converse was true for SFA. Oil from Kamuli and Luwero showed high levels of MUFA for female while that from Dokolo showed the reverse. Results suggest that both location and sex of oyster nut influence composition of fatty acids. The SFA content in oyster nut oil was similar to total unsaturated FA, hence the ratio of unsaturated mostly linoleic acid (C18.2ω6) to saturated FA mainly palmitic acid (C16.0) was approximately 1:1. Findings of this study showed similar amounts of PUFA and SFA but low MUFA compared to those reported by Minzangi et al. (2015). Overall, oyster nut oil contained a unique FA composition with high levels of saturation as compared to most vegetable oil that are highly unsaturated.

Table 19: Fatty Acid Composition of Oyster Nut Oil

Source	Gender	C16.0	C18.0	C18:1	C18:2	18.3ω3	18.3ω6	C20.1	C20.2	C23.0
Dokolo	Male	38.14±0.01^{b}	13.65±0.03^{a}	5.93±0.01^{e}	41.50±0.04^{e}	nd	nd	nd	nd	0.03±0.00^{c}
	Female	36.37±0.08^{d}	11.47±0.04^{b}	8.08±0.01^{b}	44.87±0.04^{a}	0.03±0.00^{b}	0.06±0.01^{c}	nd	0.02±0.00^{b}	0.13±0.00^{b}
Kamuli	Male	38.75±0.02^{a}	10.47±0.02^{c}	5.69±0.00^{f}	42.79±0.08^{d}	nd	nd	nd	nd	0.16±0.00^{a}
	Female	37.26±0.04^{c}	10.29±0.05^{e}	7.17±0.01^{c}	43.60±0.07^{b}	nd	nd	nd	nd	nd
Luwero	Male	35.46±0.09^{e}	13.59±0.01^{a}	6.86±0.02^{d}	43.45±0.01^{c}	nd	0.23±0.00^{b}	0.23±0.01	0.01±0.00^{b}	nd
	Female	33.58 ±0.02^{f}	9.48±0.03^{d}	8.78±0.01^{a}	44.65±0.04^{c}	0.01±0.00^{a}	0.29±0.01^{a}	0.20±0.00	0.13±0.00^{a}	nd

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values with similar superscript letters within each column are not significantly different ($p>0.05$); nd: not detected.

Table 20: Total fatty acids (%) and health lipid indices of oyster nut oil

Source	Sex	SFA	MUFA	PUFA	TUFA
Dokolo	Male	51.82±0.02[a]	5.93±0.01[f]	41.59±0.04[f]	47.51±0.03[e]
	Female	47.97±0.05[d]	8.08±0.01[b]	44.90±0.04[a]	52.97±0.04[c]
Kamuli	Male	49.39±0.03[b]	7.26±0.00[c]	42.76±0.03[e]	53.06±0.03[b]
	Female	42.98±0.03[f]	7.18±0.01[e]	43.60±0.07[c]	50.78±0.06[d]
Luwero	Male	49.05±0.08[c]	9.11±0.02[a]	43.45±0.07[d]	50.66±0.02[d]
	Female	43.06±0.02[e]	7.21±0.01[d]	44.75±0.04[b]	53.86±0.04[a]

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values within each column were not significantly different ($p>0.05$); nd: not detected; SFA: saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; TUFA: Total unsaturated fatty acid.

4.2.5 Fatty acid composition of oil from roasted and stewed groundnuts

As indicated in Figure 12 below, levels of palmitic (C16.0) and stearic acid (C18.0) with heat treatment. The increase was higher during roasting as compared to stewing. On the other hand, unsaturated fatty acids oleic (C18.1) and linoleic acid (C18.2ω6) decreased with heat treatment. The decrease in oleic acid C18.1 was less than that observed for linoleic acid C18.2ω6. This can be attributed to the difference in level of unsaturation. According to Choe and Min (2006), polyunsaturated FA such as linoleic acid (C18.2ω6) have higher oxidation rates than monounsaturated FA like oleic acid (C18.1).

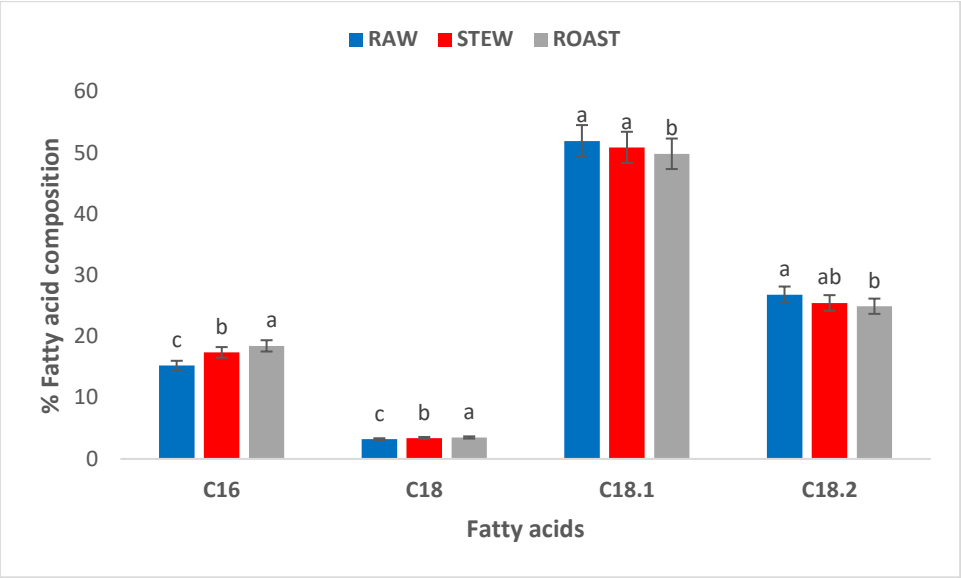

Figure 12: Effect of roasting and stewing on fatty acid composition of groundnut oil

As presented in Table 20, the total SFA, MUFA and PUFA were 17.68% to 21.40, 44.4 to 55.07% and 23.57 to 34.42%, respectively, in oil from the raw nuts. The corresponding levels for roasted nuts were 23.34 to 26.84%, 42.39 to 52.13% and 21.89 to 31.98%.

Unsaturated FA decreased while the percentage of saturated FA increased in oil from traditional and improved groundnuts. The relative changes in total FA were 2.26 to 6.62% increase in SFA, 0.89 to 2.93% decrease in MUFA and 2.85 to 7.26% decrease in PUFA. Polyunsaturated FA decreased to a higher extent compared to MUFA. The greater decline in PUFA may be ascribed to oxidative degradation of linoleic acid which unlike oleic acid; a MUFA, is more prone to oxidation. A similar observation was made by Rodrigues et al. (2011) in oil from roasted groundnuts. The overall decrease in total unsaturated FA ranged from 1.77 to 5.47% in oil from roasted nuts. The decline in levels of PUFA was high compared to the decrease in MUFA and SFA. Similar observations were made by Ayoola and Adeyeye (2010) for oil from roasted groundnuts. This can be attributed to structural differences among FA.

Table 21*:* Total fatty acids (%) in oil extracted from heat-treated groundnuts

Treatment	GNC	SFA	MUFA	PUFA	TUFA
Raw	S2	20.54±0.02^{j}	53.20±0.00^{d}	26.32±0.01^{j}	79.52±0.01^{f}
Stewed	S2	21.87±0.01^{d}	52.00±0.01^{e}	26.10±0.01^{k}	78.11±0.01^{j}
Roasted	S2	21.90±0.05^{d}	51.64±0.01^{h}	24.53±0.01^{l}	75.17±0.01^{n}
Raw	S4Tan	22.11±0.02^{c}	54.34±0.01^{a}	23.56±0.01^{m}	77.90±0.00^{k}
Stewed	S4Tan	22.54±0.01^{b}	54.21±0.01^{b}	23.18±0.01^{n}	77.39±0.02^{m}
Roasted	S4Tan	22.61±0.07^{a}	53.86±0.00^{c}	22.21±0.01^{o}	76.08±0.01^{n}
Raw	S5Red	19.10±0.01^{m}	49.14±0.01^{j}	31.77±0.01^{f}	80.92±0.01^{c}
Stewed	S5Red	20.11±0.04^{l}	48.87±0.01^{k}	31.02±0.01^{g}	79.89±0.01^{e}
Roasted	S5Red	20.12±0.05^{l}	48.54±0.01^{l}	30.55±0.01^{h}	79.09±0.01^{hg}
Raw	EGT	17.05±0.01^{p}	51.86±0.00^{f}	31.11±0.00^{g}	82.97±.0.01^{a}
Stewed	EGT	17.36±0.01^{o}	51.76±0.01^{g}	31.07±0.02^{g}	82.83±0.00^{b}
Roasted	EGT	17.44±0.08^{l}	51.40±0.00^{i}	28.85±0.02^{i}	80.25±0.03^{d}
Raw	RW	20.90±0.01^{i}	45.53±0.01^{m}	33.65±0.00^{c}	79.18±0.01^{g}
Stewed	RW	21.02±0.01^{i}	45.21±0.00^{m}	33.46±0.00^{d}	78.98±0.01^{hi}
Roasted	RW	21.40±0.01^{e}	45.02±0.00^{n}	32.34±0.00^{e}	77.55±0.01^{l}
Raw	RR	20.50±0.01^{k}	45.14±0.01^{o}	34.42±0.01^{a}	79.57±0.01^{f}
Stewed	RR	21.14±0.01^{g}	45.02±0.00^{p}	33.88±0.01^{b}	78.90±0.01^{i}
Roasted	RR	21.19±0.01^{f}	44.72±0.00^{q}	33.44±0.00^{d}	78.16±0.01^{j}

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values followed by the same superscript letter within each column are not significantly different ($p>0.05$) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: red; T: Tan; AW: Acholi white; RR: *Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; S: saturated fatty acid; M: monounsaturated fatty acid; P: polyunsaturated fatty acid; TUFA: total unsaturated fatty acid.

Total FA in oil from stewed nuts were 20.95 to 24.69% SFA, 43.43 to 53.55% MUFA and 22.37 to 32.68% PUFA. Saturated FA increased by 0.57 to 6.48% while PUFA and MUFA decreased by 0.19 to 2.26% and 0.84 to 2.36%, respectively. The reduction in TUFA ranged from 0.17 to 1.77%, which is lower that the individual losses for MUFA and PUFA. Literature about stewing studies is scanty, however, it is well known that increase in temperature enhances oxidation in oil. Overall, the relative retention of MUFA and PUFA and the relative increase in SFA was higher in oil from roasted than the stewed nuts.

There was a reduction in the FA content in all of the cultivars after stewing and roasting, but differences among some FA were not significant for some cultivars. Amounts of total SFA increased during heat processing while MUFA and PUFA decreased significantly ($p<0.05$). Similar effects were reported by Rodrigues et al., (2011); Musa and Aljuhaimi (2014). Cisneros, Paredes, Arana, and Cisneros-Zevallos (2014) reported very little change in FA composition of roasted *Plukenetia volubilis* seed oil. Tenyang et al. (2017) reported little modification of FA composition in oil from sesame roasted at 180^{o} C for 10 min. Ali, Islam, Othman, and Noor (2017) reported similar effect for oil from roasted soy beans.

According to Cisneros et al. (2014), the roasting process leads production of anti-oxidative phenolics that retard oil degradation. It is therefore possible that metabolites formed due to the Maillard reaction offered protective action against oxidation. On the other hand, Mcclements and Decker (2000) pointed out that hydrolytic degradation proceeds in presence of water and transition metals like iron, however, this reaction is very slow. In addition groundnut flour was subjected to blanching with hot water for five minutes prior to stewing. According to Belitz et al. (2009), blanching causes enzyme inactivation. It is therefore presumed that chances of lipolysis during stewing were too low to cause significant impact. It therefore implies that the blanching action and the short time heat treatment of 15 min. may have retarded lipolysis and oxidative degradation of groundnut oil thereby conserving

the unsaturated FA. There was scarcity of literature on effect of stewing on FA composition, however, its effect on FA composition was mild compared to that caused by roasting.

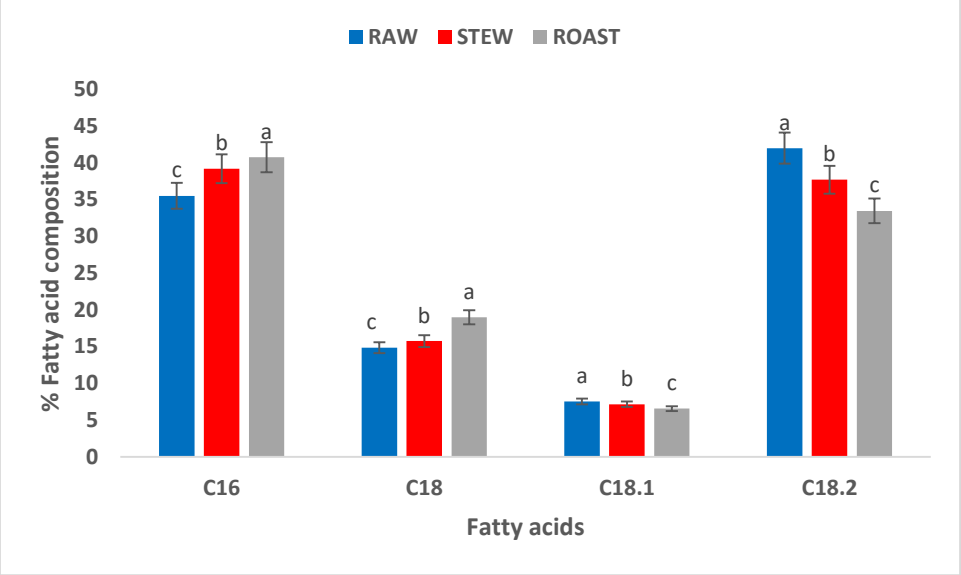

Figure 13: Fatty acid composition of oil from roasted and stewed oyster nuts

The fatty acid composition of oil from oyster nuts was modified during heat treatment. Saturated FA particularly palmitic and stearic acid increased while the unsaturated FA oleic and linoleic acid decreased (Figure 13). Total FA composition of oil from raw, stewed and roasted oyster nuts is shown in Table 21. Levels of total FA in oil showed significant differences ($p<0.05$) after thermal treatment. The total SFA, MUFA and PUFA were 50.35%, 7.58% and 42.11%, respectively, in oil from raw nuts. Oil from stewed nuts showed a decline of 0.13% and 0.57% in MUFA and PUFA, respectively, while the SFA increased by 0.1%. Corresponding decline of MUFA and PUFA in oil from roasted nuts was 0.26% and 4.66%. Total saturated FA increased by 2.15%. The decline in unsaturated FA may be ascribed to oxidation that is enhanced at high temperatures. Losses may be attributed to linoleic acid which was detected in higher quantity than oleic acid in oyster nut oil. Linoleic acid is more susceptible to oxidation than oleic due to its higher level of unsaturation

(Leyton, Drury, and Crawford, 1987). Ahmed et al. (2016), and Wasowicz, Gramza, Hes, Malecka, and Jelen, 2004) asserted that rate of oxidation increases with degree of unsaturation and occurrence of multiple bonds. Wasowicz et al. (2004) suggested that oxidation has serious health implications including lowering of nutritional value and interaction of oxidation products with other nutrients such as vitamins and minerals. Furthermore, oxidation products have been cited in damage of the intestinal mucosa due to their slow digestion and in promotion of atherosclerosis.

Table 22: Total fatty acids (%) in oil extracted from heat treated oyster nuts

Treatment	SFA	MUFA	PUFA	TUFA
Raw	50.20±0.01[a]	7.56±0.03[a]	42.24±0.02[a]	49.80±0.05[a]
Stewed	50.10±0.03[a]	7.51±0.03[a]	41.80±0.04[a]	49.31±0.01[a]
Roasted	51.35±0.02[a]	7.47±0.03[a]	41.25±0.04[a]	48.73±0.02[a]

Data are expressed as percentages of total fatty acid methyl esters; values are means of triplicate determinations, values followed by the same letter within each column are not significantly different ($p>0.05$) nd: not detected; S: saturated fatty acid; M: monounsaturated fatty acid; P: polyunsaturated fatty acid; TUFA: total unsaturated fatty acid.

Dominant FA in oil from groundnut oil were oleic acid, linoleic acid and palmitic acid. Acholi white exhibited a unique FA profile with similar proportions of PUFA and MUFA. Oyster nut oil was dominated by of palmitic and linoleic acid making the levels of both saturated and unsaturated FA balanced.

4.2.6 Heat Stability of Oil from Heat Treated Groundnuts

The change in PV during roasting and stewing is given in Table 23. There was significant increase in the PV of oil from treated groundnuts during the heating period ($p<0.05$). Oil

from the roasted groundnuts exhibited the highest PV (14.33 to 28.86 mEq/kg), followed by stewed (7.70 to 13.23 mEq/kg) and lastly raw nuts (0.20 to 1.41 mEq/Kg). The results from this study are in agreement with the findings of Falade and Oboh, (2015) where the PV of thermal-oxidized palm oil increased significantly from 3.00 to 13.00 mEq/kg after heating for 10 min. Results of this study differ from those of Ayoola and Adeyeye (2010) who reported that PV of groundnut oil remained within acceptable level after roasting. In their study, PV of oil from roasted nut was lower than that of oil from the untreated nuts. Differences may also be attributed to methods applied during processing. Whereas Ayoola and Adeyeye (2010) used oven roasting, open pan roasting was done in this study. It is likely that exposure of seeds to light air and heat enhanced oxidation. Oil from stewed Serenut 4Tan and 5Red had acceptable PV of <10 mEq/kg for vegetable oil (FAO/WHO, 2001) implying that their oil could be more stable to oxidation than other oils. Peroxide value of oil from roasted groundnuts was higher than the tolerable limit of 10 mEq/kg suggesting that oil from all roasted groundnut is more susceptible to oxidation (Tenyang et al., 2017). During heating hydroperoxides are formed as the initial products of oxidation. These may later breakdown into non-volatile secondary oxidation products which lowers the shelf life and nutritional quality of oil. Increased PV of oil may be due to oxidation.

Table 23: Peroxide value of oil obtained from heat treated groundnuts

	Peroxide value of nuts ($mEqO_2/kg$)					
Treatment	S 2	S4T	S5R	EGT	RW	RR
Raw	0.36 ± 0.01^{b}	0.20 ± 0.01^{c}	0.51 ± 0.06^{c}	0.65 ± 0.04^{c}	1.41 ± 0.08^{c}	1.40 ± 0.01^{c}
Stewed	9.33 ± 0.59^{ab}	11.41 ± 0.27^{b}	13.23 ± 0.20^{b}	7.70 ± 0.21^{b}	8.98 ± 0.04^{b}	9.21 ± 0.09^{b}
Roasted	17.54 ± 0.65^{a}	18.90 ± 0.18^{a}	21.41 ± 0.57^{a}	28.86 ± 0.11^{a}	15.07 ± 0.16^{a}	14.33 ± 0.03^{a}

Values are means of 3 replicates. S: Serenut; R: red; T: Tan; AW: Acholi white; RR: *Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; values followed by the same letter within each column are not significantly different ($p>0.05$)

Iodine value (IV) of oil from heat treated groundnuts decreased significantly ($p<0.05$) Table 24. Decrease in IV is often consistent with decrease in double bonds as oil becomes oxidized (Tenyang et al., 2017). A greater decrease was observed in oil from roasted as compared to stewed nuts. In spite of the decrease, IV for groundnut oil remained within the standard level of 15mEq/kg as specified by FAO/WHO (2005). This may be attributed to presence of alpha-tocopherols in oil which act as anti-oxidants (Kamal-Eldin, 2006).

Table 24: Iodine value of oil obtained from heat treated groundnuts

	Iodine value					
Treatment	S 2	S4T	S5R	EGT	RW	RR
Raw	89.48±0.09[a]	85.77±0.04[a]	95.89±0.05[a]	96.90±0.04[a]	96.01±0.03[a]	96.50±0.02[a]
Stewed	89.22±0.09[b]	84.50±0.04[b]	94.53±0.05[b]	96.79±0.05[a]	95.77±0.03[b]	95.71±0.02[b]
Roasted	86.24±0.09[c]	83.52±0.04[c]	93.44±0.05[c]	93.01±0.05[b]	93.58±0.04[c]	94.78±0.02[c]

Values are means of 3 replicates. S: Serenut; R: red; T: Tan; AW: Acholi white; RR: *Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; values followed by the same letter within each column are not significantly different ($p>0.05$)

Changes in the PV of oyster nut oil during heat processing are as shown in Table 25. Peroxide value increased from 3.09 mEq/kg in raw groundnuts to 7.95 and 15.73 mEq/kg in stewed and roasted oyster nuts, respectively. Peroxide value of oil from raw nuts, was desirable as recommended by FAO/WHO (2005). Significant differences ($p<0.05$) in PV were observed in oil from stewed and roasted nuts (Table 25). The PV of oil from roasted nuts was relatively higher than that of oil from stewed nuts. This may be attributed to oxidative degradation of oil which is enhanced at high temperature. According to Falade and Oboh, (2015) and Kaleem et al., (2015), increased PV may be due to production of hydro-peroxides as a result of thermal-aided oxidation during stewing and roasting. Hydroperoxides are formed in the initiation stage of oxidation chain reaction (Musa and Aljuhaimi, 2014).

Table 25: Peroxide Value and Iodine value of oil obtained from heat-processed oyster nuts

Treatment	Peroxide value (mEq/100 g)	Iodine value (g/100 g)
Raw	3.09±0.05[c]	78.98±0.01[a]
Stewed	7.95±0.66[b]	78.56±0.01[b]
Roasted	15.73±0.28[a]	75.22±1.01[c]

Values are means of three independent replicates ±standard deviations of the means. Values followed by the same superscript letter within each column are not different ($p>0.05$)

Iodine value of oil from stewed oyster nuts barely declined by <1% while that of oil from roasted nuts declined by 4.76%. A reduction in iodine value implies a decrease in the level of unsaturation (Ahmed, Pickova, Liaquat, and Jahangir, 2016). Reduction in the level of unsaturated EFA and build-up of SFA in heat treated nuts lowers the nutritional value of lipids and increases cardiovascular risk (TAO, 2015; Wasowicz et al., 2004). Various studies have shown that both hydrolysis and oxidation lead to loss of unsaturation of FA. Hydrolysis is enhanced by enzyme lipase and lipoxygenase and presence of water while oxidation is accelerated by increase in temperature, presence of oxygen and light (Wasowicz, Gramza, Hes, Malecka, and Jelen, 2004; Aladedunye, 2015; Ahmed, Pickova, Liaquat, et al., 2016). The decrease in levels of iodine value and increase in PV in this study may be ascribed to both of the above mentioned processes particularly oxidation. Such degradation may result in formation of toxic substances such as aldehydes, ketones and a possibility of trans-FA (Ahmed et al., 2016; Zahir et al., 2017). Iodine value can be lowered as a result of oxidative or hydrolytic rancidity of oil when exposed to various conditions such as air, water, heat and metallic ions (Davis et al., 2016; Mora-escobedo et al., 2015; Zahran and Tawfeuk, 2019).The results of this study suggest that the extent of hydrolysis and /or oxidative degradation in groundnut and oyster nut oil was low.

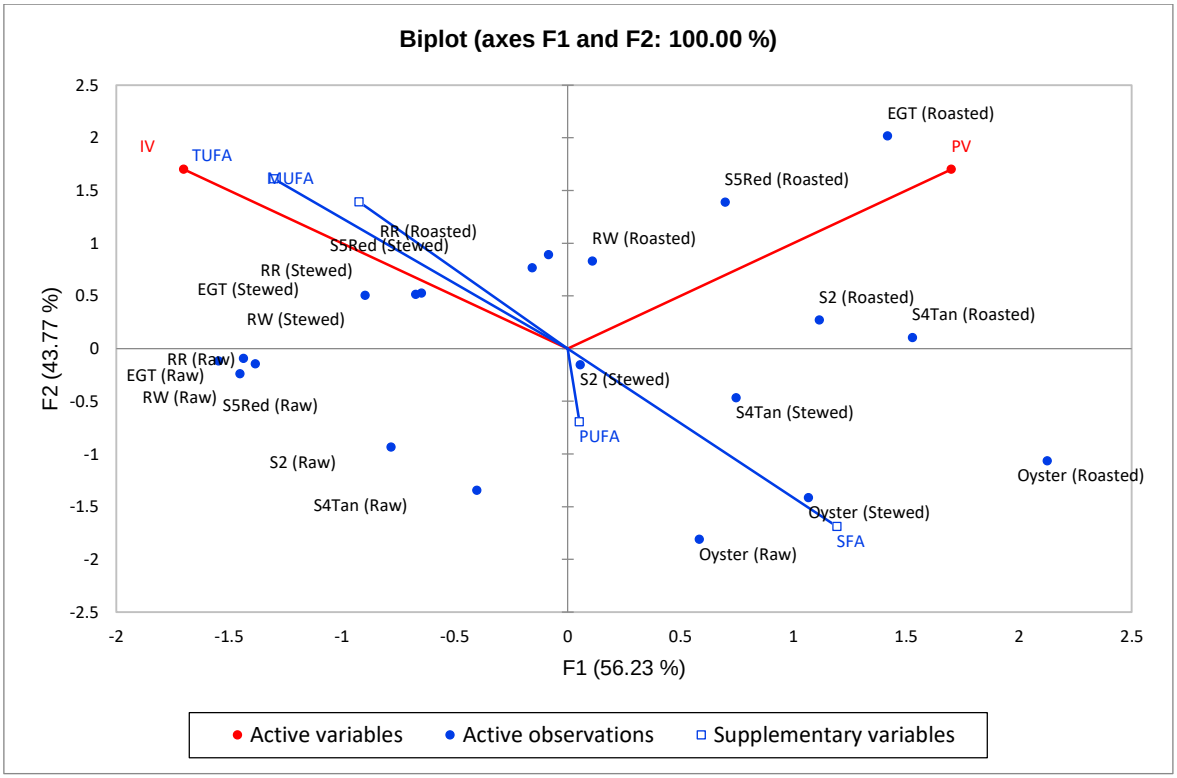

Figure 26: Heat stability of groundnut and oyster nut oil

Figure 26 above shows the negative correlation between peroxide value and iodine value. Oil from roasted nuts had the highest PV and lowest iodine value. Increase in PV is undesirable as the oxidation products formed during heat treatment may be toxic (Choe and Min, 2006). Modification of PV beyond the recommended limit of 15mEq/kg suggests loss in the unsaturation and hence nutritional value.

The amount EFA linoleic acid (C18.2ω6) in oyster nut and groundnut oil showed a decreased during roasting and stewing. Roasting caused higher losses of PUFA in both groundnut and oyster nuts oil and hence may be the least favourable method of processing for these nut oils. Stewing confers higher stability to lipid oxidation of oil than roasting. The stability and profile of FA in oil is largely dependent on type of nuts and heat treatment. Roasting severely affects unsaturated FA and oil stability.

4.3. Health Lipid Indices of Groundnut and Oyster Nut Oil

4.3.1 Health Lipid Indices of Groundnut Oil

Polyunsaturated to saturated FA ratio (P/S) measures the tendency of the diet to influence the occurrence of coronary heart disease (SˇimaT et al., 2015). This ratio is also important in determining the cholesterolemic effect of dietary lipids (Matos, Matos, and Moecke, 2019). Foods with P/S above 0.45 (FAO, 2010) are considered beneficial due to their potential to lower serum cholesterol (Kostik, Memeti, and Bauer, 2013). Oil with high P/S have higher nutritional value than ones with less value as the beneficial effect is even more significant when the P/S is >1 (Kostik et al., 2013). Polyunsaturated to saturated FA ratio of groundnut oil ranged from 1.00 to 1.95 (Table 26). Nile and Park (2013) and Johnson et al. (2009) obtained P/S of 1.8 and 2.28 for groundnut oil. The high P/S in this study suggests that consumption of diet rich in groundnut oil is beneficial for human health. Ramprasath et al. (2012) reported that serum cholesterol concentrations are hiked with consumption of diets rich in SFA while the opposite effect is provided by diets containing high levels of PUFA. Consumption of diets with a high P/S reduces plasma total and LDL-cholesterol concentrations. In order to improve the P/S, partial replacement of SFA by PUFA has been suggested by FAO (2010) and Iggman and Risérus (2017). Such intervention may reduce cardiovascular risk. The P/S in this study was above the minimum level stated above suggesting that groundnut oil is beneficial to human nutrition and cardiovascular health.

Polyunsaturated to monounsaturated FA ratio (P/M) of the oil ranged from 0.39 to 0.91. The highest ratio was exhibited by Acholi white while the lowest was observed in *Egoromoit*. Polyunsaturated and monounsaturated FA are linked to reduction of atherosclerosis and cardiovascular disease (Kris-Etherton, 1999; Harlioğlu and Yilmaz, 2011). Diets rich in monounsaturated FA are neutral while polyunsaturated FA diets have been reported to lower plasma lipids (Wen and Chao, 1998; Bos et al., 2010).

Monounsaturated to saturated FA ratio (M/S) of groundnut oil varied from 1.70 to 2.89 (Table 26). The M/S was generally higher in improved cultivars and lower in traditional ones. The M/S of this study is higher than that obtained by Nile and Park (2013). High M/S of >1 in diet decreases plasma high density lipoprotein cholesterol (HDL-C) and triacylglycerides (Yang et al., 2017) implying that improved nuts would contribute to this effect more than traditional nuts. Sinanoglou (2013) and Calder (2015) suggested that MUFA are heart protective while SFA increase risk of cardiovascular disease. The high M/S of >1 in this research suggests a healthy balance of FA in groundnut oil.

Table 26: Health Lipid Indices of Groundnut Oil

GNC	O/L	ω3/ω6	ω6/ω3	M/S	P/S	P/M	AI	TI	h/H
Improved Cultivars									
S1R	1.93	0.05	19.03	2.54	1.43	0.56	0.23	0.42	4.87
S2	2.08	0.05	18.35	2.77	1.44	0.52	0.21	0.39	5.24
S3R	2.53	0.04	22.94	2.80	1.21	0.43	0.22	0.42	5.29
S4T	2.51	0.04	23.33	2.89	1.27	0.44	0.23	0.42	5.68
S5R	1.79	0.03	28.86	2.42	1.44	0.60	0.22	0.42	5.14
S6T	1.95	0.03	40.43	2.41	1.32	0.55	0.25	0.47	4.82
S7T	1.50	0.01	73.86	2.29	1.56	0.68	0.23	0.44	5.27
S8R	1.66	0.01	82.54	2.54	1.58	0.62	0.21	0.42	5.69
S9T	1.44	0.01	77.90	2.47	1.76	0.71	0.21	0.40	5.36
S10R	2.10	0.04	27.91	2.57	1.32	0.51	0.22	0.42	5.09
S11T	1.36	0.03	35.42	2.58	1.95	0.76	0.20	0.37	5.85
S12R	1.89	-	-	2.61	1.40	0.54	0.22	0.45	5.19
S13T	1.64	-	-	2.64	1.63	0.62	0.21	0.42	5.54
S14R	1.70	-	-	2.47	1.50	0.61	0.22	0.44	5.38
Traditional Cultivars									
IGO	1.76	-	-	2.65	1.60	0.60	0.22	0.43	5.47
AW	1.12	-	-	1.70	1.54	0.91	0.29	0.58	4.42
RR	1.45	-	-	1.98	1.38	0.70	0.22	0.44	5.91
RW	1.34	0.01	157.77	2.21	1.70	0.76	0.22	0.44	6.09
EGT	2.76	0.03	30.01	2.58	1.00	0.39	0.24	0.46	5.53
RB	1.53	0.03	52.32	2.37	1.60	0.68	0.21	0.40	6.53

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values followed by the same superscript letter within each column are not significantly different ($p>0.05$) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: Red; T: Tan; AW:Acholi white; IGO: *Igola*; RR:*Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; RB: Red beauty. O/L: oleic/linoleic ratio; AI: Atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic to hypercholesterolemic index.

In general, ω6/ω3 below 4:1 is desirable to reduce cardiovascular risk (Simopoulos, 2010). In this study, the ω6/ω3 of groundnut oil from all cultivars were considerably higher than recommended (Table 26). This is ascribed to the low levels of omega-3 FA in the oil. Johnson et al. (2009) obtained ω6/ω3 of 7.5:1 for groundnut oil while Nile and Park, (2013), reported ω6/ω3 of 25:1. Omega-3 and omega-6 FA produce eicosanoids with conflicting effects in the body. Omega-3 eicosanoids are anti-inflammatory while those of omega-6 support formation of thrombus, atheromas and obesity (Alabdulkarim, Bakeet, and Arzoo, 2012; Simopoulos, 2008; Simopoulos, 2016). The high ratios imply that excessive consumption of groundnut oil may pose risk for cardiovascular disease hence, oil should be consumed sparingly. On the other hand, the ω3/ω6 for all cultivars was <1:1 suggesting that groundnut oil may not be a good dietary source of omega-3. Yehuda (2003) as well asYehuda and Carassot, (1993) studied the effect of varying proportions of alpha linolenic acid (omega-3): linoleic acid (omega-6), and reported that a range of 1:3.5 to 1:5.0 was favorable for brain development and behavior regulation. Studies on the relationship between omega-3 and omega-6 suggest that low ratios may increase risk of disease while high ratios are desirable for good health. Although both ALA and linoleic acid have essential roles in growth and development of the brain, their existence in the body in unbalanced proportions may be catastrophic to health (Yehuda and Carassot, 1993; Yehuda, 2003). This predicament may result from the suppression of omega-6 FA by the more abundant omega-3 hence inhibiting its therapeutic properties (Apte, Cavazos, Whelan, and Degraffenried, 2013). The ratio of omega-3 to omega-6 (ω3/ω6) was <2% in groundnut oil. Although consumption of balanced levels of omega-3 and omega-6 is recommended, there is no clear criteria for the ω3/ω6 in diet (EFSA, 2010) due to inadequate clinical studies. A study carried out by El-Katcha, El-Kholy, Soltan, and EL-Gayar, (2014) on the effect of dietary ω3/ω6 on performance, immune response, blood parameters and FA profile of broiler chickens suggested 1:3 as the

best ω3/ω6. According to Lunn and Theobald (2006) omega-3 is the prefferential PUFA released in cell membranes in case of arrythimia implying that omega-3 to omega-6 ratio is significant in preventing arrythimia. This report concurs with that of Simopoulos (2004) who suggested high ratios of omega-3 to omega-6 in prevention of disease.

Oleic to linoleic acid ratio (O/L), is regarded as a measure of oil stability (Asibuo, 2008; Singkham et al., 2010; Davis, Price, Patel et al., 2013; Dean, Sweigart, Cottonaro, and Sanders, 2016). High O/L is associated with higher stability to oxidation (Mora-escobedo et al., 2015) given that linoleic acid (polyunsaturated) is more susceptible to oxidation than oleic acid (monounsaturated). In addition, high O/L enhances nutritional value while the reverse is true for low ratios (Yanishlieva and Marinova, 2001; Flagella, Rotunno, Tarantino, Di Caterina, and De Caro, 2002; Mukri et al., 2012;) The O/L of groundnut oil varied from 1.11 to 2.76. Among studied oil, *Egoromoit* exhibited the highest O/L (2.76) implying that its oil may have better stability to oxidation than the other groundnut oil. Shad et al. (2012) obtained O/L of 3.53 to 19.79. In addition, Davis et al. (2016) investigated varying O/L (1.3 to 33.8) and reported an increase in shelf stability as the O/L increased. Furthermore, Campos-Mondragón et al. (2009) asserted that O/L depends on soil type; sandy type of soil promote a higher ratio. The kind of soil were the groundnuts used in this study were grown is not known as they were supplied post-harvest, however, soil type could be an area for future studies. According to Gulluoglu et al. (2016); Hashim et al. (1993); and Singkham et al. (2010), oleic and linoleic acid concentrations in groundnuts are affected by temperature during growth, drought, delay in sowing as well as genotype and environment. Automatically, O/L is affected by the above factors. In this study, however, genotype is more likely to have an effect as the factors were not studied. Results suggest that oil groundnut used in this study has moderate stability.

Atherogenic (AI), thrombogenic (TI) and hypocholesterolemic to hypercholesterolemic (h/H) indices are predictors of cardiovascular disease (Hashempour-Baltork, Torbati, Azadmard-Damirchi, and Savage, 2018; Matos et al., 2019). Atherogenic index indicates the tendency of a lipid to cause atheroma while thrombogenic index (TI) shows the tendency to form clots in the blood vessels (Ulbricht and Southgate, 1991). In addition AI and TI are used to assess the nutritional quality of lipids (SˇimaT et al., 2015). Ulbricht and Southgate (1991) suggested that AI and TI<1 could reduce the risk of cardiovascular disease. The atherogenic Index (AI) of groundnut oil ranged from 0.20 to 0.29 while the TI ranged from 0.37 to 0.58 (Table 26). There was no significant difference in AI and TI among traditional and improved groundnut oil ($p>0.05$). According to Hernández-martínez et al. (2016), AI and TI levels of <1 are favourable for cardiovascular health while Ulbricht and Southgate (1991) recommended a limit of 0.5 for each of the two indices. The TI and AI indices of all oil in this study were within recommended levels for maintaining cardiovascular health.

The hypocholesterolemic to hypercholesterolemic index (h/H) determines the effect of individual FA on cholesterol metabolism (Hashempour-Baltork et al., 2018). This index is proportional to the level of PUFA in a fat/lipid (Santos-Silva, Bessa, and Santos-Silva, 2002). The h/H for groundnut oil ranged from 4.42 to 6.53 and close to 6.14 reported for olive oil (Hashempour-Baltork et al., 2018). Hernández-martínez et al. (2016) and Osmari et al. (2011) suggested that high h/H of >1 is beneficial to human health. The high h/H observed in this work implies that consumption of groundnut oil may result in reduced cardiovascular risk.

4.3.2 Health Lipid Indices of Oyster Nut Oil

The P/S of oyster nut oil ranged from 0.80 to 1.04 (Table 27). This range is in agreement with the nutritional guidelines that show preference for P/S >0.45:1 (FAO, 2010) indicating

high nutritional value. The P/M ratio for oyster nut oil ranged from 4.15 to 7.02. The P/M oil of male nuts from Dokolo and Luwero showed higher levels than females while the converse was true for oil of nuts from Kamuli. This finding is higher than the upper recommended level of P/M of >1:1 suggesting that oyster nut oil may not have a healthy balance of FA groups recommended for consumption. Monounsaturated to saturated FA ratio (M/S) varied from 0.11 to 0.21. This is unfavourable for human health given that SFA increase cardiovascular risk while the converse is reported for MUFA (Grundy, 1997; De Souza et al., 2015; Iggman and Risérus, 2017). Pacheco et al. (2006) indicated that a low M/S of <1 results in pro-thrombic effect compared to a high ratio. Overall, the P/S and M/S were higher in oil from female nuts than the male ones. A report by Jandacek (2017) suggested that consumption of MUFA, particularly oleic acid results in more favourable levels of low density and high density lipoprotein cholesterol than linoleic acid, a PUFA. This implies that P/M should be low for good health.

Total omega-6 FA varied from 41.57 to 44.90% while omega-3 FA content was <0.3%. Accordingly, the ω6/ω3 of >100:1 was very high compared to the recommended ratio of <4:1 (FAO/WHO, 2005; Patterson et al., 2012; Rustan and Drevon, 2005; Simopoulos, 2002; Simopoulos, 2004). A study by Sartorelli, Damião, Chaim, Hirai, Gimeno, Ferreira, (2010) indicated that a diet containing high ω3/ω6 improved glucose regulation among Japanese Brazilian as opposed to a diet with a low ratio. The low ω3/ω6 of oyster nut oil may enhance symptoms of cardiovascular disease, diabetes and other non-communicable diseases (Simopoulos, 2004, 2008; Apte, Cavazos, Whelan, and Degraffenried, 2013).
Given the low levels of C18.1 compared to C18.2ω6 in oyster nut oil, O/L was low (<1.0) (Table 27). Low O/L is associated with short shelf life of oil (Carrín and Carelli, 2010; Patel et al., 2013). Short shelf life can be attributed to the higher susceptibility of linoleic acid

(PUFA) to oleic acid (MUFA) to oxidative degradation (Ahmed et al., 2016; Halvorsen and Blomhoff, 2011; Tao, 2015).

Atherogenic (AI) and thrombogenic index (TI) indicate the contribution of individual FA to cardiovascular risk. Atherogenic index of oyster nut oil ranged from 0.11 to 0.29 while thrombogenic index (TI) ranged from 1.58 to 2.18. Given that AI of <1 are desirable for cardiovascular health (Hernández-martínez et al., 2016), oyster nut oil was within the recommended range, however, TI exceeded the desirable value of <1 suggested by Ulbricht, and Southgate (1991). The higher TI could be attributed to high levels of SFA in the oil with ranges of 42.98 to 51.82%. Clearly, the TI (>1.0) for oyster nut oil in this study suggests that consumption of oyster nut oil may increase risk for cardiovascular risk. The above indices rely on the quantities of saturated FA; myristic acid (C14.0), Palmitic acid (C16.0) that are known to be thrombogenic (Attia, Al-harthi, Korish, and Shiboob, 2015).

Hypocholesterolemic to hypercholesterolemic index (h/H) ranged from 1.25 to 1.59 which is above the minimum limit of 1.0. High levels (>1) are recommended for health (Skałecki, Florek, Pyć, Kaliniak, and Staszowska, 2016).

Table 27: Health Lipid Indices of Oyster Nut Oil

Source	Sex	P/S	P/M	M/S	ω3/ω6	ω6/ω3	O/L	AI	TI	h/H
Dokolo	Male	0.80	7.01	0.11	0.00	-	0.14	0.29	2.18	1.25
	Female	0.94	5.56	0.17	0.00	-	0.18	0.22	1.80	1.45
Kamuli	Male	0.87	4.15	0.21	0.00	-	0.24	0.20	1.86	1.37
	Female	1.01	6.07	0.17	0.00	-	0.16	0.11	1.69	1.36
Luwero	Male	0.89	6.02	0.15	0.00	-	0.16	0.27	1.93	1.41
	Female	1.04	4.91	0.21	0.00	-	0.20	0.18	1.58	1.59

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values within each column were not significantly different (p>0.05); nd: not detected; ω6/ω3: Omega-6 to Omega-3 ratio;

O/L: oleic to linoleic acid ratio; AI: Atherogenic index; TI: Thrombogenic index; h/H: Hypocholesterolemic to hypercholesterolemic index.

4.3.3 Health Lipid Indices of Oil from Heat Treated Groundnuts and Oyster Nuts

4.3.3.1: Oil from Heat Treated Groundnuts

Polyunsaturated to saturated fatty acids ratio (P/S) is an indicator for nutritional value of oil (Matos, Matos, and Moecke, 2019). The P/S ranged from 1.07 to 1.82, 1.03 to 1.79 and 0.99 to 1.65 in oil from raw, stewed and roasted groundnuts showing a decline with heat treatment probably due to oxidation of PUFA. The decline in P/S was more pronounced in oil from roasted nuts than the stewed ones. This suggests that oxidation was more enhanced by roasting compared to stewing. The P/S, however, remained within the acceptable level of >0.45 (FAO/WHO, 2005) in spite of the different methods of heat treatment. Mckevith (2005) reported that heating may render some changes in the structure of fatty acids, depending on the temperature, time of process and exposure of oil to air. Heating therefore may reduce the level of PUFA. Nuts in this study were heat treated at high temperature in open air, hence, some of the above factors may explain the changes observed in FA composition of the oil.

Table 28: Health Lipid Indices of Oil Extracted From Heat-Treated Groundnuts

Treatment	GNC	O/L	P/S	P/M	M/S	ω3/ω6	AI	TI	h/H
Raw	S2	2.01	1.28	0.50	2.59	0.02	0.18	0.45	5.16
Stewed	S2	2.01	1.19	0.50	2.38	0.01	0.19	0.47	4.98
Roasted	S2	2.12	1.12	0.48	2.36	0.01	0.19	0.49	4.84
Raw	S4Tan	2.31	1.07	0.43	2.46	0.02	0.20	0.49	4.82
Stewed	S4Tan	2.31	1.03	0.43	2.40	0.01	0.20	0.51	4.80
Roasted	S4Tan	2.40	0.99	0.41	2.39	0.01	0.20	0.52	4.71
Raw	S5Red	1.53	1.66	0.65	2.57	0.01	0.15	0.37	6.36
Stewed	S5Red	1.56	1.54	0.64	2.43	0.02	0.15	0.38	6.28
Roasted	S5Red	1.57	1.52	0.63	2.41	0.02	0.15	0.38	6.17
Raw	EGT	1.67	1.82	0.60	3.04	0.01	0.15	0.34	7.36
Stewed	EGT	1.67	1.79	0.60	2.98	0.01	0.15	0.35	7.34
Roasted	EGT	1.77	1.65	0.56	2.94	0.01	0.15	0.37	7.04
Raw	RW	1.33	1.61	0.74	2.18	0.01	0.18	0.44	5.88
Stewed	RW	1.34	1.59	0.74	2.17	0.01	0.18	0.44	5.86
Roasted	RW	1.37	1.51	0.72	2.11	0.01	0.18	0.46	5.70
Raw	RR	1.29	1.68	0.76	2.20	0.01	0.17	0.42	5.72
Stewed	RR	1.31	1.60	0.75	20.13	0.01	0.18	0.44	5.57
Roasted	RR	1.31	1.58	0.75	20.11	0.01	0.18	0.45	5.51

Data are expressed as percentages of total fatty acid methyl esters. Values are means of triplicate determinations ±standard deviations of the means. Values followed by the same superscript letter within each column are not significantly different ($p>0.05$) nd: not detected; GNC: Groundnut cultivar; S: Serenut; R: red; T: Tan; AW: Acholi white; RR: *Rudu* red; RW: *Rudu* white; EGT: *Egoromoit*; S: saturated fatty acid; M: monounsaturated fatty acid; P: polyunsaturated fatty acid; TUFA: total unsaturated fatty acid; IV iodine value; O/L: oleic/linoleic ratio; AI: Atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic to hypercholesterolemic index.

4.3.3.2 Oil from Heat Treated Oyster Nuts

Polyunsaturated to saturated FA ratio (P/S) was 0.84, 0.83 and 082 in oil from raw, stewed and roasted oyster nuts, respectively. Oleic to linoleic acid ratio (O/L) increased from 0.18 in oil from raw nuts to 0.20 in that from roasted. This is attributed to the fact that of linoleic acid is more susceptible to oxidation than oleic acid due the double bonds. Lipids with low O/L tend to have low oxidative stability (Hashim, Koehlerv, Eitenmiller, and Kvien, 1993).

Table 29: Health lipid indices of oil extracted from heat treated oyster nuts

Treatment	O/L	P/S	P/M	M/S	ω3/ ω6	AI	TI	h/H
Raw	0.18	0.84	5.59	0.15	0.01	0.99	2.01	1.00
Stewed	0.18	0.83	5.57	0.15	0.01	1.01	2.02	0.99
Roasted	0.18	0.82	5.53	0.15	0.01	1.02	2.04	0.98

Data are expressed as percentages of total fatty acid methyl esters; values are means of triplicate determinations, values followed by the same letter within each column are not significantly different ($p>0.05$) nd: not detected; O/L: oleic/linoleic ratio; AI: Atherogenic index; TI: thrombogenic index; h/H: hypocholesterolemic to hypercholesterolemic index.

Health lipid indices indicated that groundnut oil is favourable for cardiovascular health. Oyster nut oil was abundant in both linoleic and palmitic acid. The ratio of total saturated FA to unsaturated FA was 1:1. The TI of oyster nut oil was high due to the high amount of palmitic acid.

4.4. Anti-Oxidant Vitamins in Groundnut and Oyster Nut Oil

4.4.1 Anti-Oxidant Vitamins in Groundnut Oil

Beta-carotene in oil from improved groundnuts varied between 0.40 and 1.09 mg/100 g in Serenut 3Red and Serenut 7Tan, respectively (Table 30). Oil from traditional cultivars had a range of 0.21 to 1.72 mg/100 g of beta-carotene in *Egoromoit* and *Rudu* red, respectively. Significant differences ($p<0.05$) were observed. The results obtained in this study are close to the 0.82 mg/100 g beta-carotene reported by Rafalowski et al. (2008) for unrefined groundnut oil. Falade and Oboh (2015) obtained a lower level of 0.05 mg/100 g beta-carotene in fresh groundnut oil. Pattee and Purcell (1967) and Nautiyal (2002) noted that stage of maturity affects beta-carotene level in groundnut kernels citing that the highest amount (6 µg/100 g) occurs in immature nuts. Techniques such as high performance liquid chromatography (Seppanen, Rahmani, and Saari Csallany, 2003) have been applied in other food matrices and can be employed to give more detailed insights of the beta-carotene content of oil from various groundnut cultivars at different stages of maturity. In addition, a comparative study of different oil extraction methods on yield and composition of soy bean oil, Nikolic and Stankovic (2009) reported that extraction method may affect composition.

In contravention of the hypothesis, results demonstrated that groundnut oil from traditional and improved cultivars was low in beta-carotene. The lowest and highest amounts, however, were found in oil from traditional cultivars. Regardless of the statistical differences ($p<0.05$), there was no clear pattern in variation of beta-carotene between oil from improved and traditional cultivars. A major limitation to this study is the scarcity of information in literature about beta-carotene content in groundnut oil.

Table 30: Vitamin A, E and Beta-carotene content of oil from groundnuts

Cultivar	Vitamin A (µg/100 g)	β-carotene (mg/100 g)	α-tocopherol (mg/100 g)
Improved Cultivars			
Serenut1Red	148.13±0.01^{d}	0.63±0.00^{e}	1.19±0.06^{c}
Serenut2	40.03±0.00^{fg}	0.74±0.00^{d}	1.27±0.15^{bc}
Serenut3Red	10.01±0.00^{i}	0.40±0.01^{f}	1.49±0.11^{bc}
Serenut4Tan	1.02±0.00^{i}	0.69±0.02^{de}	2.86±0.12^{b}
Serenut5Red	2.13±0.00^{i}	0.72±0.03^{d}	1.59±0.10^{bc}
Serenut6Tan	2.07±0.00^{i}	0.43±0.01^{f}	5.54±1.07^{a}
Serenut7Tan	21.01±0.00^{h}	1.09±0.06^{bc}	5.85±0.18^{a}
Serenut8Red	37.17±0.04^{g}	0.73±0.02^{d}	1.82±0.28^{bc}
Serenut9Tan	208.08±0.05^{b}	1.04±0.01^{c}	1.25±0.03^{bc}
Serenut10Red	45.03±0.05^{f}	0.45±0.01^{f}	1.25±0.12^{bc}
Serenut11Tan	85.62±0.00^{e}	1.06±0.00^{c}	1.34±0.19^{bc}
Serenut12Red	172.52±0.03^{c}	0.44±0.00^{f}	1.74±0.36^{bc}
Serenut13Tan	167.01±0.01^{c}	1.06±0.00^{c}	7.19±1.21^{a}
Serenut14Red	167.09±0.01^{c}	0.65±0.05^{b}	1.33±0.22^{bc}
Traditional cultivars			
Acholi white	559.21±0.10^{a}	0.31±0.00^{g}	0.88±0.03^{c}
Igola	Nd	0.45±0.00^{f}	1.81±0.04^{bc}
Rudu red	Nd	1.72±0.01^{a}	6.30±1.01^{a}
Rudu white	16±0.00^{h}	0.24±0.03^{h}	7.05±1.26^{a}
Egoromoit	19±0.01^{h}	0.21±0.00^{h}	2.21±0.06^{bc}
Red beauty	Nd	1.22±0.02^{b}	2.22±0.14^{bc}
RDI		na	15 mg/day

Values are means ± standard deviations of the means for three independent replicates. Means followed by the same superscript letter are not significantly different (p>05). na: not available; nd: not detected

Vitamin A levels in the oil ranged from not detected (nd) to 559.21 µg/100 g (Table 30). Acholi white, a traditional cultivar, exhibited the highest amount of vitamin A. All other

traditional cultivars had levels <20 µg/100 g. Among the improved cultivars, Serenut 9Tan showed the highest level of vitamin A amounting to 208.05 µg/100 g. According to Pattee and Purcell (1967), groundnut oil is not a good source of vitamin A. Since beta-carotene is a precursor for vitamin A in diet (Nagendran et al., 2000; Alabdulkarim et al., 2012),it is implied that of vitamin A quantity was affected by presence of low levels of beta-carotene in the oil. It is noteworthy that the level of vitamin A in oil from Acholi white doubled the highest amount in oil from improved cultivars. Results suggest that oil from Acholi white may be incredibly beneficial to diet with respect to vitamin A. Apart from a few cultivars; Serenut 1Red, 9Tan, 12Red, 13Tan, 14Red and Acholi white, other cultivars had only traces of vitamins A in their oil. In spite of the high variation, results of this study provide a clearer understanding of the vitamin A content of oil from different cultivars of groundnuts in Uganda.

Vitamin E (α-tocopherol) in groundnut oil varied from 0.88 to 7.19 mg/100 g (Table 30). Significant differences ($p<0.05$) were observed in the levels of α-tocopherol in the groundnut oil with minimal discrimination between improved and traditional cultivars. The lowest amount (0.88 mg/100 g) was exhibited by Acholi white and the highest (7.19.mg/100 g) by Serenut 13Tan. The range of results obtained from the current study was close to the 6.1 mg/100 g of vitamin E in groundnut oil as reported by Kornsteiner et al. (2006). Rafalowski et al. (2008) and Silva, Martinez, Casini, and Grosso (2010) reported α-tocopherol levels amounting to 21.30 mg/100 g and 20.21 mg/100 g, respectively, in raw groundnut oil. Similarly, Ejoh and Ketiku (2013) reported 48.1 mg/100 g vitamin E in oil from Nigerian grown groundnuts. This study registered lower levels of vitamin E. Differences may be associated with stage of maturity, genotype and environmental conditions during growth (Gunstone, 2002; Hashim, Koehlerv, Eitenmiller, and Kvien, 1993).

According to FAO and WHO (2001), the daily requirement of α-tocopherol is 15 mg/100 g. The vitamin E content in this study was low compared to daily dietary requirements. Although most of the groundnut oil in this study were low in vitamin E, oil from Serenut 6TanTan, 7Tan, 13Tan, *Rudu* red and *Rudu* white could contribute about 30 to 50% to the RDI if consumed in quantities >200 g of oil per day. Vitamin E has been reported to offer protective roles against ageing and non-communicable diseases like cardiovascular disease, stroke, diabetes and certain cancers (Rizvi et al., 2014). The link between disease prevention and anti-oxidant vitamins is associated with dietary consumption or serum concentration of the anti-oxidant vitamin (Rock, Jacob, and Bowen, 1996). Groundnut oil from the cultivars with high levels of vitamin E should therefore be popularised for use in dietary formulations for people with or at risk for chonic illnesses. In order to promote health, daily consumption of a table spoon of groundnut oil per day is suggested. Although a daily intake of 10 mg α-tocopherol equivalents (or 15 IU/day) is currently advised as an adequate level for healthy men, clinical trials typically involve daily administration of 134 mg (or 200 IU) or more in supplement form (Rock, Jacob, and Bowen, 1996).

Regarding shelf life, vitamin E is an important antioxidant and confers stability against oxidative deterioration of the oil (Nagendran et al., 2000; Gromadzka and Wardencki, 2011; Shad et al., 2012). Vitamin E and other substances such as carotenoids, polyphenols, squalenes could work synergistically to enhance stability of vegetable oil against oxidation and peroxidation (Gunstone, 2002; Kalogeropoulos, Mylona, Chiou, Ioannou, and Andrikopoulos, 2007; Mahatma, Thawait, Bishi, Khatediya, and Rathnakumar, 2016).

In line with the hypothesis, data confirms that oil from groundnut cultivars (Serenut 6 Tan, 7 tan, 13Tan, *Rudu* red and *Rudu* white) are good sources of vitamin E. Other cultivars contained comparatively low levels of the vitamin.

4.4.2 Anti-Oxidant Vitamins in Oyster Nut Oil

Beta-carotene content of oyster nut oil varied from 2.65 to 3.69 mg/100 g (Table 31). Levels of beta-carotene in oil varied significantly ($p<0.05$) for location but not for sex of nut. Oil extracted from nuts obtained from Kamuli nuts showed the highest levels of beta-carotene while those from Dokolo and Luwero had similar amounts. There was no literature to compare with, regarding beta-carotene content of oyster nut oil. However, most vegetable oil except palm oil contain <10 mg/100 g beta-carotene (Nagendran et al., 2000). Given the limited scholarly resources on the composition of oyster nut oil, it was not possible to compare findings of this study with literature. These findings will contribute to the existing database of crops grown in Uganda. The presence of beta-carotene in edible oil is of importance since it is an effective anti-oxidant (Rafa, 2017). Beta-carotene stabilizes oil against oxidation by acting as singlet oxygen quencher (Sanders, 2002; Su, 2003; Rafa, 2017). Beta-carotene is converted into vitamin A in-vivo (Tang, 2010, 2012). According to Tang (2010), part of the dietary beta-carotene is converted to retinol prior to use in the body while other proportions may carry out their activities as intact molecules. The above conversion depends on physiological requirement such that any amounts that are higher than the requirement may not exercise their functionality. Due to their inter conversion therefore, no RDI for beta-carotene is specified. Based on the above reports even small amounts of beta-carotene may be useful to the body. Regarding anti-oxidant activity however, the beta-carotene in oil could work synergistically with other anti-oxidants to reduce chances of oxidative degradation.

The levels of beta-carotene in oil from oyster nuts did not support the stated hypothesis. Data demonstrated that oyster nut oil is low in beta-carotene.

Vitamin A content of oyster nut oil varied from 23.84 to 29.17 µg/100 g (Table 31). Significant differences ($p<0.05$) were observed for all geographical sources and sex of nuts. Compared to the recommended daily intake (RDI) of vitamin A (900 to 1500 µg for men and 690 to 1200 µg for women) (FAO/WHO, 2004), vitamin A content in oyster nut oil was low. Johnson et al. (2009) reported that most vegetable oil and fats are not good sources of vitamin A. It was therefore not surprising that oyster nut oil had low levels of the vitamin. Vitamin A is essential for normal functioning of the visual system, growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction (Bourre, 2006). Other roles include involvement in gene expression and production of the human growth hormone (Oruch and Pryme, 2012).

The levels of vitamin A in oyster nut oil did not support the hypothesis. These findings will add to the existing body of information on oyster nuts grown in Uganda.

Content of vitamin E in oyster nut oil ranged from 1.03 to 1.77 mg/100 g (Table 27). Oil from Dokolo nuts showed the highest content while those from Kamuli had the lowest levels. Vitamin E levels were not statistically different ($p>0.05$) in nuts from different locations and sex of nut. With an average content of 1.44 mg/100 g, oyster nut oil may provide 9.58% of the daily requirement for vitamin E. Amounts of vitamin E were low in oyster nut oil. This finding disagreed with the observation by Ros (2010) that nut oil contain high levels of vitamin E. Studies about this parameter in oyster nut oil are few.

Table 31: Vitamin A, E and Beta-carotene content of oyster nut oil

Location	Gender	Vitamin A (µg/100 g)	β- carotene (mg/100 g)	α-tocopherol (mg/100 g)
Dokolo	Male	23.93±0.01^{e}	2.77±0.01^{b}	1.58±0.24^{a}
	Female	24.17±0.01^{d}	2.65±0.02^{b}	1.77±0.02^{a}
Kamuli	Male	28.47±0.01^{b}	3.68±0.04^{a}	1.22±0.11^{a}
	Female	29.16±0.02^{a}	3.69±0.06^{a}	1.03±0.03^{a}
Luwero	Male	23.84±0.01^{f}	2.69±0.06^{b}	1.43±0.15^{a}
	Female	26.23±0.01^{c}	2.74±0.02^{b}	1.59±0.01^{a}

Values are means ± standard deviations of the means for three independent replicates. Values in columns followed by the same superscript letter are not significantly different ($p>0.05$).

Results showed that groundnut and oyster nut oil had low content of beta-carotene and vitamin A compared to RDI. Oyster nut oil had low levels of vitamin E while some groundnut cultivars were rich in vitamin E.

4.5 Mineral Content of Groundnut and Oyster Nut Oil

4.5.1 Minerals in Groundnut Oil

Levels of magnesium (Mg) and calcium (Ca) in oil were relatively high while iron (Fe) and zinc (Zn) occurred in trace amounts (Table 32). Variations depended on the individual minerals. Magnesium levels were higher in traditional than improved cultivars while the reverse was observed for Ca. Both types of groundnut oil had only traces of Fe and Zn.

Levels of Ca ranged from 0.05 to 3.41 mg/100 g. There was significant difference in Ca content among traditional and improved nut oil ($p<0.05$) Serenut 3Red and 14Red contained significantly ($p<0.05$) higher amounts of Ca than the other oil (Table 32). Oil from Serenut

1Red had the lowest level of Ca amounting to 0.88 mg/100 g among improved cultivars. On the other hand, oil from all traditional cultivars were low in Ca with amounts <1 mg/100 g. The iron content in this study was similar to that observed by Asemave, Ubwa,Anhwange, and Gbaamende (2012) in groundnut oil. The levels of Zn were similar to those of Carrín and Carelli (2010) who reported trace levels in groundnut oil. Results indicated that the Fe content was below the maximum limit of 0.5 mg/100 g set by Codex for other oil (FAO/WHO, 2005). Although the tolerable amounts of Ca and Mg in oil were not specified in literature, oil is expected to contain these ions in trace amounts as they are pro-oxidants. Calcium and magnesium promote the final stages of lipid oxidation (Shahidi, 2005). These interactions cause changes in flavour, colour and odour of edible oil. Findings differed from the assertion by Inuwa, Aina, Gabi, Aimola, and Toyin (2011) that groundnut oil was a rich source of minerals. This could be explained by the fact that Fekria et al. (2012) found residual amounts of minerals in oil cake. Fekria et al. (2012) asserted that minerals tend to bind to the fibre during the extraction.

Traditional cultivars contained higher levels of magnesium compared to improved nuts except Serenut 2 which contained similar proportions to traditional cultivars. Regarding iron and zinc, low amounts <1 mg/100 g were detected in groundnut oil. Levels of iron in oil from traditional cultivars were significantly ($p<0.05$) lower than that from improved nuts with amounts <0.01 mg/100 g. Traditional cultivars, *Egoromoit* and Red beauty had the highest level of zinc (0.53 mg/100 g and 0.55 mg/100 g) respectively. Acholi white had the lowest zinc level overall.

Table 32: Mineral content (mg/100 g) of groundnut oil

Cultivar	Calcium	Magnesium	Iron	Zinc
Improved Cultivars				
Serenut 1Red	0.88±0.01^{fg}	1.03±0.01^{k}	0.28±0.02^{b}	0.21±0.02^{c}
Serenut 2	1.39±0.00^{defg}	2.12±0.01^{d}	0.27±0.01^{b}	0.12±0.01^{f}
Serenut 3Red	3.41±0.00^{a}	1.43±0.01^{h}	0.14±0.06^{e}	0.14±0.00^{ef}
Serenut 4Tan	1.26±0.02^{efg}	1.17±0.01^{k}	0.35±0.01^{a}	0.07±0.00^{hi}
Serenut 5Red	1.73±0.01^{cde}	0.86±0.01^{n}	0.10±0.01^{f}	0.09±0.01^{gh}
Serenut 6Tan	1.64±0.03^{cedf}	1.86±0.01^{g}	0.35±0.01^{a}	0.36±0.02^{b}
Serenut 7Tan	2.67±0.01^{ab}	1.19±0.01^{j}	0.24±0.01^{c}	0.07±0.01^{hi}
Serenut 8Red	1.53±0.00^{cdef}	0.17±0.01^{q}	0.07±0.00^{g}	0.04±0.01^{j}
Serenut 9Tan	1.25±0.02^{efg}	0.98±0.02^{c}	0.10±0.01^{f}	0.05±0.00^{ij}
Serenut 10Red	2.07±0.02^{bcd}	1.42±0.01^{l}	0.19±0.02^{d}	0.09±0.01^{g}
Serenut 11Tan	1.56±0.00^{cdef}	0.66±0.01^{p}	0.02±0.00^{h}	0.05±0.01^{ij}
Serenut 12Red	2.20±0.02^{bc}	0.81±0.01^{0}	0.02±0.00^{h}	0.08±0.01^{gh}
Serenut 13Tan	1.46±0.01^{cdef}	0.94±0.01^{m}	0.16±0.01^{de}	0.17±0.01^{d}
Serenut 14Red	3.03±0.03^{a}	1.19±0.01^{j}	0.10±0.01^{f}	0.15±0.03^{de}
Traditional Cultivars				
Acholi white	0.66±0.10^{gh}	1.01±0.01^{l}	0.28±0.00^{b}	0.07±0.00^{ghi}
Igola	0.06±0.00^{h}	2.06±0.02^{e}	0.02±0.00^{h}	0.23±0.00^{c}
Rudu red	0.05±0.00^{h}	2.24±0.01^{c}	0.02±0.00^{h}	0.23±0.01^{c}
Rudu white	0.06±0.00^{h}	2.49±0.01^{a}	0.03±0.00^{h}	0.22±0.01^{c}
Egoromoit	0.06±0.00^{h}	2.05±0.01^{e}	0.02± 0.01^{h}	0.53±0.01^{a}
Red beauty	0.05±0.00^{h}	2.31±0.00^{b}	0.02±0.00^{h}	0.55±0.01^{a}
RDI (FAO/WHO, 2004)	1000 to 1300 mg	190 to 420 mg	7.5 to 19.6 mg	3 to 6 mg

Values are means ± standard deviations of the means for three independent replicates. Values in columns followed by the same superscript letter are not significantly different ($p>0.05$).

Mineral levels were lower than data reported by Ayoola and Adeyeye (2012) who observed high levels of mineral elements (1,180 mg/100 g Ca, 180 mg/100 g Mg, 420 mg/100 g Fe and 440 mg/100 g Zn) in groundnut kernels. In the human body, minerals are required as

enzyme cofactors in many biochemical reactions in the body (Adjepong et al., 2017). Calcium content of oil from improved groundnut cultivars was higher than that of oil from the traditional cultivars while the latter presented relatively higher content of magnesium. Magnesium content in oil from Serenut 2 (2.12 mg/100 g), an improved cultivar was consistent with that in oil from the traditional cultivars (1.01 to 2.49 mg/100 g) except Acholi white which was significantly lower ($p<0.05$). Traditional cultivars had higher levels of Zn and Fe than improved cultivars. The high quantity of zinc may be beneficial for human nutrition. Zinc plays a role in growth and development as well as metabolism of essential nutrients. This includes the conversion of linoleic acid to arachidonic acid and the conversion of alpha linolenic acid to eicosapentanoic acid and docosahexanoic acid in the body (FAO/WHO, 2004; Adjepong et al., 2017). Iron on the other hand is essential in formation of and hence the prevention of anaemia (FAO/WHO, 2001, FAO/WHO, 2008)

4.5.2 Minerals in Oyster Nut Oil

High amounts of Ca and Mg were detected in all samples of oyster nut oil (Table 33). The respective ranges were 16.55 to 31.57 mg/100 g and 42.15 to 64.04 mg/100 g. Oil from Luwero nuts had the highest level of calcium while that from Kamuli had the lowest. Oil from Kamuli nuts had the highest Mg content while that from Dokolo had the lowest. (Table 33). Sex of nut had no significant effect on Ca and Mg composition of oyster nut oil ($p>0.05$). Iron and zinc were low in all oyster nut oil with amounts <0.3 and <0.5 mg/100 g, respectively. Results indicated that the Fe content was below the maximum limit of 0.5 mg/100 g set by Codex for other oil (FAO/WHO, 2005). Iron is a known pro-oxidant which may enhance oil degradation in the initiation stage of the oxidation chain reaction (Mcclements and Decker, 2000, Ahmed et al., 2016; Madhujith and Sivakanthan, 2018;). Low levels therefore, may be beneficial to shelf life and nutritional quality of oil. Minerals

besides Fe have potential to cause damage of oil at different stages of oxidation reactions (Davis et al., 2016).

Minerals Mg and Ca occurred in considerable amounts while Fe and Zn were low in oil. Variations in mineral content of oil amongst three districts of Dokolo, Luwero and Kamuli where nuts were sourced and differences in sex of nut were significant ($p<0.05$). Lutz, Álvarez, and Loewe (2017) reported variations in the Ca, Fe and Zn composition of pine nuts from different geographical zones in Chile. Results of Ca and Mg content confirmed the hypothesis that oyster nut oil contains high levels of minerals, however, contents of Fe and Zn did not support the hypothesis. Due to the inadequacy of literature on oyster nut oil composition, findings were compared with other vegetable oil.

From the nutritional point of view, Fe , Zn, Mg and Ca, all have important functions in the body that may promote health (Carrín and Carelli, 2010). None of these minerals, however, occurred in large enough levels to meet the RDI for adult males and females (Table 32 and 33). In order to meet the RDI, one must consume a large amounts of oil, but, this is not advisable from the nutritional perspective. Oil should contain low amounts of minerals unless purposefully fortified to meet nutrition needs. Data from this study will contribute information that can be added to the data base of nutrients in oil seeds and their products.

Table 33: Mineral composition (mg/100 g) of oyster nut oil

District	Gender	Ca	Mg	Fe	Zn
Dokolo	Male	24.83±0.01^{d}	42.15±0.01^{d}	0.17±0.01^{b}	0.43±0.01^{a}
	Female	24.90±0.01^{c}	42.28±0.01^{c}	0.17±0.01^{b}	0.40±0.01^{b}
Kamuli	Male	16.55±0.01^{f}	63.48±0.01^{b}	0.21±0.01^{a}	0.33±0.01^{d}
	Female	16.91±0.01^{e}	64.04±0.01^{a}	0.22±0.01^{a}	0.36±0.01^{c}
Luwero	Male	31.57±0.01^{a}	42.54±0.01^{e}	0.20±0.01^{a}	0.27±0.01^{e}
	Female	28.06±0.02^{b}	43.07±0.01^{f}	0.19±0.01^{ab}	0.27±0.01^{e}
RDI (FAO/WHO, 2004)		1000 to 1300 mg	190 to 420 mg	7.5 to 19.6 mg	3 to 6 mg

Values are means ± standard deviations of the means for three independent replicates. Values in columns followed by the same superscript letter are not significantly different ($p>0.05$).

Trace amounts of metals are absorbed by plants during the growing season and introduced in fats and oil during processing due to tear and wear of machines (Sanders, 2002). Mineral elements in oil catalyse oxidation, a factor in edible oil deterioration (Sanders, 2002; Shahidi, 2005; Strayer et al., 2006; Xia and Budge, 2018). Groundnuts were decorticated prior to oil extraction and the resultant levels of minerals were low compared to amounts (114.80, 4.72 4.81 mg/100 g of Ca, Fe, Zn, respectively, and 24.50 to 262.00 mg/100 g of Mg) reported in whole nuts (Rodrigues et al., 2013; Mustapha, Mohammed, Adeosun, Mathew, and Muhammed, 2015). Findings from this study suggested that high amounts of the mineral elements were retained in the testa and groundnut cake. Sanders et al. (1992), analysed pressed cake of groundnuts from different countries and different crop years and reported values of 3.79 to 8.03 mg/100 g iron which were far higher than results detected in oil. Limited information is available on the maximum residual limits for Mg and Zn in vegetable

oil. The requirements of Mg, Ca, Fe and Zn to enable the body perform important functions is minimal. Thus, the levels detected in this study could be sufficient for human nutrition. Moreover, these elements are heat stable (Shahidi, 2005) which would guarantee minimal losses after conventional food preparations involving heat treatment. Moreover, exceeding the RDI of these elements may result in toxicity. Farzin and Moassesi (2014) suggested that concentrations of minerals in edible oil should not reach levels that reduce shelf life of oil and cause toxicity in the human body.

Levels of Mg, Ca, Fe and Zn in groundnut and oyster nut oil were too low compared to the RDI. The oil cannot be used as sources of the above minerals in diet. The low mineral content of oil is a positive attribute towards oxidative stability of oil.

4.5.3 Tannin Content of Groundnut and Oyster Nut Oil

4.5.3.1 Groundnut Oil

Tannin levels in groundnut oil ranged from 531.80 to 852 µg/100 g (Table 34). In improved cultivars, Serenut 10 Red had the lowest level whereas Serenut 1Red had the highest (Table 34). Among traditional cultivars, Red beauty had the highest (940.48 µg/100 g) whereas Acholi white had the lowest concentration. Overall, oil from traditional cultivars *Rudu* red *Rudu* white and Red beauty exhibited higher amount of tannins than the other cultivars (Table 34). There were significant differences ($p<0.05$) in the tannin composition among cultivars. In this study tannin levels were lower than the findings of Inuwa et al. (2011), who reported a tannin level of 370 mg/100 g in groundnut oil and a lethal dose of 3 mg/100 g. Ventachalam and Sathe (2006), reported a level of 160 to 290 mg/100 g tannins in Virginia groundnuts. Similarly, Satish and Shrivastava (2011), obtained 412 mg/100 g tannins in groundnut variety JL- 24 in India most of which was found in the skins. According to Nautiyal (2002); Sobolev and Cole (2004) and Mackown, Brown and Walker (2011),

substantial amounts of tannins and catechol-type compounds are located in groundnut testa and are responsible for imparting color to the testa of groundnuts. Similarly, Ejigui, Savoie, Marin, and Desrosiers (2005) stated that condensed tannins are located in the seed hull and their quantity is reduced when groundnuts are processed after decortication. This suggests that the low levels of tannins in oil could be ascribed to the removal of groundnut skin prior to oil extraction as was done in chapter 3. The level of tannins in this study was within the recommended range of 1500 µg/100 g stipulated by EFSA (2014). This implies that groundnut and oyster nut oil is safe for human consumption. In line with the hypothesis, findings provide evidence that groundnut oil contains low levels of these tannins compounds.

Table 34: Tannin content of groundnut oil

Cultivar	Tannins (µg/100 g)
Improved Cultivars	
Serenut 1Red	852.25±0.04^{e}
Serenut 2	816.08±0.12^{f}
Serenut 3Red	715.12±0.01^{i}
Serenut 4Tan	725.75±0.12^{h}
Serenut 5Red	662.55±0.45^{l}
Serenut 6Tan	540.43±0.11^{s}
Serenut 7Tan	776.01±0.08^{g}
Serenut 8Red	545.43±0.03^{r}
Serenut 9Tan	555.24±0.00^{q}
Serenut 10Red	531.80±0.01^{t}
Serenut 11Tan	629.60±0.01^{n}
Serenut 12Red	569.40±0.01^{p}
Serenut 13Tan	703.26±0.01^{j}
Serenut 14Red	679.38±0.03^{k}
Traditional Cultivars	
Acholi white	586.74±0.00^{o}
Igola	645.70±0.00^{m}
Rudu red	936.65±0.01^{b}
Rudu white	929.53±0.01^{c}
Egoromoit	919.63±0.01^{d}
Red beauty	940.48±0.01^{a}

Values are means ± standard deviations of the means for three independent replicates. Values in columns followed by the same superscript letter are not significantly different ($p>0.05$).

4.5.3.2 Oyster nut oil

Tannin levels in oyster nut oil ranged from 340.85 to 440.06 µg/100 g (Table 35). Oil from nuts sourced from Luwero had the highest while that from Kamuli had the lowest level. Significant differences ($p<0.05$) were observed in the tannin composition among sex of nut

and districts. Tannin level in oyster nut oil was lower than the upper tolerable limit of 1500 µg/100 g set by EFSA (2014). The low levels are attributed to the fact that the fibrous skin and bast of the oyster nut were removed prior to oyster nut oil extraction. According to (Okoli, 2007), the bast contains abundant levels of tannins.

A mini review by Kuca and Opletal (2008) suggested that tannins in low amounts act as anti-oxidants in prevention of oxidation and inflammation and hence reducing risk of non-communicable diseases. The same author suggested that tannins supressed oxidative stress which is influential in cancer pathogenesis. This evidence suggests that presence of small amount of tannins is beneficial to health. Furthermore, tannins have been demonstrated to have anti-inflammatory ability through their anti-oxidant properties by preventing LDL oxidation, ability to cause vasodilation and hence reducing of cardio vascular risk. It is important to note that in high amounts, hydrolysable and condensed tannins inhibit the enzymes activity and interfere with protein digestion. Tannins in high concentrations bind with proteins, cellulose, hemicellulose and pectin, to form stable complexes hence impairing protein, calcium and iron bioavailability (Hagerman, et al.,1992; Mcmahon et al., 2000; Inuwa et al., 2011; Ashok and Upadhyaya, 2012; Talabi et al., 2016). On the other hand, tannins, as metal chelators prevent oxidation which action may the shelf life of oil In low concentration, however, tannins may retard lipid peroxidation (Gemede and Ratta, 2014). This suggests that the low levels of tannins observed in oyster nut oil in this work could be favourable for its stability to peroxidation and enhance the oil quality.

The study shows that oil from groundnuts and oyster nuts are low in tannins compared to the safe dose of 1500 µg/100 g (EFSA, 2014).

Table 35: Tannin content of oyster nut oil

Location	Gender	Tannins (µg/100 g)
Dokolo	Male	365.05±0.02[c]
	Female	363.66±0.01[d]
Kamuli	Male	341.44±0.02[e]
	Female	340.85±0.01[f]
Luwero	Male	438.76±0.01[b]
	Female	440.06±0.02[a]

Values are means ± standard deviations of the means for three independent replicates. Values in columns followed by the same superscript letter are not significantly different ($p>0.05$).

CHAPTER FIVE

5 Conclusions and Recommendations

5.1 Conclusions

1. Groundnut cultivars had high oil yield. Traditional and improved cultivars were all high in the amounts of the oil extracted. It was observed that variation occurred in the oil yields among the different cultivars. Oil from the groundnuts were of good quality given that the peroxide and acid values were within limits approved for oils meant for human consumption. The acid, peroxide and iodine values were within the limits prescribed by international standards for vegetable oil.
2. Oil from traditional and improved groundnut cultivars was abundant in oleic, linoleic and palmitic acids. Unsaturated FA were high compared to the saturated. Levels of these FA did not vary between improved and traditional cultivars. Oil from improved cultivars contained cis 11-eicosenoic acid (C20.1), cis 11, 14 eicosadienoic acid (C20.2) and cis 11, 14, 17 eicosatrienoic acid (C20.3ω3) which were not detected in traditional cultivars. Lipid health indices indicate that consumption of groundnut oil may lower cardiovascular risk.
3. Oyster nuts contained high amounts of linoleic and palmitic acid. Due to the high levels of both, the total saturated and unsaturated FA were nearly 50:50. The high concentration of linoleic acid, however, makes oyster nut oil susceptible to oxidation. The FA composition of oyster nut oil differed from that of most nut oil that are high in oleic acid and low in palmitic acid. The thrombogenic index of oyster nut oil gave a negative indication for cardiovascular risk and the hypocholesterolemic / hypocholesterolemic index were lower than most vegetable oil.

4. Vitamin A and beta-carotene amounts in the oil from traditional and improved groundnut were high. Vitamin E was the most abundant in oil from Serenut 6Tan, 7Tan and 13Tan as well as *Rudu* white and *Rudu* red hence oils from these groundnuts could be utilized as good dietary sources of vitamin E. Levels of vitamin A, beta-carotene and vitamin E in oyster nut oil was low.
5. Levels of mineral elements calcium, magnesium, iron and zinc were low in groundnut oil. Minerals levels in oil were too low compared to RDI. Low amounts of minerals elements is good in that it may reduce, risk of auto-oxidation of the oil. Oyster nut had low levels of calcium, magnesium, iron and zinc were in oil.
6. Tannin concentration was generally low in oil from improved and traditional groundnuts. Oyster nut oil had low levels of tannins irrespective of gender or source of nuts. The low level of tannins indicates that consumption of groundnut and oyster nut oil may be beneficial for health as they possess anti-oxidant properties at low levels.
7. The FA composition of oyster nut oil was modified with higher losses of linoleic acid and higher elevation of palmitic and stearic acid evident during roasting than stewing. The peroxide value of oil increased while the iodine values of oil decreased with heat treatment.

5.2. Recommendations

1. Large scale production of oils from groundnuts and oyster nuts can be explored by producers and industrialists based on the high oil yield and superior quality of the oils.

2. Oils from groundnuts and oyster nut require blending with a small proportion of omega-3 rich oil such as flax seed oil for better health benefit due to the low levels of omega-3 fatty acids.
3. Given the low levels of vitamin A, groundnut and oyster nut oils may require fortification to enhance the levels of this vitamin. Oil that is rich in vitamin E could be popularized for consumption.
4. Results suggest that stewing at 92±2^{o}C is a better treatment than roasting at 179.3±2^{o}C for both groundnut and oyster nuts. It is therefore recommendable that preparation methods for the groundnuts and oyster nuts adopt the stewing method over the roasting one.
5. Considering that tannins in low quantities act as anti-oxidants, further research is needed to determine their behavior in presence of other anti-oxidants such as vitamin E.

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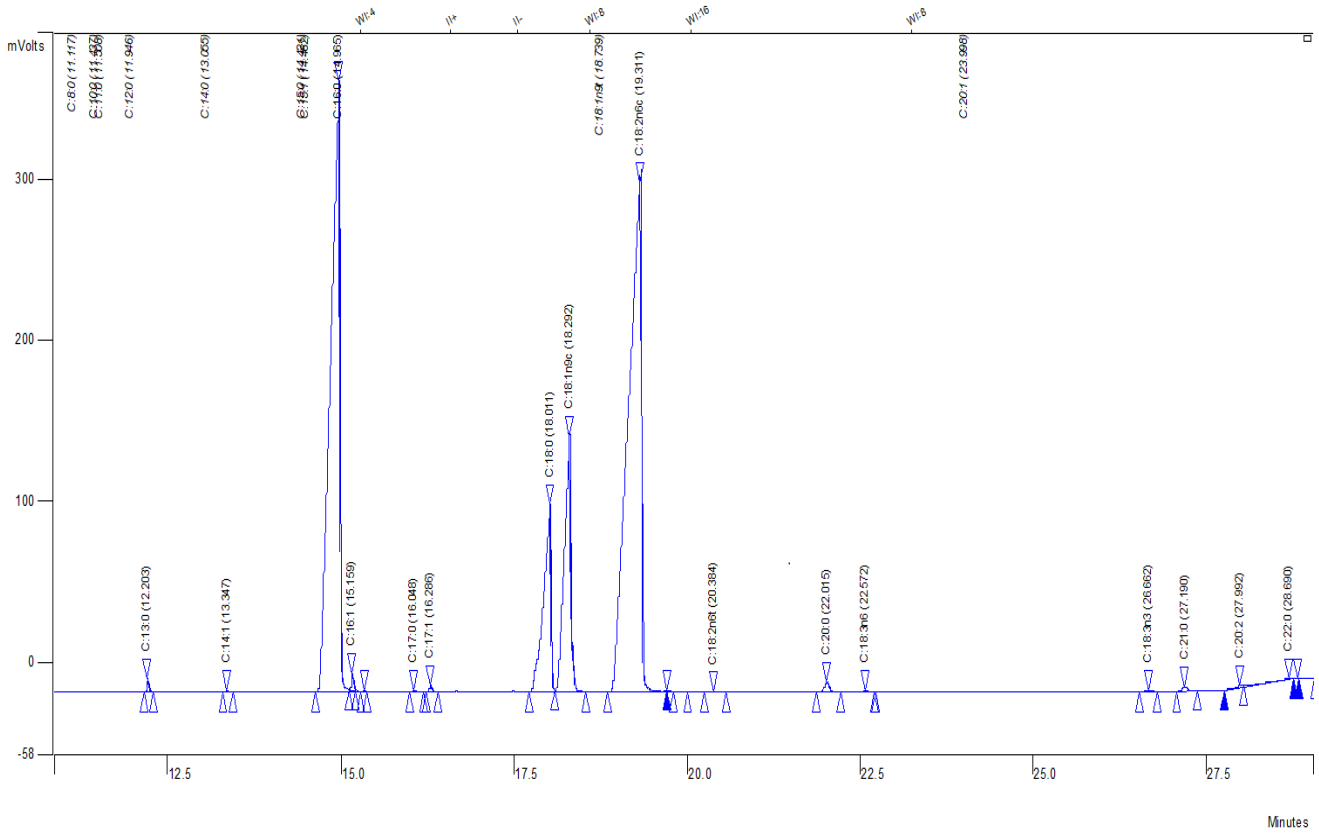
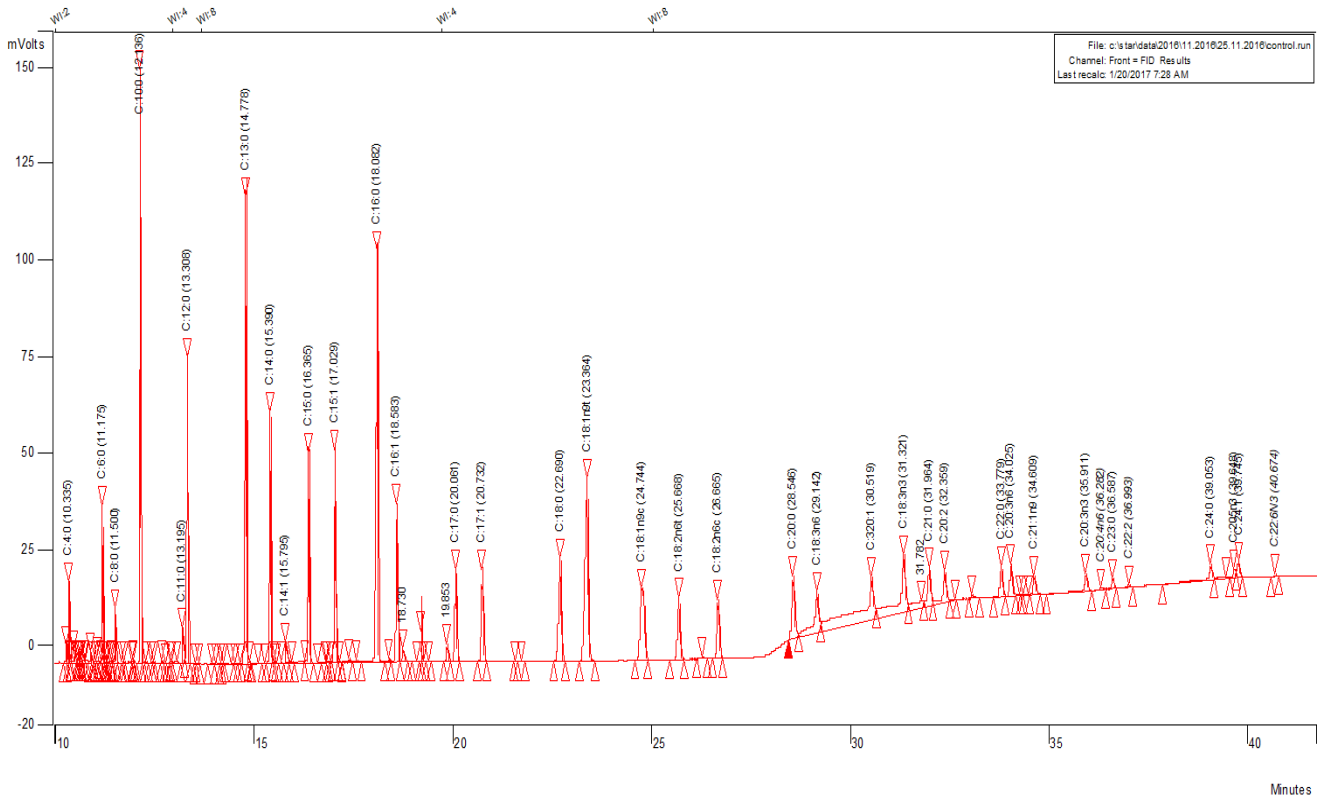
Appendices

Appendix 1a: Chromatogram for fatty acids composition

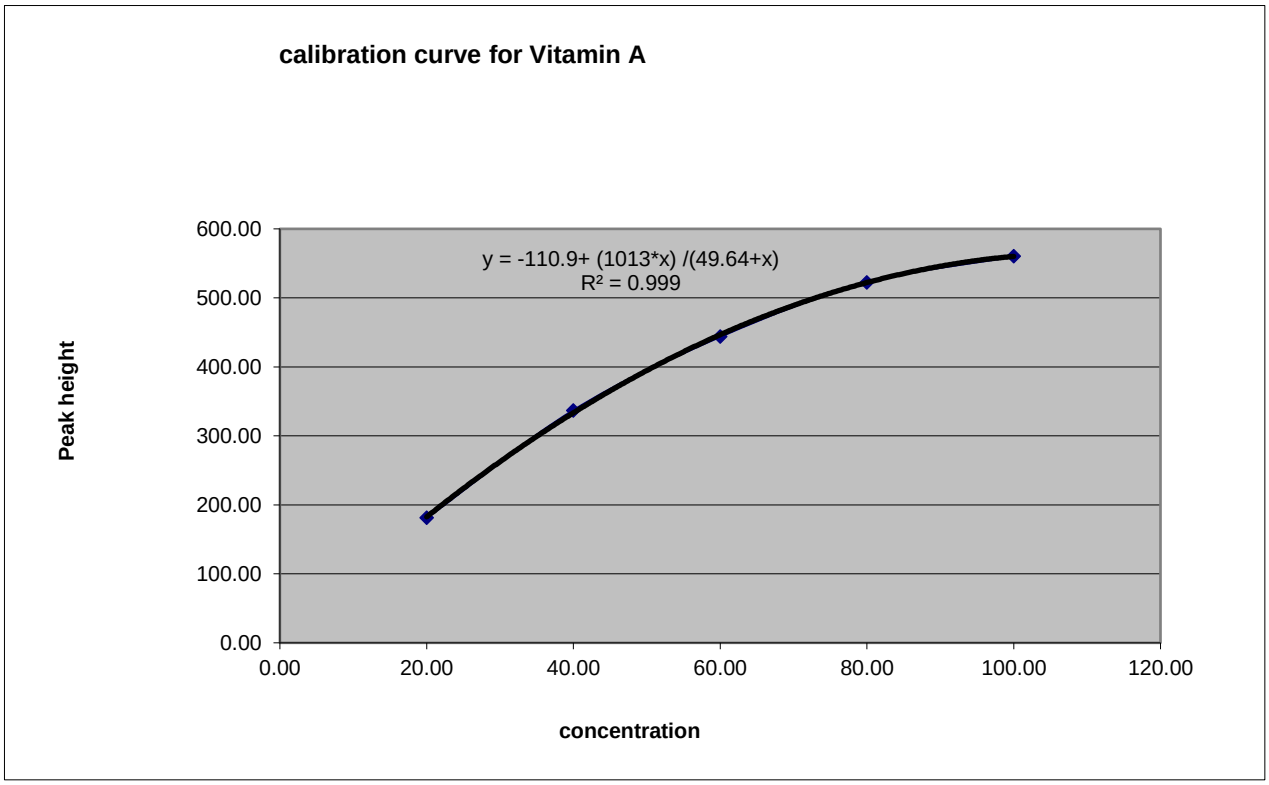


Appendix 1b: The control for fatty acids



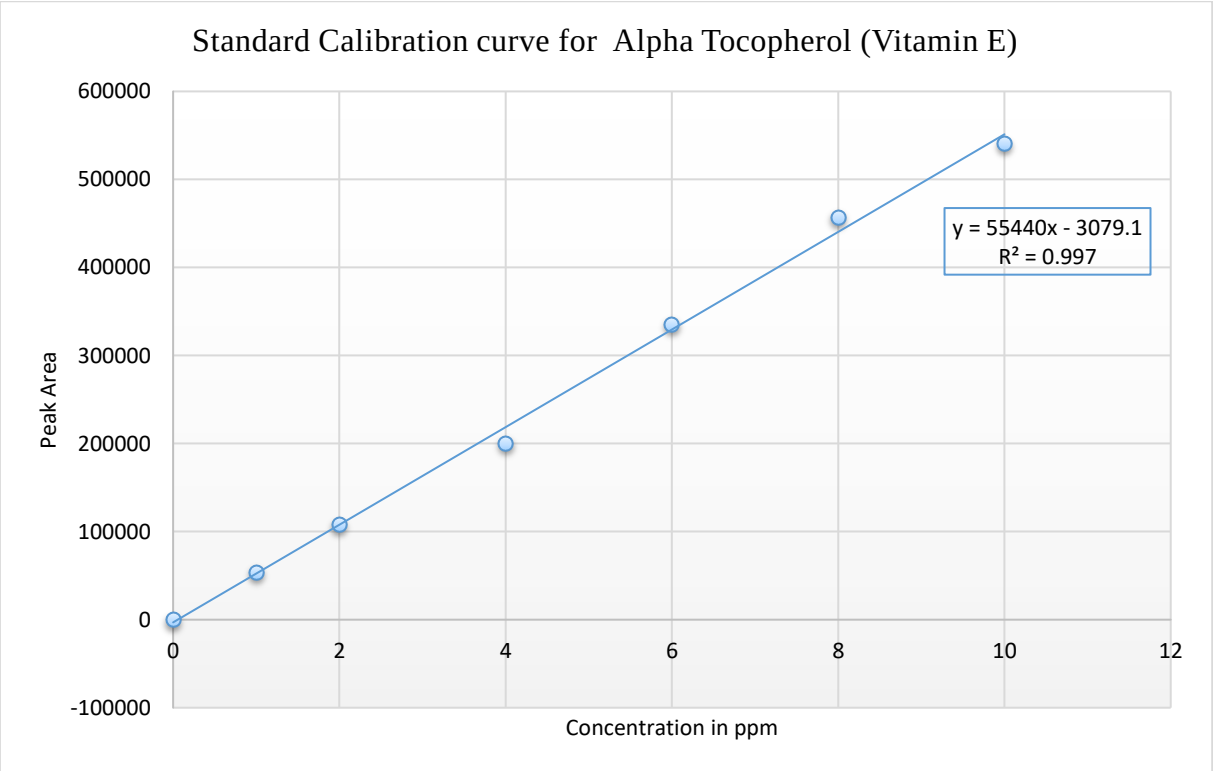
Appendix 2: Calibration Curve for vitamin A

Concentration	**Peak Area**
20.00	181.28
40.00	336.80
60.00	443.83
80.00	522.51
100.00	560.40

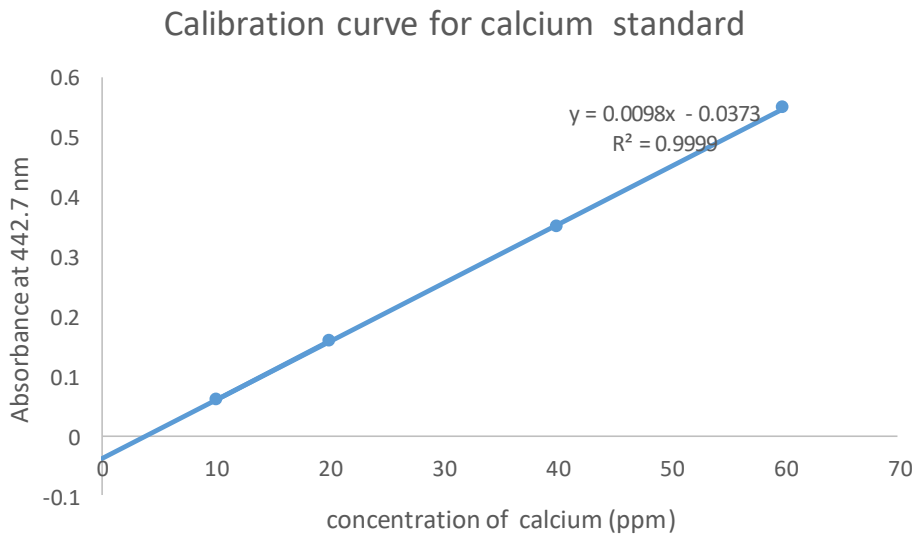


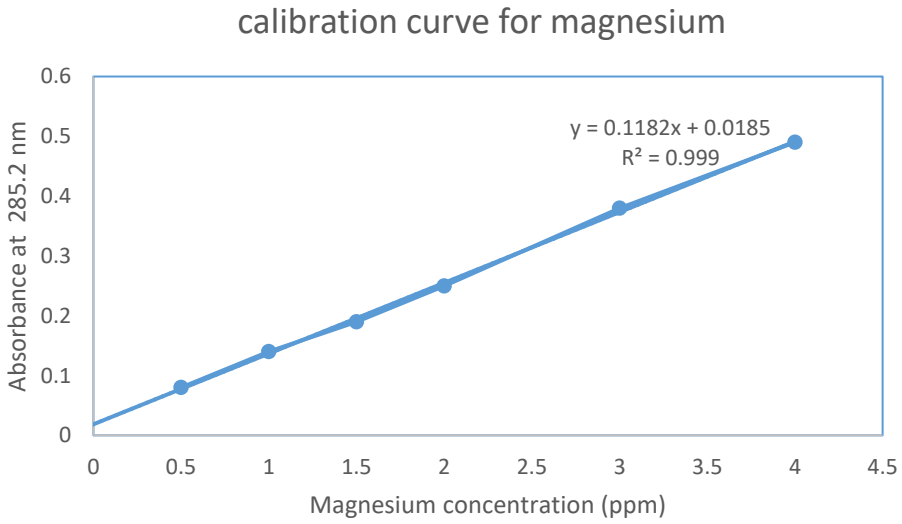
Appendix 3: Calibration curve for vitamin E

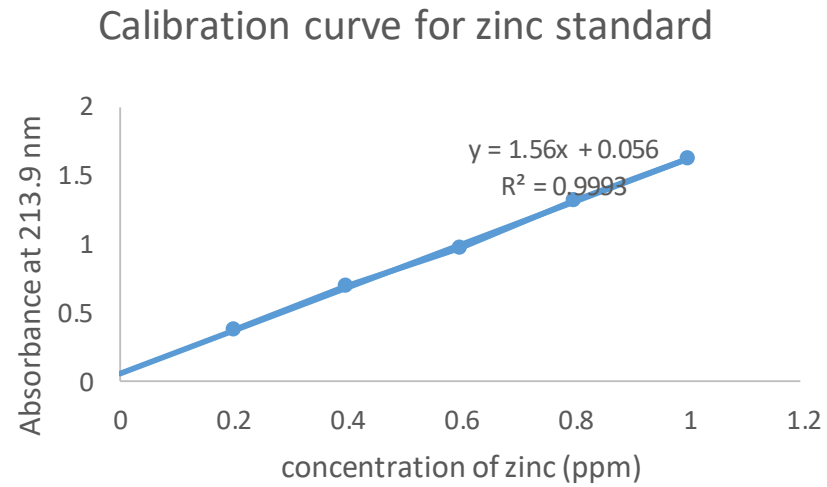
Concentration in ppm	**Area of the peak**
0	**0**
1	54114.69
2	108318.34
4	200548.6
6	335695.53
8	457231.78
10	541191.88

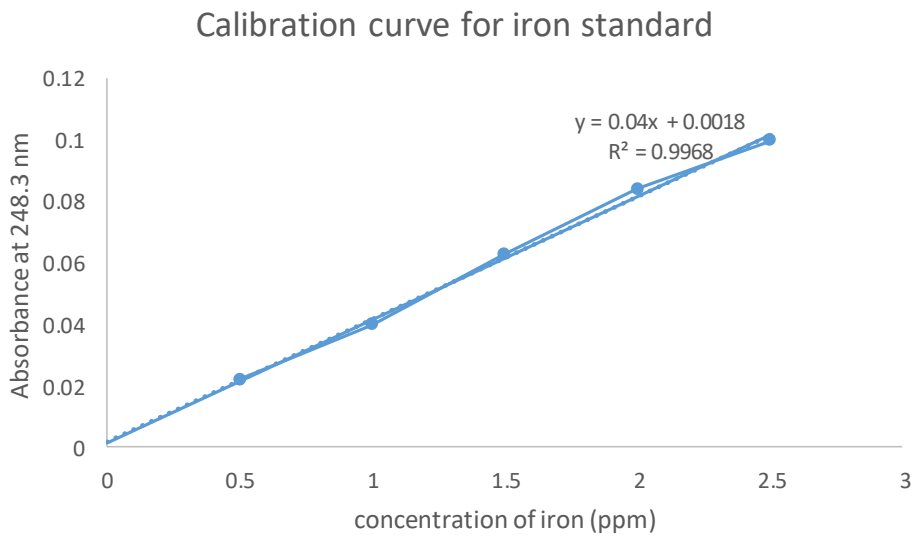


Appendix 4: Calibration curves for minerals





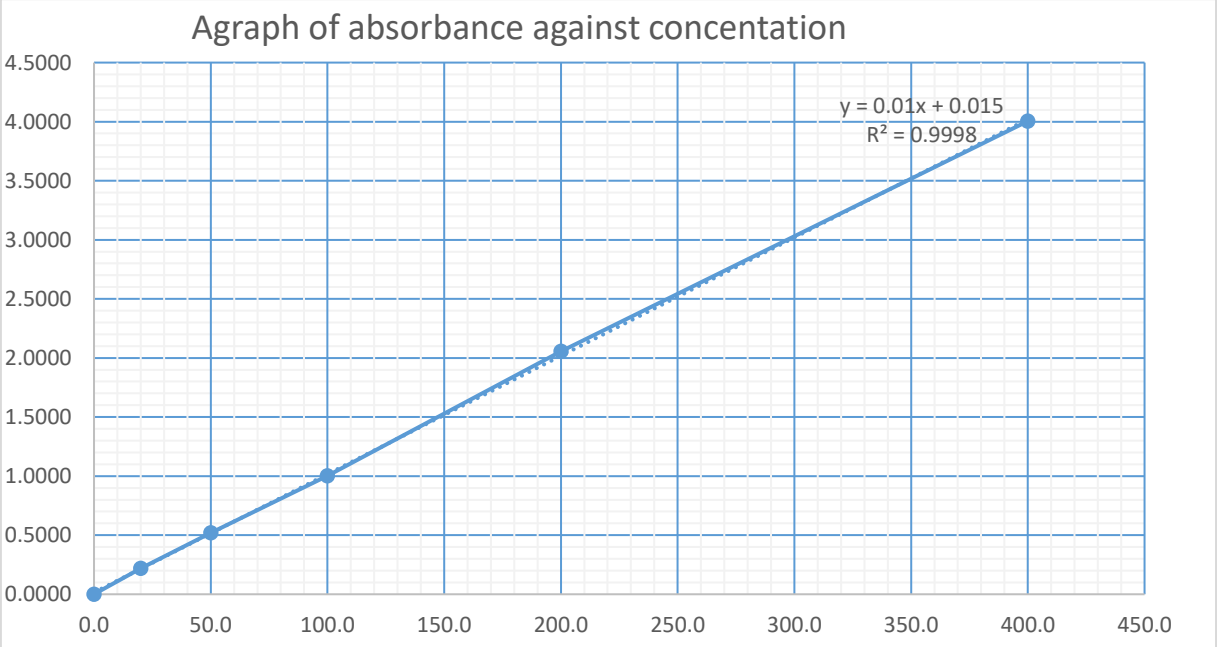

Calibration curve for zinc standard
Absorbance at 213.9 nm
y = 1.56x + 0.056
R² = 0.9993
0
0.5
1
1.5
2
0
0.2
0.4
0.6
0.8
1
1.2
concentration of zinc (ppm)


Calibration curve for iron standard
Absorbance at 248.3 nm
y = 0.04x + 0.0018
R² = 0.9968
0
0.02
0.04
0.06
0.08
0.1
0.12
0
0.5
1
1.5
2
2.5
3
concentration of iron (ppm)

Appendix 5: calibration curve for Tannins

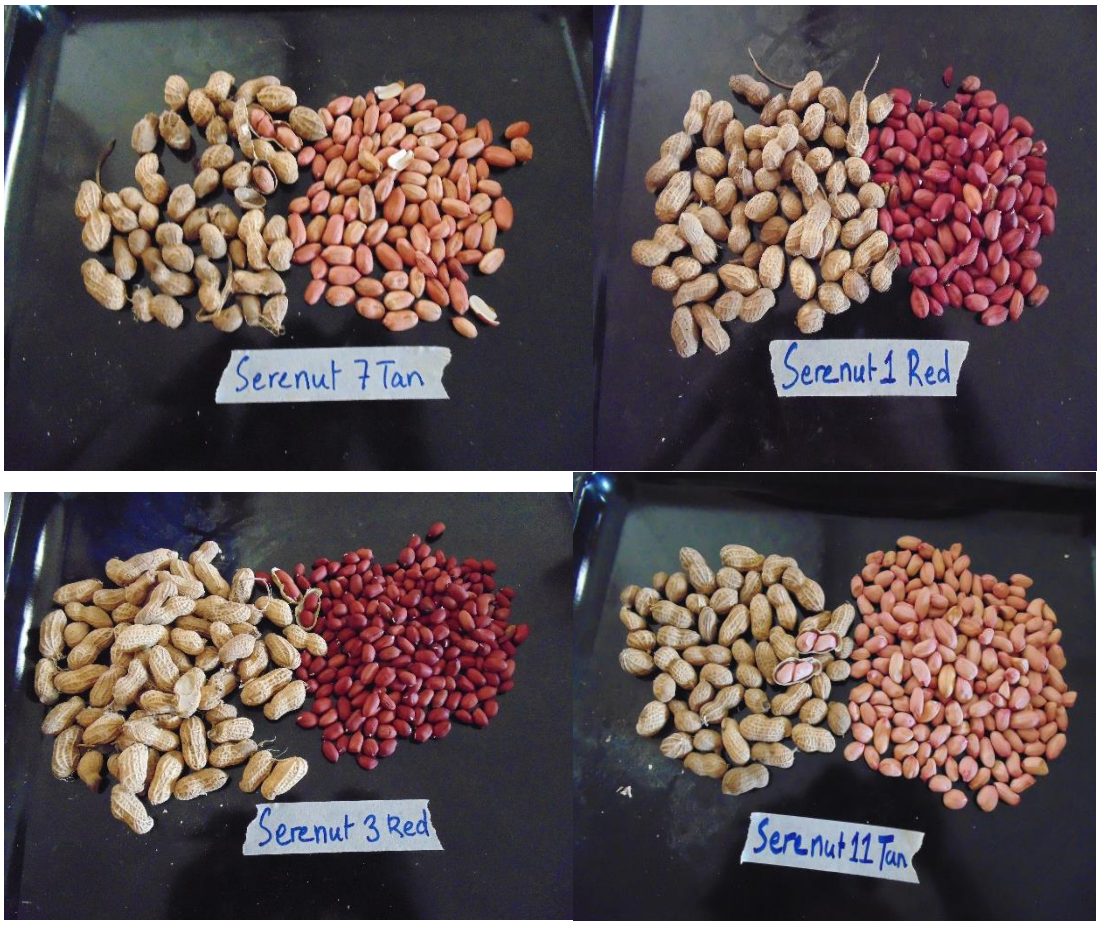
Concentration (ug)	**Absorbance**
0.0	0.0003
20.0	0.2177
50.0	0.5198
100.0	1.0032
200.0	2.0551
400.0	4.0045

Procedure blank 0.0003



Groundnuts


Acholi White
Acholi White
Serenut 4 Tan
Serenut 14 Red
Serenut 2 Tan
Serenut 13 Tan


Serenut 7 Tan
Serenut 1 Red
Serenut 3 Red
Serenut 11 Tan