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Variations in nutrient composition and oil stability of oyster nuts (telfairia pedata) across different agro- climatic conditions in Northern Tanzania

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VARIATIONS IN NUTRIENT COMPOSITION AND OIL STABILITY OF OYSTER NUTS (*Telfairia pedata*) ACROSS DIFFERENT AGRO-CLIMATIC CONDITIONS IN NORTHERN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

In sub-Saharan Africa, nutrient deficiency remains a challenge. The challenge is partly due to the underutilization of available nutrient sources and failure to optimize nutrients based on agro-climatic conditions. Oyster nuts (*Telfairia pedata*) are the under-utilized oilseeds with promising food and non-food applications. This study investigated oyster nut's nutrient composition and variations across different sites and elevation levels encompassing different agro-climatic conditions. Additionally, the stability of crude oyster nuts oil, flaxseed oil, and sunflower oil were compared. Fats were the most abundant contents of the nuts with 68%, followed by 25% protein, 5% carbohydrates, and 2% ash. Fatty acid contents were dominated by linoleic acid (47%), while magnesium was the most abundant mineral (150 mg/100 g). Protein and fat contents increased significantly with declining elevations, while sites did not show any significant effects. In contrast, linoleic acids and minerals such as magnesium (Mg) and phosphorus (P) of the nuts decreased significantly in low elevations. Other nutrients such as carbohydrates, potassium (K), palmitic acid, iron (Fe), and stearic acid in the nuts were not significantly affected by neither site nor elevations. Finally, oyster nut oil produced high peroxide value (PV) and free fatty acids (FFA) compared to flaxseed and sunflower oil at room temperature. Our results highlight that oyster nuts oil stability should be improved before storage. Likewise, in lower elevations associated with high temperature and low precipitation, oyster nuts' quality is optimized. Hence, oyster nuts can improve nutritional status and income generation to farmers and communities in Tanzania.

DECLARATION

I, Emmanuel Mwakasege, do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entitled "Variations in nutrient composition and oil stability of oyster nuts (*Telfairia pedata*) across different agro-climatic conditions in northern Tanzania" as a fulfillment of the requirement for the degree Master of Life Sciences at the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This work is firstly dedicated to my God, who has given me life and strength to complete this work. In addition, I dedicate this to my family, who has been supporting me in every possible way.

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LIST OF ABREVIATIONS AND SYMBOLS

AN	Acid number
ANOVA	Analysis of variance
Conc.	Concentration
FAME	Fatty acid methyl esters
FID	Flame ionization detector
FFA	Free fatty acids
GC	Gas chromatography
HCl	Hydrochloric acid
ICP - OES	Inductive coupled plasma – optical emission spectrometry
mEq O_2/kg	Milliequivalent of oxygen per kilogram
M.a.s.l	Meters above sea level
MUFA	Monounsaturated fatty acids
N	Number of samples
PV	Peroxide value
PUFA	Polyunsaturated fatty acids
R^2	R – Squared
SFA	Saturated fatty acids
NaOH	Sodium hydroxide
SD	Standard deviation

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Nutrient deficiency in sub-Saharan Africa remains a challenge despite local and international institutions' efforts (Bain *et al*., 2013; Ecker *et al*., 2010). Although nutrient-rich sources are available, utilization and nutrient optimization in different climatic conditions are still challenging (Mbwana *et al*., 2017). Oyster nuts (*Telfairia pedata* Hook) is one of the under-utilized oilseed plants of the family *Cucurbitaceae* in sub-Saharan Africa (Ajayi, 2015; Asiegbu, 1987). These nuts are grown for their valuable edible nuts, which are consumed fresh, roasted, ground, or added as thickeners in vegetables, soups, or meat dishes (Ajayi, 2015; Musalima *et al.*, 2019). The flour made from oyster nut seeds is supplemented in baked products, while its pressed oil is used for cooking by local communities (Minzangi *et al.*, 2015; Okoli & Nyananyo, 1988). Its vines are drought resistant, have low cultivation input requirements, and high yield per area (Agatemor, 2006; Ajayi, 2015; Garrity, 2004; Musila, 2018). Additionally, due to their lactogenic and medicinal properties, oyster nuts are used by local pregnant and lactating mothers to increase milk production and fast healing after delivery (Ajayi *et al.,* 1990; Kazadi *et al.*, 2015). Despite these benefits, the nuts' nutritional composition has not been adequately quantified, limiting their utilization and contribution to health among rural communities (Musila, 2018).

Moreover, oilseeds are affected by internal and external factors that result in variations in their nutrient compositions (Ayerza & Coates, 2011; D'Imperio *et al.*, 2007; Vollmann *et al*., 2007). Under constant agro-ecological conditions, different cultivars show variations in nutrient contents, seed size, weight, and agricultural performances (Borges *et al., 2007;* Vollmann *et al.*, 2007). Likewise, farming practices and agro-climatic conditions such as temperature, rainfall, precipitations, and geographical sites affect plants' physiological reactions, causing variations in nutrient compositions (D'Imperio *et al.,* 2007; Romero *et al.,* 2016). Due to existing differences in soil composition and agro-climatic conditions in different geographical sites used for cultivation, the same oilseeds cultivars were reported to contain nutrients in different proportions (Borges *et al.,* 2007; D'Imperio *et al.,* 2007; Romero *et al.,* 2016). Additionally, oyster nuts are mostly grown in agroforests of high hills and mountains at 800 to 2000 m.a.s.l elevations ranges (Ajayi, 2015; Okoli, 2007). These differences in elevation range cause variations in temperature and precipitations. D'Imperio *et al.* (2007) and Hemp (2006) reported

a decline in temperature with a lapse rate of 0.56 ℃ or 1 ℃ for every 100 m upwards, while the precipitation rates were also decreasing from low to high elevation levels. As a result, deviations in temperature and precipitations might affect enzymatic kinetics in plants resulting in variations of nutrient contents such as linoleic acid, oleic acid, protein, and fat saturation in oilseeds (Izquierdo *et al.,* 2006). Therefore, there is a need to identify and characterize agro-climatic conditions to produce high-quality nutrient sources.

Oil quality and stability are also affected by variations in fatty acid compositions and antioxidant contents (Casal *et al.*, 2010; Velasco & Dobarganes, 2002). Despite their nutritional benefits, polyunsaturated fatty acids (PUFA) are more prone to oxidative deterioration during storage and high heat cooking (Zhang *et al*., 2010). The double bond structure in linoleic and alpha-linoleic acids (PUFAs) is unstable. It readily reacts with singlet oxygen forming oxidative by-products such as free radicals and hydroperoxides aldehydes, causing detrimental health effects to consumers (Bozan & Temelli, 2008). Therefore, for long-time storage and high heat applications, oilseeds with high monounsaturated fatty acids (MUFA) contents are preferred over oilseeds with high PUFA contents.

1.2 Statement of the problem

Nutritional composition in oyster nuts has not been adequately studied and quantified, despite being wide consumed by pregnant and lactating mothers in northern Tanzania (Ajayi, 2015). For generations, oyster nuts have formed an essential traditional and heritage food for local tribes in the North of Tanzania and East Africa (Ajayi, 2015; Musila, 2018; Okoli & Nyananyo, 1988). Various nutritional and medicinal properties associated with oyster nuts have been locally claimed with no scientific proof (Musila, 2018). Hence, the quantification and establishment of nutritional contents in oyster nuts will enhance informed food choices and local communities' consumption.

Furthermore, oyster nuts are grown in diverse agro-ecological conditions in agroforests and mountain ranges, which affects their Physico-chemical quality (Ajayi *et al.*, 1990). Differences in elevational ranges result in variations in agro-climatic parameters such as temperature, rainfall, and precipitations in growing sites (Ayerza & Coates, 2011; D'Imperio *et al*., 2007; Hemp, 2006). Consequently, nutrient composition and oil quality are affected. Therefore, a study on the performance of different sites and elevational ranges will enhance the optimization of oyster nuts quality by selecting locations with optimum conditions.

Finally, despite being the main product from oyster nuts seeds, oyster nut oil quality and stability are unknown (Musalima *et al.,* 2019). High PUFA and low antioxidants contents have been reported to affect the stability of oils (Casal *et al.*, 2010; Frega *et al.*, 1999; Zhang *et al.*, 2010). Hence, a comparison study on oyster nut oil stability compared to other common vegetable oils can provide insights into oyster nut oil's quality and stability.

1.3 Rationale of the study

This study's results and findings will provide nutritional information about oyster nuts related to their health benefits. This will enhance informed food choices and consumption among local communities in northern Tanzania and East Africa. This study will also provide insights into suitable agro-climatic conditions for producing and improving protein and omega-6 contents in oyster nuts. Elevational ranges associated with high or low nutrient contents have to be stipulated to optimize beneficial nutrients. Furthermore, a comparison of oyster nut's oil stability will enable proper utilization and enhancement of oyster nut oil stability.

1.4 Objectives of the study

1.4.1 General objective

To determine variations in nutrient composition and oil stability of oyster nuts (*Telfairia pedata*) across different agro-ecological conditions in Northern Tanzania.

1.4.2 Specific objectives

(i) To identify the nutrient composition and quantify nutrient contents of oyster nuts from Northern Tanzania.

(ii) To evaluate existing variations in nutrient contents in samples from different sites and elevations in the North of Tanzania.

(iii) To compare unrefined oyster nuts oil's quality and stability to unrefined flaxseed oil, unrefined, and refined sunflower oil at room temperature.

1.5 Hypotheses

(i) Oyster nuts contain a highly beneficial nutritional profile.

(ii) Nutrient contents in oyster nuts will differ across different sites and elevations.

(iii) Oyster nut oil will display low oxidative stability at room temperature conditions than flaxseed oil and sunflower oil due to high PUFA contents.

1.6 Significance of the study

Despite their nutritional and medicinal benefits, oyster nuts are rarely utilized and are among the disappearing species (Ajayi, 2015). This research will promote and enhance consumption and utilization of oyster nuts in the communities. Utilization of oyster nut as an altenative crop will enable income generation to the farmers and improve their livelihoods. In addition, the community health including childrens, pregnant and lactating mothers will be improved through nutritional benefits obtained from consumption of oyster nuts.

1.7 Delineation of the study

This study analyzed nutrient contents in oyster nuts across different sites and elevation levels. Oyster nut samples were collected in the agro-forests of the Usambara, Meru, and Kilimanjaro mountains at different elevation levels from 800 to 2000 m.a.s.l. Samples were analyzed for proximate, fatty acid, and mineral contents using AOAC, (1990) methods, gas chromatography, and ICP-OES, respectively. Elevation levels with optimum cultivation conditions for nutrients optimization were highlighted and recommended for more cultivation. For the oil stability study, oyster nut oil was compared to unrefined flaxseed oil, crude, and refined sunflower oil by measuring PV and FFA levels in 6 weeks using titration methods. The stability of oyster nut oil at room conditions was highlighted, and improvement methods were recommended.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual framework

The study assumption is that, different elevation levels and sites (regions) have different agro-climatic conditions such as temperature, rainfall, humidity, sunlight and precipitations. Variations in agro-climatic conditions in growing areas results in differences in nutrient compositions and quality of oyster nuts. Consequently, the nutrient composition also affects the keeping quality of the crude oysternuts oil.

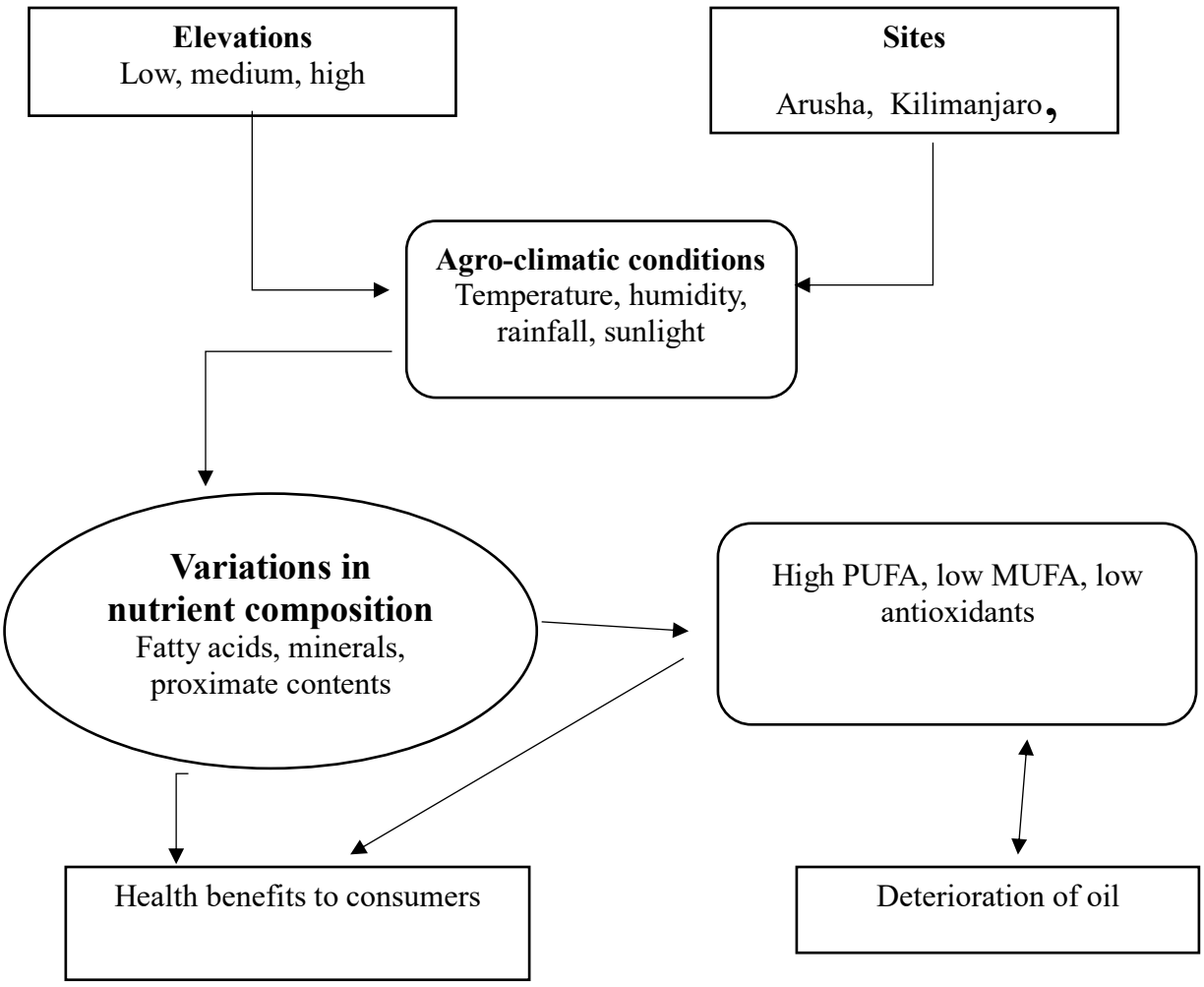

Figure 1: The conceptual framework showing the effects of sites and elevation levels on the agro-climatic conditions of growing areas which then affects the nutrient compostion of the oysternuts

2.1 Oysternuts (*Telfairia pedata*)

2.1.1 Origin, diversity, and utilization

Oysternuts (*Telfairia pedata*), similarly referred to as Kweme or Zanzibar oil vines are a dioecious African liana from genus *Telfairia* in the family Cucurbitaceae (Achu *et al.*, 2005; Ajayi *et al.*, 1990; Okoli & Nyananyo, 1988). The genus *Telfairia* is comprised of two members; *Telfairia occidentalis* (fluted pumpkin) and *Telfairia pedata* (Oyster nuts). Oyster nuts are native to mainland Tanzania and Mozambique but, are cultivated in South Africa, Malawi, Zambia, Ethiopia, Rwanda, Kenya, and Uganda. Fluted pumpkin is famously cultivated for its nutritious leaves in west Africa, especially in Guinea, Ghana, Nigeria, and Cameroon (Achu *et al.*, 2005; Ajayi, 2015; Kazadi, 2015; Okoli, 2007).

Oyster nuts are found in diverse ecological and agro-climatic conditions on lowlands and highlands of up to 2000 m.a.s.l with an annual rainfall of 1000 mm or more (Ajayi, 2015). The oyster nuts grown on higher elevation sites are claimed to produce more yield than those on lower elevation (Gardens, 2018). Oyster nuts produce a high yield per area (150 nuts in one gourd), are drought resistant, and can thrive well on drained medium loam soils (Ajayi *et al.*, 1990; Okoli, 2007). In Tanzania, oyster nuts are cultivated along with coffee trees and banana plantations in the country's northeast parts. They are also found growing in home gardens on the edges of the agroforests of Pare, Meru, Kilimanjaro, and the Usambara mountains (Ajayi, 2015).

Oyster nuts are cultivated for valuable and nutritious nuts found within a large gourd in the agroforests (Okoli, 2007). According to Ajayi *et al.* (1990) and Okoli (2007), one gourd can contain 70-140 nuts, protected with net-like outer covering. The nut’s kennels are 3-4 cm in diameter and contain a high amount of oil used by local communities in East Africa. The taste of oyster nuts resembles Brazilian nuts and almonds (Ajayi *et al.*, 1990). The extracted oil is used for multiple purposes such as cosmetics, candles, soap making, and household cooking. The ground oilseeds are added to vegetables, soups, meat dishes, or consumed fresh. Its flour is supplemented in baking products to increase protein contents and improve taste (Ajayi *et al.*, 1990; Kazadi, 2015; Okoli & Nyananyo, 1988).

Oyster nuts form an essential medicinal, traditional, and cultural heritage food in East Africa (Kazadi *et al.*, 2015). Oyster nuts are given to nursing mothers and pregnant women to improve lactation due to their lactogenic properties, enhancing milk secretion (Ajayi, 2015; Okoli, 1988). The local tribes in northern Tanzania use oyster nuts as a tonic for fast healing and recovery after childbirth (Ajayi, 2015). In East Africa, the oil is used to treat rheumatism and stomach

ailments. (Kayode & Kayode, 2011). Hence, oyster nuts have potential food and industrial applications to improve health and income for communities in Sub-Saharan Africa.

2.2 Variations of nutrients in oilseeds

2.2.1 The effects of temperature on nutrient quality

Oil quality is determined by the amount and type of fatty acids compositions (Ayerza & Coates, 2011). Fatty acid saturation in oilseeds depends on specific enzymatic activities. These enzymatic activities depend on temperature to reach maximum or minimum catalysis levels. During flowering and fruit filling stages, the optimum temperature for enzymatic activity lead to maximum conversions and saturations of beneficial fatty acids (Borges *et al*., 2007; Mannina *et al.*, 2001). Izquierdo *et al.* (2006) observed a linear relationship between temperature and oleic acid saturation in hybrid sunflowers (*Helianthus annuus* L.), with a steady increment in oleic acid saturation with increasing night temperatures. The oleic acid contents were maximum at 22℃, which was identified as the optimum temperature for oleate saturates enzymes associated with the synthesis of oleic acids. Besides, fatty acid composition in other oilseeds such as Chia (*Salvia hispanica* L.), camelina seeds (*Helianthus annuus* L.), and chestnuts (*Castanea sativa* L.) have all been reported to be affected by temperature regimes of growing locations (Ayerza & Coates, 2011; Borges *et al*., 2007; Vollmann *et al.,* 2007). Hence, despite the internal factors such as genotypes and cultivars, the temperature has been the main factor for variations of fatty acid contents in oilseeds.

These reflect the possibility of optimizing valuable fatty acid contents by cultivating oyster nuts and other oilseeds in locations with optimum temperature. This can be benefit consumers' assurance of consuming high-quality seeds containing all expected nutrients in the right amounts. Essential fatty acids such as linoleic acids (omega-6) and alpha-linolenic acid (omega-3) can be optimized and maintained in a good ratio of 1:2 for maintenance of good health (Abedi & Sahari, 2014).

2.2.2 Variations of nutrient contents in different cultivars

Under constant ecological conditions, different cultivars contain different nutrient compositions (Vollmann *et al.,* 2007). Genetic variations among cultivars result in deviations in seed performance and nutritional contents. Seeds with more weight, high yield, beneficial fatty acids (oleic acids, linolenic acids (omega-3)), and high-quality protein contents are often selected for breeding (Borges *et al*., 2007). Genetic breeding is, therefore, aiming at improving seed quality

by optimizing desirable characteristics. In a study by Borges *et al*. (2007) investigated the variations of nutrient contents in different chestnut cultivars indicated significant variations in fatty acid contents. Cultivars with more beneficial fatty acids such as oleic acids were selected and promoted for use. Despite differences due to cultivars, growing sites' ecological conditions also may contribute to significant variations in crude fat and fatty acid contents. Hence, similar cultivars should be applied during the determination of effects caused by environmental factors. These can prevent interference since both factors have a significant impact on chemical compositions.

2.2.3 Effects of different geographical sites on nutrient composition

Variations in nutrient compositions in oilseeds from different geographical locations have been reported (Ayerza & Coates, 2011; Borges *et al.*, 2007; D'Imperio *et al*., 2007). In most cases, existing variations are due to variations in environmental parameters such as temperature, sunlight intensity, precipitations, and soil components (Wruss *et al.*, 2015). Depending on the type of oilseed or crop, the effects of environmental factors can be desirable or undesirable. These reflect on the need to characterize nutrient contents according to geographical locations, facilitating high-quality oilseeds production (Borges *et al.*, 2007; D'Imperio *et al*., 2007). Nutritional variations were exhibited in virgin olive oils (*Olea europea*) from North, center, and Southern geographical positions in Lazio, Italy by D'Imperio *et al*. (2007). Specifically, these variations were detected in linolenic acids, β-sitosterol, oleic acid, and squalene contents. Variations were also reported from different areas and elevation levels using different irrigation practices. These highlight the effects of geographical locations on nutrient compositions in oilseeds.

The influence of geographical positions on olive oil compositions was also reported by Romero *et al.* (2016). Geographical positions were found to influence phenolic compounds than cultivars and ripening periods in samples from Limari valley and Molina sites in Chile (Romero *et al.*, 2016). These variations were caused by differences in temperature and irrigation practices of particular geographical locations. These indicate geographical areas' potential in discriminating nutritional composition in oilseeds when other factors are kept constant.

2.2.4 Effects of elevations on nutrient composition

Different elevation levels are associated with variations in environmental factors such as temperature, rainfall, precipitation, humidity, and soil compositions (Hemp, 2006). Variations of these environmental factors have caused, different elevation levels to exhibit different

chemical compositions within similar cultivars (Ayerza & Coates, 2011; D'Imperio *et al.*, 2007). Changes in temperature and precipitations were reported in a study conducted on slopes of Mount Kilimanjaro and Usambara by Hemp (2006). The study intended to investigate climatic conditions' variations due to changes in elevations from the bottom to the mountains' top. The temperature declined at a rate of 0.56 ℃ per every 100 m upwards. These were from the bottom of 800 m with 23 ℃ to 5000 m with -7 ℃ on top. Due to evaporation rates, as temperature decreases, precipitation increased from bottom to top. These results reflect the expected variations in nutritional contents in oyster nuts from samples collected at different elevations along ridges of Mount Kilimanjaro, Usambara, and Meru.

Differences in elevation levels were also reported to cause variations in linoleic acid, squalene, β-sitosterol, and oleic acids in olive oil samples from Lazio (Mannina *et al.*, 2001). The difference of 100 m to 400 m was enough to cause significant variations in nutritional contents. These indicate the strength of elevation levels in discrimination of nutrient contents compared to cultivars, agricultural practices, sowing seasons, ripening periods, harvest season, and physiological maturation (Ayerza and Coates, 2011; D'Imperio *et al.*, 2007; Romero *et al.*, 2016). As pointed out by Ayerza and Coates (2011), variations in chemical composition in chia seed from different elevations can help determine the seeds' origin. Hence, elevations are an essential factor in optimizing beneficial nutritional contents in oyster nuts and other oilseeds.

2.2.5 Other factors causing variations in nutrient compositions

Soil mineral concentrations, agricultural practices, sunlight, harvest period, and physiological maturation are other factors for variation of nutrient compositions (Borges *et al.*, 2007; Junior *et al.*, 2017; Romero *et al.*, 2016; Wruss *et al.*, 2015). Mineral composition in oilseeds has been affected by soil mineral concentrations (Kabata-pendias, 2004). The uptake of minerals from the soil to plants depends on soil mineral concentration and soil pH, which influences the transfer gradient (Romero *et al.*, 2016). Selenium concentration in Brazilian nuts (*Bertholletia excels*) was reported to depend directly on soil pH and mineral concentration (Junior *et al.*, 2017). High selenium concentration in the soil led to more selenium concentrations in the nuts; vice versa was true for soil pH where high soil pH affected uptake of minerals leading to lower selenium concentrations. These reflect the importance of soil mineral richness to plant physiology.

Precipitation and rainfall regimes are the other potential contributors to chemical variations in oilseeds (Mannina *et al.*, 2001; Nenadis *et al.*, 2015). Physiological activities in plant cells and

tissues require a balance in electrolytes and transfer medium for nutrients. High precipitation and rainfall result in a balance in electrolytes and physiological activities, causing increased secretion of essential molecules and nutrients (D'Imperio *et al.*, 2007). Nenadis *et al.* (2015) reported reduced phenolic compounds and antioxidant activity on *Arbutus unedo* plants due to reduced precipitation. This highlights the importance of water to physiological activities in plants. Agricultural practices such as irrigation can result in a high concentration of critical biological molecules and healthy plants (Nenadis *et al.,* 2015). Hence, the precipitation and rainfall regimes are likely causes for variations in nutrient contents in oilseeds.

2.3 Oil stability

During storage, oils are susceptible to oxidative or enzymatic deterioration, resulting in chemical, sensory, and nutritional changes (Abdalla & Roozen, 1999; Casal *et al.,* 2010; Hasiewicz-Derkacz *et al.*, 2015). These changes are undesirable and result in detrimental health effects to consumers, such as developing cardiovascular diseases and cancer due to the formation of free radicals (Hasiewicz-Derkacz *et al.*, 2015). Additionally, rancid oil is associated with off odors that signal a deterioration of oil or oil-containing foods (Zhang *et al.*, 2010). Deterioration of fats is also associated with depleting essential nutrients such as antioxidants (Bozan & Temelli, 2008). Stable oils such as virgin olive oils have a long shelf life and retain their nutritional and organoleptic properties (Casal *et al.,* 2010). Therefore, determination of oil stability and shelf life is important before it can be stored.

2.3.1 Internal factors affecting oil stability

Oil structures contain three fatty acids (FA) attached to the glycerol molecule making a triglyceride (Abuzaytoun & Shahidi, 2006). These polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) are more susceptible to oxidation. This is due to the reactive double bond structure. During the oxidation process, singlet oxygen or free radicals tend to attack the double bonds resulting in alkyl peroxides and water molecules (Casal *et al.,* 2010). High amount of PUFA such as linoleic acids and alpha-linolenic fatty acids results in more oxidation reactions resulting in the deterioration of fats. In a study by Casal *et al.* (2010) to determine olive oil changes under deep-frying conditions of up to 170 °C, fatty acid contents correlated with oxidation indication parameters. More deterioration was observed in oil with high PUFA contents compared to MUFA and SFA. Free fatty acids (FFA) are also more prone to oxidation reactions than triglycerides (Zhang *et al.*, 2010). The neutralization process during oil refining reduces the amount of FFA making oils more stable.

The amount of antioxidants also affects oils' stability (Bozan & Temelli, 2008; Casal *et al.*, 2010; Hasiewicz-Derkacz *et al.*, 2015). Antioxidants are bioactive compounds found naturally or added to oils that act as scavengers for free radicals (Hasiewicz-Derkacz *et al.*, 2015). Stable oils contain vitamin E (tocopherols), carotenoids, sterols, and phenolic compounds that donate electrons to reactive free radicals to neutralize and form stable compounds (Velasco & Dobarganes, 2002). For these reasons, the amount of antioxidants affects oils' stability, and more commercial antioxidants such as tocopherols, β-carotenes, and quercetin are added to enhance oils stability (Łukaszewicz *et al.*, 2004).

2.3.2 External factors affecting oil stability

Apart from internal factors (FA, enzymes, metals, phospholipids, and antioxidants), deterioration rates are accelerated by external factors such as temperature, oxygen, and light (Velasco & Dobarganes, 2002). Temperature affects reaction kinetics for the oxidation process, causing a high amount of peroxide end products. Under high temperatures, antioxidant activity is affected, and the autoxidation process is accelerated (Zhang *et al.*, 2010). In a study by Casal *et al.* (2010) determining olive oils' stability compared to vegetable oils under frying conditions (170 °C), olive oil was more stable than vegetable oils. Despite having a high amount of vitamin E, vegetable oils contain a high amount of PUFA compared to olive oils, hence at high-temperature antioxidant activity is reduced, and high deterioration rates occur. This highlights the suitability of low PUFA oils for high-temperature deep-frying.

Oxygen also accelerates rates of oil deterioration (Velasco & Dobarganes, 2002). Under room temperature conditions, oxygen dissolves in oil and forms more reactive singlet oxygen, readily reacting with fatty acid double bonds forming alkyl peroxides. The oxidation process is more rapid in the presence of oxygen; hence, oxygen is replaced by nitrogen during the packaging of oil and oil-containing food (Zhang *et al.*, 2010).

Absorption of light energy by electrons in outermost orbitals in oil pigments results in excitation and oxidation (Abuzaytoun & Shahidi, 2006). This results in the formation of free radicals, which accelerates further oil oxidation. These pigments (chlorophyll), which cause photosensitization, are removed during the refining process making oil more stable (Velasco & Dobarganes, 2002). Hence, to prevent further oxidation, oils are stored under dark conditions or in amber bottles.

2.3.3 Methods for evaluation of oil stability

Various methods are used to determine oil stability (Łukaszewicz *et al.*, 2004). These methods are used to evaluate the extent of oil deterioration by measuring indicative parameters. Factors such as percentage of free fatty acids (FFA), peroxide value (PV), amounts of β-carotene, type of fatty acids, and specific extinction coefficient are widely used. Others are changes in the number of phenols, tocopherols, and oxidative stability (Velasco & Dobarganes, 2002). In most cases, these parameters are determined simultaneously to enable precision in the evaluation of oil stability.

Free fatty acids (FFA) are long-chain fatty acids detached from triglycerides molecules (Bozan & Temelli, 2008). These FFA is indicative of the extent of deterioration of oils and has been reported to be more prone to oxidation than triglycerides chains (Frega *et al.*, 1999). Though the oil's acidity is associated with microbial stability, high free fatty acids result in high deterioration rates (Bozan & Temelli, 2008). During oil refining, the neutralization process reduces the FFA contents making the oil more stable. Hence, FFA contents in oil are an essential parameter for the measurement of oil deterioration.

Peroxide value (PV) is another critical parameter that gives the number of hydroperoxides present in oil (Casal *et al.*, 2010). The oxidation process involves three stages, which are initiation, propagation, and termination stages. In the initial stage, the double bonds are attacked by singlet oxygen or other radical compounds resulting in the formation of alkyl peroxy radicals, which further propagates the oxidation process. The process involves forming other polymeric and volatile end products such as hydrocarbons, ketones, and aldehydes. At the termination, stage peroxides are formed along with water molecules (Abuzaytoun & Shahidi, 2006; Casal *et al.*, 2010; Velasco & Dobarganes, 2002). Hence, the determination of peroxide value indicates the extent of deterioration and the storage period. The oils which have been stored for more extended periods are expected to have high peroxide values depending on the type of fat. According to European Union standards, virgin oils should have PV values below 20 mEq O_2/kg, 10 mEq O_2/kg for vegetable oils, and 15 mEq O_2/kg for refined oils (Casal *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

The study was conducted in northern Tanzania, where oyster nuts are mostly grown (Fig. 2). Three oysternut growing sites (Kilimanjaro, Tanga, and Arusha) were purposefully selected as described in Appendix 2. In Kilimanjaro, samples were collected along agroforests of Mount Kilimanjaro in Moshi (Uru 1, Uru 2, Materuni, Materuni kwa Helman, and Marangu 1, Marangu 2) from 800 up to 2000 m.a.s.l. In Tanga, the study was conducted along agroforests of the Usambara Mountains located in Korogwe district (Fune kiwandani, Kwekibomi, Kwashemshi, Kwamanoro, Bungu village, and Msiga village) from 900 up to 2000 m.a.s.l. Arusha region, samples were collected along edges of agroforests of mount Meru located in Arumeru disctrict (Nkoaranga kwa sumari, Nkoaranga-2, Songolo, Sura, Nshing'a, and Sura kibaoni) from 900 to 2000 m.a.s.l. These sites were selected along mountain edges from bottom to top because they span different elevations (m.a.s.l) associated with variations in climatic factors such as temperature,rainfall, and precipitations regimes (Hemp, 2006).

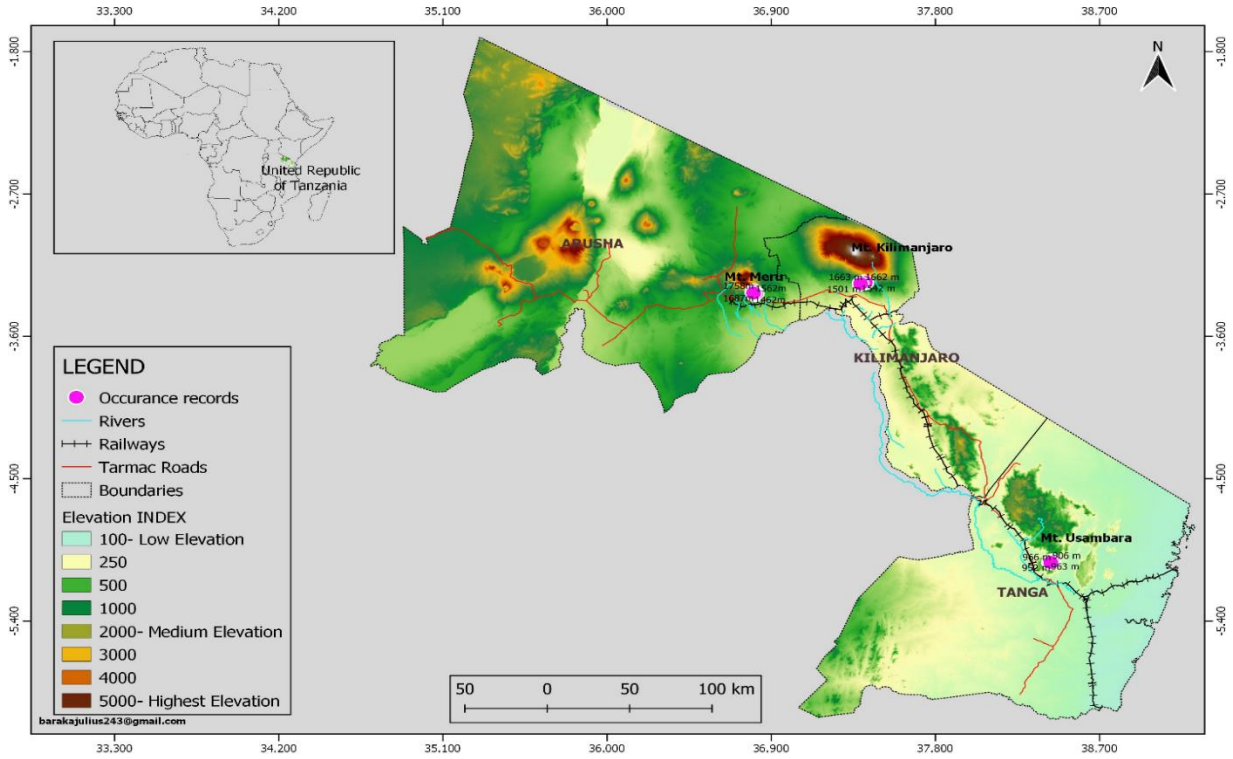

Figure 2: Map showing study sites in three regions of Arusha (agro-forests of mount Meru), Kilimanjaro (agro-forests of Mount Kilimanjaro), and Tanga (agro-forests of Usambara mountains) in northern Tanzania

3.2 Sample collection

Oyster nut samples were collected in May, June, and July 2019, during harvest season in Kilimanjaro, Arusha, and Tanga. Only naturally grown *Telfairia pedata* species ewere used in this study to prevent nutrient variations due to differences in spcies or farming practices. At each site, growing areas were allocated into three fields based on elevational ranges categorized into "low elevation" (800-1200 m.a.s.l), "medium-elevation" (1200-1600 m.a.s.l), and "high elevation" (1600-2000 m.a.s.l). Six samples were collected from each site (region), as described in Table 1. Samples were cleaned, sun-dried as described by Musalima *et al.* (2019), and transported to the laboratory for analysis.

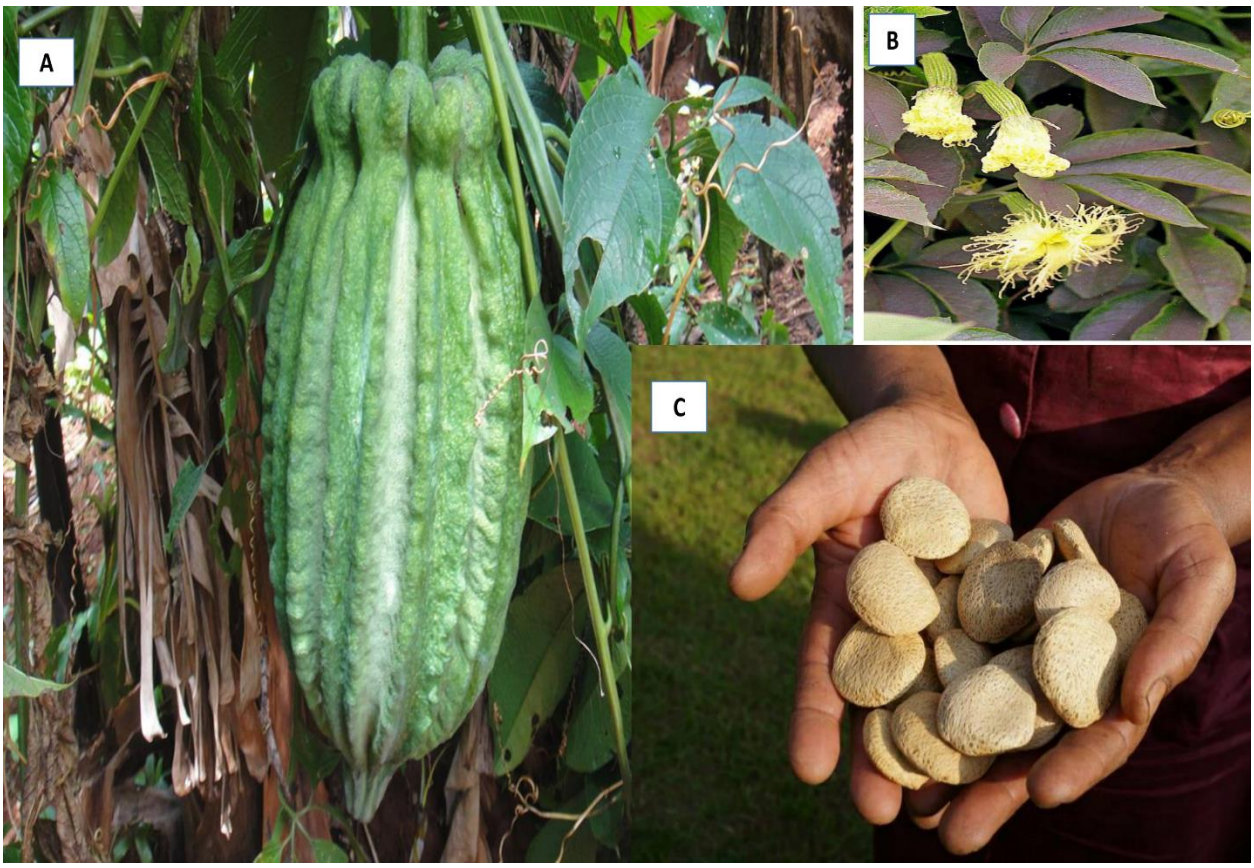

Plate 1: Oyster nut showing; "A" oyster nut gourd, "B" oyster nut plant leaves and flowers, "C" dried oyster nut seeds

Table 1: Description of collection sites and their environmental conditions (i.e., average annual temperature range, average annual rainfall range, and elevational gradient) in the year 2019 in northern Tanzania

Region	Number of samples	Environmental conditions		
		Temperature (°C)	Rainfall (mm)	Elevation (m.a.s.l)
Arusha	6	17 - 20	800 - 1200	900 – 1600
Kilimanjaro	6	21 - 27	200 – 2300	800 - 2400
Tanga	6	30 - 32	1100 - 1400	700 – 1200

(TMA, 2018)

3.3 Samples for comparison of oil stability

The stability of unrefined oyster nut oil was compared with the stability of unrefined flaxseed oil (*Linum usitatissimum*, L.) and two brands of sunflower (*Helianthus annuus*) vegetable oil (refined and unrefined). Oyster nut oil was extracted directly from oyster nut kernels using a laboratory oil extractor (scheler w85, deniz scheler, Germany). The other three oil samples (Nativ Leinol, Osana, and nativ Osana) were bought from the local market in Wels, Austria. Each oil sample was stored in a 500 ml amber bottle in the dark at room temperature of 27 °C for six weeks. Peroxide value (PV) and free fatty acid (FFA) contents were evaluated after every 7 days for 6 weeks.

3.4 Chemical analyses

3.4.1 Proximate analyses

Oysternut samples from individual plants were prepared by removing the fibrous outer shell and splitting the inner shell to obtain the kernels (Fig. 2). Each sample was ground to flour using a blender (krups KB4351, Germany) and stored at 4 °C before analysis (Nielsen, 2014).

(i) Moisture contents

Moisture contents were determined according to Yerlikaya *et al.* (2012) by drying 5 g of each sample in a hot-air oven (Cascade Tek CTF112, USA) at 105 °C overnight to reach the constant weight. The following formular was used to calculate moisture contents.

$$\%\ Moisture = \frac{(A - B) * 100}{\mathrm{A}}$$

Where; A = Weight of the fresh sample (g), B = Weight of dry sample (g), % Dry matter = 100-% moisture.

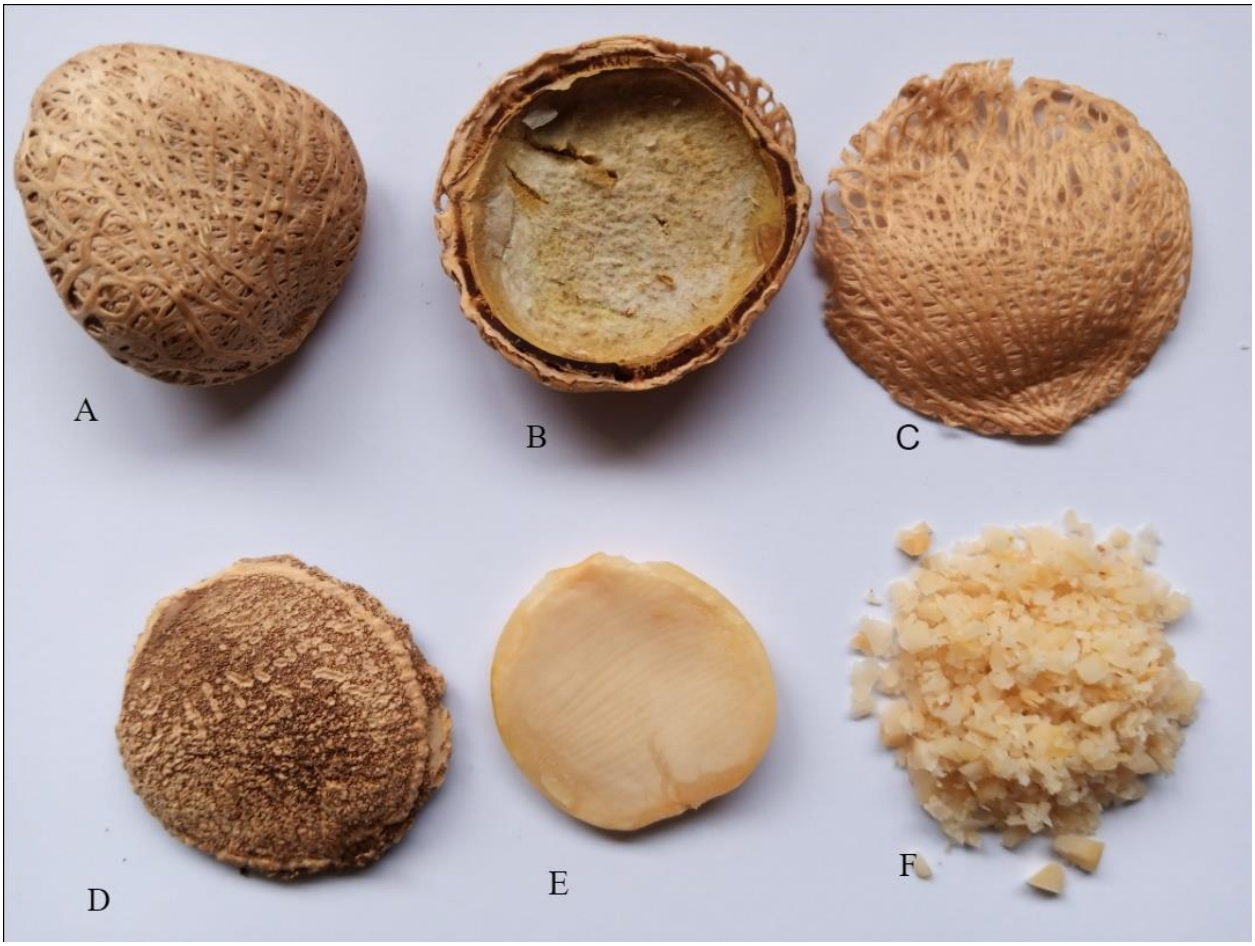

Figure 3: The oyster nut (Telfairia pedata) showing (A) closed oyster nut seed, (B)opened shell, (C) fibrous outer shell, (D) inner shell, (E) kernel after being split in half, (F) ground oyster nut kernel

(ii) Ash contents

Ash content was determined according to AOAC (1990) method no 950.49. The empty crucibles were prepared by being burned in a muffle furnance (Tmax-1750DD, China) at 500 ℃ for 5h to reach a constant weight and then put in the desiccator to cool the temperature. Then, 5 g of the sample was poured into crucibles and put in the oven overnight at 500 ℃ to burn all the organic matter, and the inorganic ashes were weighed after being cooled in a desiccator (Nielsen, 2014).

$$\%\ ash = \frac{weight\ of\ ash\ (g)}{\text{weight of dry samples (g)}} * 100$$

(iii) Crude fat contents

The crude fat content of oyster nuts samples was determined according to AOAC (1990) method no 934.50. Crude fat was determined using the Soxhlet apparatus. First, the 250 mL round-bottomed flask was prepared by being dried in the oven (Cascade Tek CTF112, USA) for 1 hour at 105 ℃ before being weighed and introduced into the Soxhlet setup. Afterward, 15 g of a fat-free thimble sample was subjected to continuous extraction with petroleum ether (AppliChem GmbH, Germany) for 6 hrs at 120 ℃ heating plate. Then, the solvent was recovered, and the oil sample in 250 ml round-bottomed flask was dried in the oven (Cascade Tek CTF112, USA) at 105 ℃ for 3 hrs. Dry oil was cooled in a desiccator and weighed. Crude fat content was determined using the following formula.

$$\% \text{ crude fat} = \frac{\text{weight of crude fat (g)} * 100\%}{\text{weight of the dry sample (g)}}$$

(iv) Crude protein contents

Crude protein content was determined by using the Kjeldahl method, AOAC 981.10 (AOAC, 1990). First, 0.5 g of oyster nut samples in the digestion tube was added with 1 Kjeldahl tablet (Sigma-Aldrich, USA), 20 mL of H_2SO_4 (95%) (AppliChem GmbH, Germany), spatula of stearic acid (AppliChem GmbH, Germany), and five boiling stones (Sigma-Aldrich, USA). The tube was then heated for digestion (10 min x 40 watts, 20 min x 80 watts, 40 min x 100 watts, and 10 min for 40 watts). After digestion, the tube was cooled for 30 minutes. Then, distillation was done by Kjeldahl system (Hanon K9860, China) using 35% NaOH (AppliChem GmbH, Germany) set at 80 mLs for 7 minutes with 3% H_3BO_3 (AppliChem GmbH, Germany). The resulting solution was titrated with 0.1 HCl (AppliChem GmbH, Germany) in the burette.

$$\% \text{ N} = \frac{14.01 \text{ (titrer volume} - \text{blank volume) conc. of acid}}{\text{sample wt x } 10}$$

The factor of 5.4 was used to determine the protein percent from the nitrogen value for oily samples (AOAC, 1990).

Carbohydrates were calculated according to Grosso *et al*. (2000) as the difference of other contents from 100%. All analyses were done in triplicates.

3.4.2 Mineral analyses

Samples were prepared as described by Saracoglu *et al*. (2007), and Xie *et al*. (2013) using the dry ashing digestion procedure with few modifications. All the reagents were prepared from analytical grade chemicals and double-distilled deionized water was employed. All the equipments were washed using 4 mol/L hydrochloric acids and rinsed with deionized water before being used. First, 2 g of sample material in a porcelain container was burned in a muffle furnace oven (Tmax-1750DD, China) set at 500 °C for six hrs. Then, 5 mL of 4 mol/L hydrochloric acids (AppliChem GmbH, Germany) was added, followed by distilled water and filtered. For dilution, 1 mL of the sample was added in a tube of 1 mL of 4 mol/L hydrochloric acids (AppliChem GmbH, Germany) and dissolved in 8 mL distilled water to make a 10 mL dilution. The mineral analysis was done using pre-calibrated ICP-OES (model Icap7200 Duo, Thermo Fisher, Waltham, USA), equipped with an auto-sampler (Teledyne ASX-280, Omaha, NE 68144, USA). Magnesium (Mg), potassium (K), calcium (Ca), copper (Cu), iron (Fe), zinc (Zn), phosphorus (P), and sodium (Na) were analyszed. All analyses were done in triplicates. For calibration procedures, standard solutions of analytes were prepared by dissolving a stock of 1000 mg/L of all analyzed elements to generate a calibration curve.

3.4.3 Fatty acids analyses

Fatty acid methyl esters (FAME) were prepared as described by Teh and Birch, (2013) where, 10 mg of dry oyster nut oil sample was methylated and suspended with a 5 mL mixture of acetyl chloride (Sigma-Aldrich, USA) and methanol (AppliChem GmbH, Germany) in the ratio of 1:50. The mixture was left for 4 hrs at 60 ℃. The reaction was stopped by adding 2.5 mL of 0.6 g/mL sodium carbonate (AppliChem GmbH, Germany). Fatty acid methyl esters were extracted by 2 mL hexane (AppliChem GmbH, Germany) and 1 mL of the upper clear phase transferred to a GC vial. The appropriately diluted hexane extract was inserted in a thermo-trace gas chromatography (thermo Elecron Corp, Italy) equipped with an auto-sampler (AS, 2000). The detection was carried out with a flame ionization detector (FID) (Agilent Tech Inc., Wilmington, DE, USA). The chromatographic conditions were composed of an injection volume of 2 µL, and the injector temperature 240 ℃. Helium was applied as carrier gas with 120 kPa constant pressure and a flow of 30 mL/min. while, the Agilent capillary column DB23 60 m, 0.25 mm ID and, film thickness of 0.25 µm were used for analytical separation. The oven temperature gradient was set at 0-3 minutes at 130 °C, then 6.5 °C per minute to 170 °C, followed by 2.8 °C per minute to 215 °C, and then was maintained for 10 minutes. Finally, 3 °C per minute to 240 °C and maintained for 15 minutes. The FID was set at a temperature of 280 °C and an airflow

of 450 mL/min with hydrogen flow of 45 mL/min and makeup gas of nitrogen held at 40 mL/min (Gao *et al.*, 2015). Data were analyzed using Chrom card data system version 2.8 from Thermo Finnigan (Fig. 4). Each oyster nut oil sample was analyzed in duplicates. For calibration, an external standard method was used where standard solutions (Agilent Tech Inc, USA) with known analyte concentrations and fixed volume were prepared and injected in the column. The calibration plot was prepared and used to determine the concentration of the fatty acids in oyster nut samples.

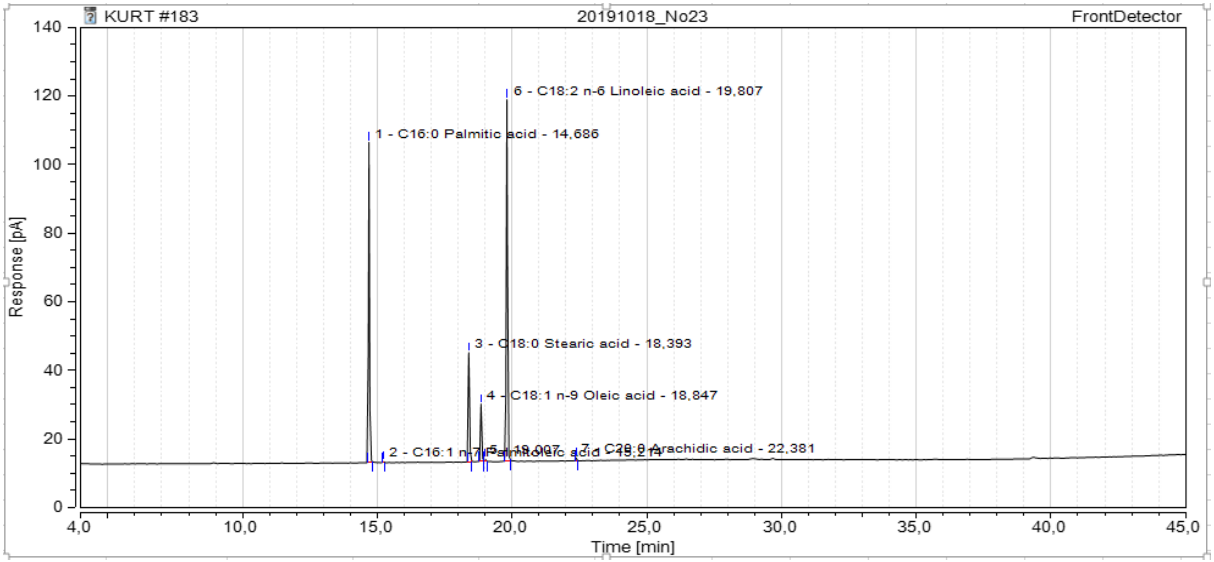

Figure 4: A chromatogram showing response (pA) against time (min) for the fatty acids analyses using GC-FID

3.4.4 Oyster nut's oil stability

(i) Peroxide value

Peroxide value was analyzed as described by Casal *et al*. (2010) where 5 g of oil samples were measured in an Erlenmeyer flask and dissolve the sample by adding 25 mL of the 3 volumes of glacial acetic acid (Fresenius GmbH, Austria) and 2 volumes of chloroform (AppliChem GmbH, Germany) solvent mixture. Then, 0.5 mL of the saturated potassium iodide (AppliChem GmbH, Germany) solution was pipetted to the sample and swirled on a magnetic mixer (IKA RCT, Germany) for precisely 60 seconds. Then, the solution was diluted with 25 mL of distilled water to stop the reaction. Then 1 mL of 1% starch solution (Fresenius GmbH, Austria) was added, followed by titration using the 0.01 sodium thiosulfate solution (AppliChem GmbH, Germany) in the burette until the blue color disappears.

$$PN = \frac{(a\ -\ b) * c * 1000}{E}$$

Where a = total consumption of sodium thiosulphate standard solution, b = consumption of sodium thiosulphate in a blind test, c = Concentration of used sodium thiosulphate solution, E = lipid weight.

(ii) The acid number and free fatty acids

The FFA was analyzed according to Frega *et al.* (1999), where 15 g of the oil sample was weighed into an Erlenmeyer flask and dissolved in 50 mL (1 volume ethanol + 1 volume toluene) solvent mixture (AppliChem GmbH, Germany). Few drops of the phenolphthalein indicator solution (Fresenius GmbH, Austria) were added then, titrated with the potassium hydroxide (AppliChem GmbH, Germany) standard solution until the titration endpoint (permanent red coloring).

$$AN = \frac{a * c * 56.1}{E}$$

Where, a = consumption (mL) of potassium hydroxide solution, c = concentration of the potassium hydroxide solution (mol/L), E = lipid weight, 56.1 = molar mass (g/moL) potassium hydroxide.

$$FFA\% = AN * \frac{M(FS) * 100}{56.1 * 1000}$$

Where, M (FS) = Molar mass of free fatty acids = 282 g/moL.

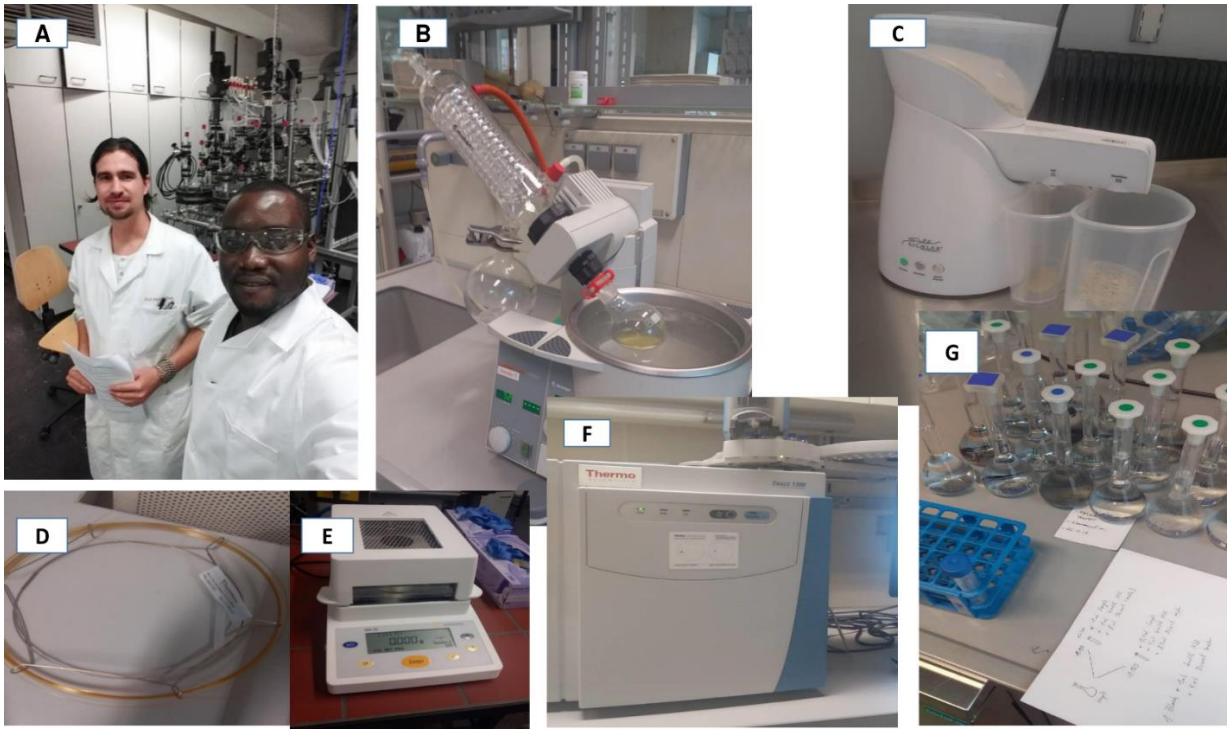

Plate 2: Laboratory analyses showing "A" researcher during analyses, "B" Petroleum ether recovery during the analysis of fat, "C" oyster nut oil cold extraction using Sichler extractor, "D" gas chromatography column used for methyl esters separation, "E" rapid moisture analyzer, "F" gas chromatograph used for methyl esters analysis, "G" 10 mL volumetric flasks during mineral analyses

3.5 Statistical analysis

Two-way analysis of variance (ANOVA) was used to compute the effects of elevations, categorized into "low elevation" (800-1200 m.a.s.l), "mid-elevation" (1200-1600 m.a.s.l), and "high elevation" (1600-2000 m.a.s.l), the three growing sites, and their interactions on nutrient compositions (proximate, minerals, and fatty acid contents) in oyster nuts (Romero *et al.*, 2016).

T-test was used to compare oyster nut oil's stability to flaxseed oil, refined, and unrefined sunflower oils (Zhang *et al.*, 2010). Person correlation was used to establish the effects of storage time on the peroxide value (PV) and Free fatty acid (FFA) contents in the four oil samples (Casal *et al.*, 2010). Additionally, data diagnostic tests such as the normality test (using Shapiro-Wilk) were run before analysis (Angelini, 2018). Post-hoc tests using Turkeys' multiple comparison tests were done to compare means. The criterion for significance was set at $p < 0.05$. All analyses were run using R-software Version 3.3.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate contents (fat, protein, ash, and carbohydrates)

Descriptive statistics for proximate results for oyster nuts indicated that fat was the most abundant content, followed by protein, carbohydrates, and ash contents on a dry matter basis (Table 2). Protein was the second most abundant content ranging from 23.1% to 25.3% while, ash and carbohydrates were the least abundant contents with 2.5% and 4.4% averages respectively. The energy contribution by consumption of 100 g of oysternuts is expected to be 690 to 719 callories with most from fat contents.

4.1.2 Fatty acid contents

Fatty acid content analysis revealed that the polyunsaturated fatty acid (PUFA), made of linoleic acid (C18:2 n-6), was with 47% the most abundant component (Table 3). Saturated fatty acids (SFAs) were 43%, mainly composed of palmitic acid and stearic acid (Table 3). Another fatty acid of interest was oleic acid (C18:1 n-9), which constituted low amounts of monounsaturated fatty acids (MUFA). Other important fatty acid such as linolenic acid (omega-3) was not detected during fatty acid analysis.

4.1.3 Minerals contents

Trace element analysis found that magnesium (Mg) and phosphorus (P) contents were about four times higher than other mineral elements such as potassium (K), Calcium (Ca), and Iron (Fe) (Table 4). Magnesium and phosphorus had averages of 172 mg/100 g and 179 mg/100 g respectively. Potassium (K), calcium (Ca), and iron (Fe) contained in averages of 16 mg/100 g, 14 mg/100 g, and 12 mg/100 g respectively. Other mineral elements such as selenium (Se), sodium (Na), Manganese (Mn), zinc (Zn), and copper (Cu) were found in minimum amounts ranging from 0.5 mg/100 g to 4 mg/100 g.

4.1.4 Variations of nutrient contents across different sites

There was no significant difference in all proximate contents, including fat ($F_{2, 9}$ = 3.98, P = 0.072) and protein contents ($F_{2, 9}$ = 0.71, P = 0.527) across sites. Still, there were slightly higher protein and fat contents in the Tanga region than Kilimanjaro and Arusha (Table 2). Ash and

carbohydrates trends were slightly but not significantly higher in Arusha compared to Kilimanjaro and Tanga regions ($F_{2,9} = 1.72$, $P = 0.25$ and $F_{2,9} = 1.34$, $P = 0.68$, respectively; Table 2). Furthermore, determination of oil quality across sites showed that oleic acid (C18:1 n-9) was slightly higher in Tanga compared to Kilimanjaro and Arusha ($F_{2,9} = 5.91$, $P = 0.035$; Table 3) while linoleic acid (C18:2 n-6) was not significantly different across sites ($F_{2,9} = 0.47$, $P = 0.668$).

In addition, the most abundant minerals magnesium (Mg) and phosphorus (P) were not significantly different across sites ($F_{2,9} = 0.16$, $P = 0.854$ and $F_{2,9} = 1.54$, $P = 0.287$, respectively; Table 4).

4.1.5 Variations of nutrient contents across different elevation levels

In contrast, ash, protein, and fat compositions significantly differed across elevations. Both protein ($F_{2,9} = 69.38$, $P = 0.001$) and fat ($F_{2,9} = 24.75$, $P = 0.002$) contents were significantly lower in higher elevations compared to lower elevation levels while the opposite was true for ash contents ($F_{2,9} = 28.13$, $P = 0.001$; Fig. 2). Moreover, oil quality based on fatty acid contents differed significantly only for linoleic acid ($F_{2,9} = 51.31$, $P < 0.001$) by increasing in high elevation levels (Fig. 2). Other fatty acid contents found in oysternuts were not significantly different across elevations.

Table 2: Average (± SD) of oyster nut ash, fat, protein, carbohydrate, and energy contents across different sites in northern Tanzania collected in the year 2019

Sites	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Energy (kcal)
Arusha	3.0 ± 0.1^a	63.2 ± 1.5^a	23.1 ± 1.9^a	7.1 ± 3.6^a	690.1 ± 23.1^a
Kilimanjaro	2.8 ± 0.1^a	66.4 ± 2.6^a	22.8 ± 1.2^a	4.8 ± 3.9^a	707.9 ± 19.2^a
Tanga	2.3 ± 0.3^a	68.1 ± 1.2^a	25.3 ± 1.0^a	1.4 ± 1.6^a	719.2 ± 17.6^a

Different letters in superscript indicate significantly different means within columns (sites) by Turkey's multiple comparison test at $P = 0.05$.

Table 3: Average (± SD) of fatty acid contents of oyster nuts across different sites from samples collected in June – August 2019 in northern Tanzania

Fatty acids (%)	Sites		
	Arusha	Kilimanjaro	Tanga
Linoleic acid (C18:2 n-6)	49.8 ± 2.4[a]	48.1 ± 0.5[a]	45.7 ± 0.2[a]
Palmitic acid (C16:0)	32.7 ± 1.5[a]	33.5 ± 0.5[a]	32.4 ± 1.3[a]
Stearic acid (C18:0)	9.8 ± 1.8[a]	9.5 ± 0.6[a]	11.4 ± 2.1[a]
Oleic acid (C18:1 n-9)	7.6 ± 1.1[a]	7.8 ± 0.7[a]	8.1 ± 1.1[b]
Palmitoleic acid (C16:1 n-7)	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0[a]
Myristic acid (C14:0)	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
Arachidic acid (C20:0)	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.0[a]
Saturated fatty acids (SFAs)	43.1 ± 1.2[a]	43.4 ± 0.7[a]	44.3 ± 1.1[a]
Mono-unsaturated fatty acid (MUFAs)	7.7 ± 1.1[a]	8.0 ± 0.7[a]	8.3 ± 1.0[a]
Poly-unsaturated fatty acids (PUFAs)	49.8 ± 2.4[a]	48.1 ± 0.5[a]	45.7 ± 0.2[b]

Different letters in superscript indicate significantly different means within rows by Turkey's multiple comparison test at $P = 0.05$.

Table 4: Average (± SD) of mineral contents in oyster nuts across different sites in northern Tanzania collected from June – August 2019

Minerals (mg/100 g)	Sites		
	Arusha	Kilimanjaro	Tanga
Magnesium (Mg)	220.8 ± 80.1[a]	210.9 ± 50.9[a]	94.7 ± 21.2[a]
Phosphorus (P)	205.2 ± 35.1[a]	202.5 ± 26.6[a]	131.7 ± 65.2[a]
Potassium (K)	17.4 ± 4.8[a]	18.0 ± 2.9[a]	16.2 ± 3.1[a]
Calcium (Ca)	19.9 ± 5.1[a]	12.3 ± .3[a]	9.9 ± 2.8[a]
Manganese (Mn)	0.2 ± 0.05[a]	0.1 ± 0.05[a]	0.1 ± 0.04[a]
Iron (Fe)	12.9 ± 1.0[a]	12.2 ± 1.2[a]	11.1 ± 1.0[a]
Copper (Cu)	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.5 ± 0.1[a]
Zinc (Zn)	1.4 ± 0.5[a]	1.2 ± 0.6[a]	1.3 ± 0.4[a]
Selenium (Se)	0.01 ± 0.0[a]	0.01 ± 0.0[a]	0.01 ± 0.0[a]
Sodium (Na)	4.3 ± 0.9[a]	4.2 ± 2.2[a]	4.2 ± 3.6[a]

Different letters in superscript indicate significant differences within rows by Turkey's multiple comparison test at $P = 0.05$.

Only magnesium (Mg) contents increased from low to high elevations ($F_{2, 9} = 85.50$, $P < 0.001$; Fig. 5) while phosphorus (P) was higher in lower elevations ($F_{2, 9} = 41.29$, $P < 0.001$). Other mineral contents were not significantly different across different elevations. The interaction between sites and elevation was not significantly different in most cases except for Phosphorus contents ($F_{2, 9} = 11.01$, $P = 0.009$).

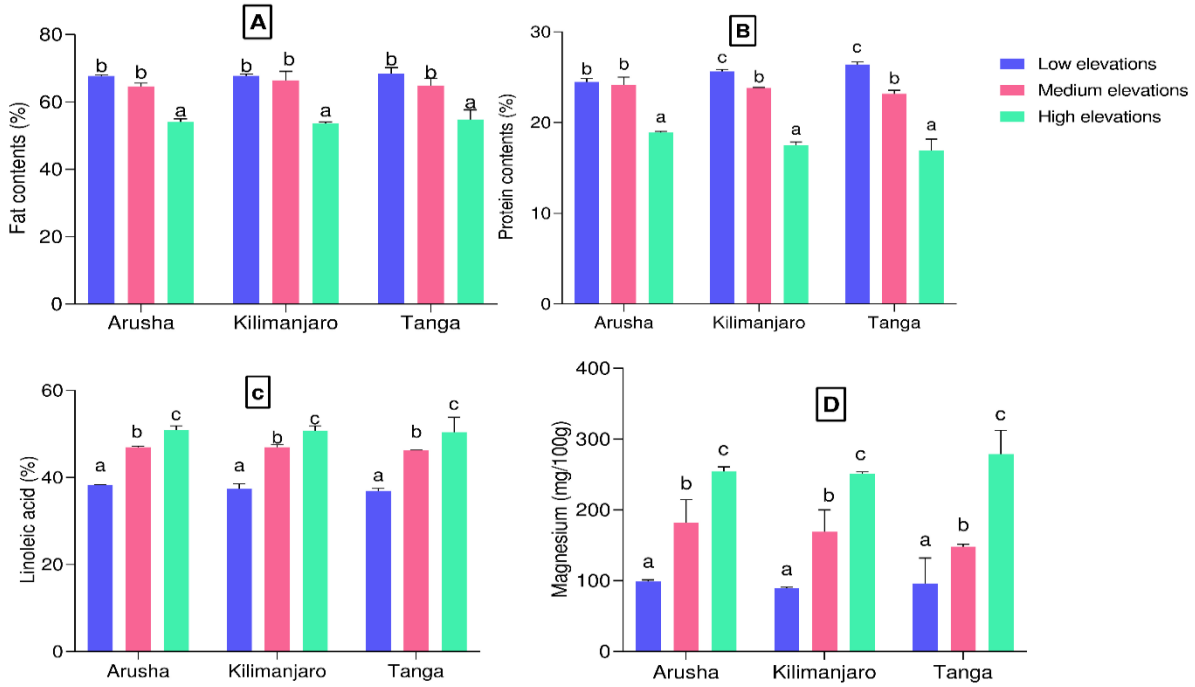

Figure 5: Bar graphs showing average (±SD) for variations of nutrient compositions (A) fat, (B) protein, (C) linoleic acid, and (D) magnesium in oysternuts across different sites and elevation levels collected in northern Tanzania in 2019. Different letters above column groups of three indicate significant difference across elevations. "Low" = low elevation (800-1200 m.a.s.l), "medium" = medium elevation (1200-1600 m.a.s.l), and "high" = high elevation (1600-2000 m.a.s.l). N = 18

4.1.6 Oil stability

Peroxides and free fatty acid contents significantly correlated with time in all four oil samples (Table 5). Oxidation rates increased with time in all oil samples (Fig. 6). Unrefined oyster nut and flaxseed oil recorded higher FFA contents, by an increase of 0.13% and 0.9%, compared to 0.04% and 0.02% for unrefined and refined sunflower oils during the six weeks. The peroxide values (PV) increased by 4.35 mEq O2/kg in oyster nut oil sample while, flaxseed oil, unrefined, and refined sunflower oils recorded 7.88 mEq O2/kg, 1.61 mEq O2/kg, and 1.13 increase respectively.

No significance difference was observed in average produced FFA contents of oyster nut compared to flaxseed oil ($p = 0.362$), unrefined sunflower oil ($p = 0.220$) or refined sunflower oil ($p = 0.142$) in six weeks. Additionally, oyster nut produced significantly higher peroxide values PV compared to unrefined sunflower ($p = 0.034$) and refined sunflower oil ($p = 0.021$). There was no significant difference in the amount of PV produced by oyster nuts oil compared to flaxseed oil ($p = 0.269$).

Table 5: Correlation results of peroxide value (PV) and free fatty acid (FFA) for unrefined oyster nut oil, unrefined flaxseed oil, unrefined sunflower oil, and refined sunflower oil with time (weeks)

	Peroxide value (PV) in mEq O_2/kg							
	Oysternut		Flaxseed		U-sunflower		R-sunflower	
Time (weeks)	R^2	P	R^2	P	R^2	P	R^2	P
6	0.99	< 0.001	0.93	0.001	0.87	0.006	0.82	0.013
	Free fatty acids (FFA) in %							
	Oysternut		Flaxseed		U-sunflower		R-sunflower	
Time (weeks)	R^2	P	R^2	P	R^2	P	R^2	P
6	0.87	0.006	0.70	0.037	0.93	0.002	0.92	0.002

Oysternut = unrefined oyster nut oil, flaxseed = unrefined flaxseed oil, U-sunflower = unrefined sunflower oil, and R-sunflower = refined sunflower oil.

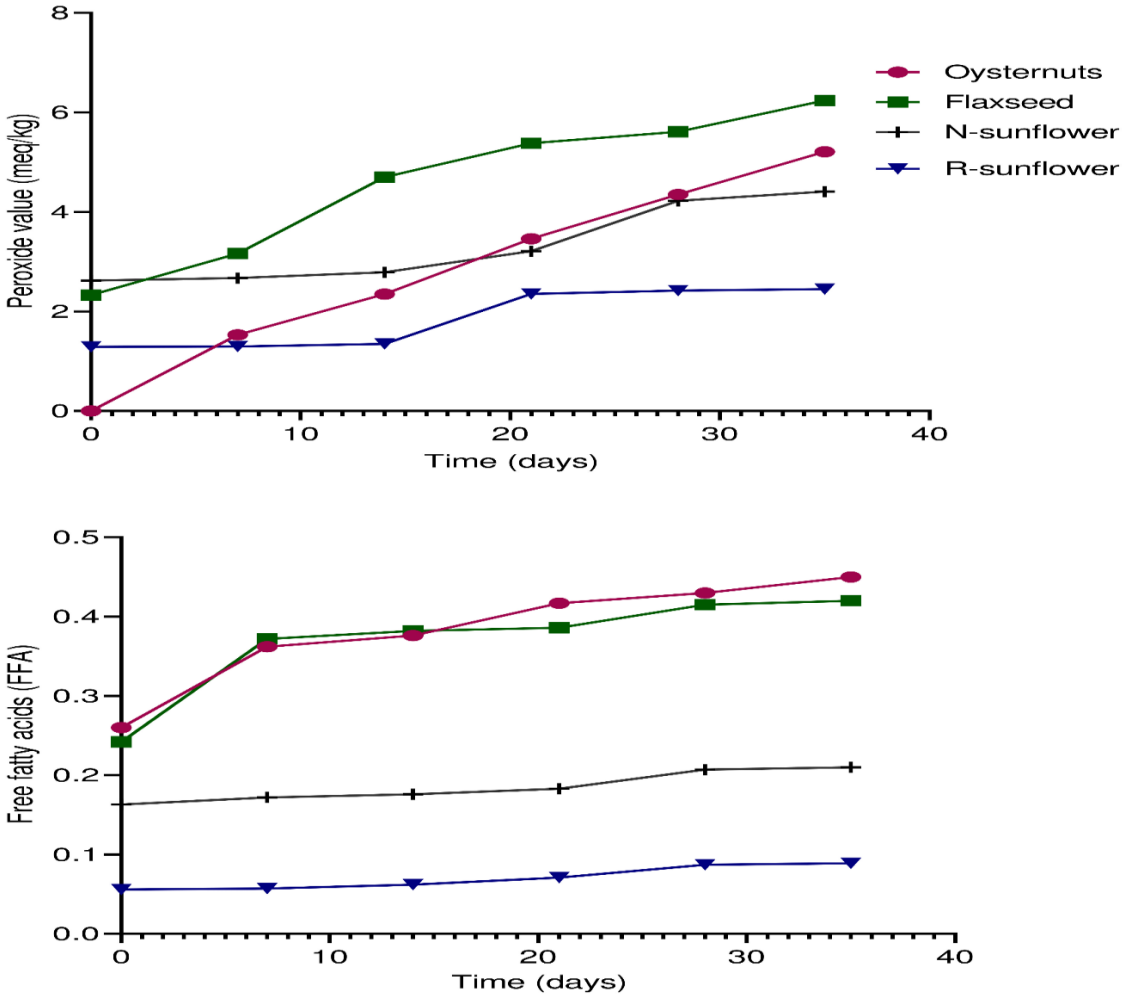

Figure 6: Line graphs showing variations in peroxide value (PV) and free fatty acid (FFA) of unrefined oyster nut oil, unrefined flaxseed oil, unrefined sunflower oil, and refined sunflower oil samples in six weeks storage period

4.2 Discussion

4.2.1 Proximate contents

According to the results, oyster nuts can be regarded as a good source of nutrients. Oyster nut contained about 25% proteins (Table 2), which was equal to the amount reported in peanuts (*Arachis hypogaea*) (25%) and even higher than that in cashew nuts (*Anacardium occidentale*) (21%), walnuts (*Juglans* spp.) (17%), and sesame seeds (*Sesamum indicum*) (19%) (USDA, 2015). According to WHO (2010), pregnant and lactating women have a recommended daily allowance (RDA) for the protein of 1.1 g/kg/day. This large proportion can be acquired through oyster nuts' consumption. Additionally, oyster nuts contained high amounts of fat (68%), mostly used by local communities for food, cooking, hair, and skin tonic (Ajayi *et al*., 1990).

4.2.2 Fatty acid contents

Fat in oyster nuts contains high MUFA and PUFA, which are highly beneficial for improving health. These can also negatively affect the oil's stability and shelf life since polyunsaturated fatty acids, including linoleic acids, are unstable when exposed to heat, light, and oxygen (Borges *et al*., 2007). The quality of the oil is determined by fatty acid composition (Izquierdo *et al.*, 2006). Oyster nuts contained monounsaturated fatty acids (MUFA) composed of 8.1% oleic acids **(**C18:1 n-9), which contributes to the stability of the oil and reduces the onset of cardiovascular diseases to consumers (Abedi & Sahari, 2014). Additionally, oyster nut oil contained about 47% linoleic acid (C18:2 n-6), an essential polyunsaturated fatty acid. A ratio of 1:2 for alpha-linolenic acid (C18:3 omega-3) and linoleic acid (C18:2 omega-6) contents have been reported to be vital for maintaining good health as they can reduce rates of inflammation and cardiovascular diseases when consumed (Abedi & Sahari, 2014; Gibson *et al.*, 2011; Salter, 2013; Simopoulos, 2011).

4.2.3 Minerals contents

Oysternuts contained high amount of magnesium and phosphorus, which are essential for maintaining muscle and bone strength, boosting the immune system, and aiding in energy production (Fender, 2014). The recommended daily intake for magnesium to maintain body health is 310 – 420 mg (King *et al*., 2005), which can be attained by consuming about 150 g of oyster nuts per day. According to Ajayi (2015), unshelled oyster nuts have a shelf life of up to eight years. They also have rich nutrient composition, simple and low input requirements during cultivation as fertilization is not required. With these factors, Oyster nuts can be used as an

altenative source of minerals in food formulations. In addition, oysternuts can, replace other common oilseeds such as peanuts, which are highly susceptible to microbial infestations and aflatoxins, often due to storage difficulty (Blesa *et al.*, 2003).

4.2.4 Variations of nutrients across different sites

Generally, genotypes and cultivars have been shown to affect oilseeds' chemical contents (Borges *et al*., 2007; Vollmann *et al.*, 2007). However, several studies have recently identified that agro-climatic conditions and location can also affect oilseeds' chemical contents (Ayerza & Coates, 2011; D'Imperio *et al*., 2007). In this study, only oleic acid showed variations based on sites. Oleic acid was higher in samples from western sites (Kilimanjaro and Arusha) than eastern sites (Tanga). Existing variations are probably due to differences in growing conditions, with western sites experiencing slightly lower annual temperatures and rainfall conditions (Thornton *et al.*, 2009). Hence, the westernmost sites seemed most beneficial for attaining high oleic acid content, which has more health and storage benefits (Łukaszewicz *et al.*, 2004). Similar observations were reported in the protein and fat composition of chestnuts (*Castanea sativa* L.), where variations in nutrients were found across different sites rather than elevations and seeds origin (Borges *et al*., 2007).

4.2.5 Variations in protein and fats contents across different elevation levels

Protein and fat contents declined in higher elevations, which indicates that lower elevations might be preferred for optimization of protein contents. Differences in elevations are associated with changes in environmental conditions such as temperature and precipitation (Hemp, 2006). Due to variations of these environmental factors, different elevation levels have been reported to exhibit different chemical compositions within similar cultivars (Ayerza & Coates, 2011; D'Imperio *et al*., 2007). Changes in temperature and precipitations were reported in a study conducted on slopes of Mount Kilimanjaro and Usambara by Hemp (2006). These study intended to investigate climatic conditions' variations due to changes in elevations from the bottom to the mountains' top (Hemp, 2006). The temperature was found to decrease by 0.56 ℃ per 100 m.a.s.l upwards. These changes were from the bottom 800 m.a.s.l with 23 ℃ to the top 5000 m.a.s.l with -7 ℃ (Hemp, 2006). Due to differences in evaporation rates as temperature decreases, precipitation increased from bottom to top. These results reflect on existing variations in nutritional contents in oyster nuts from samples collected at different elevations along the edges of Mount Kilimanjaro, Usambara, and mount Meru. In similar studies, variations of fat contents in chestnuts were associated with differences in elevation, temperature, and soil

composition. These factors affect rates of enzyme reactions causing desaturation or saturation of fat contents (Ayerza & Coates, 2011; Borges *et al.*, 2007). Hence, high protein and fat contents optimization in oyster nuts can be achieved in lower elevation levels with comparatively higher temperatures.

4.2.6 Variations in fatty acid contents across different elevations

In fatty acid contents, the significant increase of linoleic acids from lower to higher elevations agrees with studies by Ayerza and Coates (2011); D'Imperio *et al.* (2007), and Izquierdo *et al.* (2006), who identified temperature as the main factor for the determination of fatty acid compositions. The observed differences might reflect desaturase enzyme activity for oyster nuts, responsible for converting palmitic acid, oleic acid, and stearic acid to linoleic acid. Furthermore, fatty acids are synthesized within plastids containing 16-18 carbon atoms, then transported to the cytosol, where desaturation occurs causing linoleic and linolenic acid formation (Łukaszewicz *et al*., 2004). This is supported by a study on hybrid sunflowers (*Helianthus annuus* L.) by Izquierdo *et al.* (2002), where enzymatic activity catalyzing oleic acid desaturations were found to be higher in cooler night temperature than in high day temperatures (Izquierdo *et al*., 2006). The same variations were also reported in chia seeds (*Salvia hispanica* L.) by Ayerza and Coates (2011), where linoleic acids were found to increase in higher elevations. Besides, Vollmann *et al*. (2007) also described temperature as the main factor for fatty acid variations in camelina seeds (*Camelina sativa* L), pointing out the significance of optimum temperature for enzymatic activity.

High elevations receive more annual precipitations (You *et al*., 2010). Hence, high amounts of fatty acid and mineral contents in oyster nut samples from high elevations might also be associated with the generally higher annual precipitation in higher elevations apart from temperature (Hemp, 2006; Winiger, 2017). Similarly, Nenadis *et al.* (2015) reported reduced phenolic compounds and antioxidant activity on *Arbutus unedo* plants due to reduced precipitation, emphasizing the importance of high precipitation to plant nutrient contents and physiology. Hence, the optimization of omega-6 fatty acids in oyster nut oily can be achieved by cultivating at high precipitation sites in high elevations or irrigation methods.

4.2.7 Variations in mineral contents across different elevations

Mineral analyses showed variations in magnesium (Mg) and phosphorus (P) contents, which increased in high elevations. These variations agree with studies on selenium concentration in Brazilian nuts (*Bertholletia excels*) by Junior *et al.* (2017). However, plants' uptake of mineral

elements also depends on soil mineral concentration and soil acidity levels (Kabata-pendias, 2004; Romero *et al*., 2016). Hence, these variations could be contributed by differences in mineral concentrations in different elevations. High mineral contents in oyster nut samples from high elevations also indicate mineral-rich soil in the top of volcanic mountains such as Kilimanjaro (Hemp, 2006). Hence, additional studies should also focus on the contribution of soil mineral concentrations and pH to oyster nut mineral compositions.

4.2.8 Comparison of oyster nut's oil stability

Auto oxidation of oil occurs when oil is stored for a certain period, resulting in hydroperoxides, aldehydes, and hydrolysis of triglycerides (Casal *et al.*, 2010). Deterioration of oil affects the organoleptic and nutritional characteristics of oils and makes oil un-safe for consumption. The stability of oil depends on its structural composition (the type of fatty acids), antioxidant contents (tocopherol and phenolic), and storage conditions (oxygen, light, temperature) (Frega *et al.*, 1999; Hasiewicz-Derkacz *et al*., 2015; Łukaszewicz *et al*., 2004; Zhang *et al*., 2010). In this study, PV and FFA contents in all four samples were positively correlated with time. The steady increment of these parameters was expected at room temperature (23 ℃), but the extent of deterioration was expected to differ depending on each oil's nature and contents. The samples were stored at room temperature in an amber bottle and closed with a tight lid to prevent extra oxygen from penetrating, but still, PV and FFA increased with time. These indicate the importance of effective methods to avoid deterioration, such as adding more vitamin E and replacing oxygen with nitrogen (Frega *et al.*, 1999).

Due to high temperature, enzymatic activities, or high moisture contents in oil, hydrolysis of triglycerides occurs due to the detachment of fatty acid tails from glycerol molecule resulting in the formation of FFAs (Velasco & Dobarganes, 2002). The FFAs are highly susceptible to oxidation; hence, they indicate deterioration in oils (Casal *et al.*, 2010). During the six-week storage period, all four oil samples showed no significant difference in the amount of produced FFA. Still, the trends indicated high FFA values in unrefined oyster nut oil and flaxseed oil. The FFA contents are highly reduced by saponification during the refining process, so FFA contents were in low amounts in refined sunflower oil compared to oyster nut and flaxseed oil. High FFA contents in flaxseed oil were also reported by Łukaszewicz *et al*. (2004). Flaxseed oil from different cultivars was highly susceptible to deterioration except for samples with added antioxidants. Although crude oyster nut oil was freshly prepared, its FFA contents were higher than crude sunflower oil and flaxseed oil, indicating instability of oyster nut oil.

Peroxide value (PV) is a popular oil oxidation indicator that reflects on the formed hydroperoxide compounds (Zhang *et al.*, 2010). In this study, the PV for oyster nut oil was not significantly different from that of flaxseed oil. Existing similarities might cause this. Both oil samples were unrefined and more prone to oxidation. Additionally, both oil samples contain high polyunsaturated fatty acids (PUFA), which are more prone to oxidation. According to our results, oyster nut oil contains 47% linoleic acid (PUFA) and only 8% oleic acid (MUFA), while; flaxseed oil contains 55% alpha-linolenic acid, 15% linoleic acid (PUFA), and only 16% oleic acid (MUFA) (Goyal *et al.*, 2014). Flaxseed oil and oyster nut oil were more prone to oxidation due to high PUFA and low MUFA contents, vital for oil stability.

Oyster nut oil produced significantly higher amounts of peroxides compared to sunflower oil samples. Both refined and unrefined sunflower oil samples exhibited more stability compared to oyster nut oil. Again, the existing differences in instability are due to fatty acid contents. As reported by Zhang *et al*. (2010), sunflower oil is high in MUFA contents at 57.34% while linoleic acid (PUFA) is only 28.97% compared to 47% PUFA contents in oyster nuts. Likewise, sunflower oil contains 41.08 mg/100 g vitamin E compared to 2.03 mg/100 g vitamin E, 23.34 mg/100 g vitamin A, and 2.68 mg/100 g beta-carotenes reported in oyster nut oil by Musalima *et al*. (2019). These results reflect on the implications of high PUFA contents in oyster nut oil stability and highlight the need for refining and antioxidants supplementation during long-time storage.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

We conclude that oyster nuts are a rich source of nutrients, contributing to communities' health and social-economic benefits due to the nut's rich nutritional profile. In particular, high protein, fat, linoleic acid, and oleic acid compositions make oyster nuts a potential alternative to common oilseeds used in different food formulations. Additionally, elevation levels in cultivation areas are an essential factor for improving nutrient compositions in oyster nuts. Cultivation of oyster nut in lower elevation sites improves protein contents while reducing linoleic acid (PUFA) and increasing oleic acid (MUFA). Furthermore, crude oysternut oil is unstable during storage which was expected due to high PUFA contents which are relatively easy to oxidize during storage. Due to high protein and omega-6 fatty acid contents, it is highlighted that oysternuts can be incorporated in improving health and livilihoods in Tanzania.

5.2 Recommendations

Oyster nut nutritional quality is affected by agro-climatic conditions; hence, attention is required during the selection of cultivation sites. Protein contents should be optimized by cultivating oyster nuts on low elevation sites. At the same time, oil quality might be improved by balancing oleic acid (MUFA) and omega-6 contents at low elevation sites with high temperatures and high precipitations. Additionally, due to instability of oyster nuts oil during storage, utilization of oyster nuts should not be limited to its oil but rather ground flour which is a good source of protein and fatty acids.

REFFERENCES

Abdalla, A. E., & Roozen, J. P. (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chemistry*, *64*(3), 323–329. https:// doi. org/10. 1016/S0308-8146(98)00112-5

Abedi, E., & Sahari, M. A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition*, *2*(5), 443–463. https://doi.org/10.1002/fsn3.121

Abuzaytoun, R., & Shahidi, F. (2006). Oxidative stability of flax and hemp oils. *JAOCS, Journal of the American Oil Chemists' Society*, *83*(10), 855–861. https:// doi. org/10.1007/s11746-006-5037-7

Achu, M. B., Fokou, E., Tchiégang, C., Fotso, M., & Mbiapo, F. (2005). Nutritive value of some Cucurbitaceae oilseeds from different regions in Cameroon. *African journal of biotechnology*, 4(11), 1329–1334.

Agatemor, C. (2006). Studies of selected physicochemical properties of fluted pumpkin (Telfairia occidentalis Hook F.) seed oil and Tropical almond (*Terminalia catappia* L.) seed oil. *Pakistan Journal of Nutrition*, *5*(4), 306–307. https:// doi. org/10. 3923/pjn. 2006. 306. 307

Ajayi, S. A., Dulloo, M. E, Vodouhe, R. S., Berjak, P., & Kioko, J. I. (2015). *Conservation status of Telfairia spp in sub-Saharan Africa*. http:// www. un. org/ sustainabledevelopment/ sustainable-development-goals/

Angelini, C. (2018). Regression analysis. *Encyclopedia of Bioinformatics and Computational Biology: Bioinformatics*, *1–3*, 722–730. https://doi.org/10.1016/B978-0-12-809633-8.20360-9

Asiegbu, J. E. (1987). Some biochemical evaluation of fluted pumpkin seed. *Journal of the Science of Food and Agriculture*, *40*(2), 151–155. https:// doi.org/ 10.1002/jsfa. 2740400207

Ayerza, R., & Coates, W. (2011). Protein content , oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L). *Industrial Crops and Products 34*, 1366–1371. https:// doi. org/10. 1016/j. indcrop. 2010.

12.007

Bain, L. E., Awah, P. K., Geraldine, N., Kindong, N. P., Sigal, Y., Bernard, N., & Tanjeko, A. T. (2013). Malnutrition in Sub - Saharan Africa: Burden, causes and prospects. *Pan African Medical Journal*, *15*, 1–9. https://doi.org/10.11604/pamj.2013.15.120.2535

Blesa, J., Soriano, J. M., Moltó, J. C., Marín, R., & Mañes, J. (2003). Determination of aflatoxins in peanuts by matrix solid-phase dispersion and liquid chromatography. *Journal of Chromatography A*, *1011*(1–2), 49–54. https://doi.org/10.1016/S0021-9673(03)01102-6

Borges, O. P., Soeiro Carvalho, J., Reis Correia, P., & Paula Silva, A. (2007). Lipid and fatty acid profiles of Castanea sativa Mill. Chestnuts of 17 native Portuguese cultivars. *Journal of Food Composition and Analysis*, *20*(2), 80–89. https:// doi. org/10. 1016/j.jfca.2006.07.008

Bozan, B., & Temelli, F. (2008). Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource Technology*, *99*(14), 6354–6359. https://doi.org/10.1016/j.biortech.2007.12.009

Casal, S., Malheiro, R., Sendas, A., Oliveira, B. P. P., & Pereira, J. A. (2010). Olive oil stability under deep-frying conditions. *Food and Chemical Toxicology*, *48*(10), 2972–2979. https://doi.org/10.1016/j.fct.2010.07.036

D'Imperio, M., Mannina, L., Capitani, D., Bidet, O., Rossi, E., Bucarelli, F. M., Quaglia, G. B., & Segre, A. (2007). NMR and statistical study of olive oils from Lazio: A geographical, ecological and agronomic characterization. *Food Chemistry*, *105*(3), 1256–1267. https://doi.org/10.1016/j.foodchem.2007.02.045

Ecker, O., Weinberger, K., & Qaim, M. (2010). Patterns and determinants of dietary micronutrient deficiencies in rural areas of East Africa. *Afjare*, *4*(6), 175–194.

Fender, G. (2014). *Calcium metabolism and its disorders. Reproductive Endocrinology for the MRCOG and Beyond, Second Edition*. https:// doi. org/10.1017/CBO9781139696920.019

Frega, N., Mozzon, M., & Lercker, G. (1999). Effects of free fatty acids on oxidative stability of vegetable oil. *Journal of the American Oil Chemists' Society*, *76*(3), 325–329. https://doi.org/10.1007/s11746-999-0239-4

Gao, F., Yang, S., & Birch, J. (2015). Physicochemical characteristics, fatty acid positional

distribution and triglyceride compostion in oil extracted from carrot seeds using supercritical CO_2. *Journal of Food Composition and Analysis,194(5) 884-890*. https://doi.org/10.1016/j.jfca.2015.09.004

Jeffery, R. B. (2018). A review of the Cucurbitaceae. *Botanical Journal of Linnean Society*, 81, 233-247.

Garrity, D. P. (2004). Agroforestry and the achievement of the Millennium Development Goals August 2002. *Agroforestry Systems* 61, 5–17. https://doi.org/10.1007/978-94-017-2424-1_1

Gibson, R. A., Muhlhausler, B., & Makrides, M. (2011). Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. *Maternal and Child Nutrition*, *7*(2), 17–26. https://doi.org/10.1111/j.1740-8709.2011.00299.x

Goyal, A., Sharma, V., Upadhyay, N., Gill, S., & Sihag, M. (2014). Flax and flaxseed oil: An ancient medicine & modern functional food. *Journal of Food Science and Technology*, *51*(9), 1633–1653. https://doi.org/10.1007/s13197-013-1247-9

Grosso, N. R., Nepote, V., & Guzmán, C. A. (2000). Chemical composition of some wild peanut species (*Arachis* L.) seeds. *Journal of Agricultural and Food Chemistry*, *48*(3), 806–809. https://doi.org/10.1021/jf9901744

Hasiewicz-Derkacz, K., Kulma, A., Czuj, T., Prescha, A., Zuk, M., Grajzer, M., Łukaszewicz, M., & Szopa, J. (2015). Natural phenolics greatly increase flax (*Linum usitatissimum*) oil stability. *Biotechnology*, *15*(1), 1–14. https://doi.org/10.1186/s12896-015-0178-0

Hemp, A. (2006). Continuum or zonation? Altitudinal gradients in the forest vegetation of Mt . Kilimanjaro. *Plant Ecology*, 184, 27–42. https://doi.org/10.1007/s11258-005-9049-4

Izquierdo, N., Aguirrezábal, L., Andrade, F., & Pereyra, V. (2002). Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. *Field Crops Research*, *77*(2–3), 115–126. https://doi.org/10.1016/S0378-4290(02)00060-6

Izquierdo, N. G., Aguirrezábal, L. A. N., Andrade, F. H., & Cantarero, M. G. (2006). Modeling the response of fatty acid composition to temperature in a traditional sunflower hybrid.

Agronomy Journal, *98*(3), 451–461. https://doi.org/10.2134/agronj2005.0083

Junior, E. C. S., Wadt, L. H. O., Silva, K. E., Lima, R. M. B., Batista, K. D., Guedes, M. C., Carvalho, G. S., Carvalho, T. S., Reis, A. R., Lopes, G., & Guilherme, L. R. G. (2017). Chemosphere Natural variation of selenium in Brazil nuts and soils from the Amazon region. *Chemosphere*, *188*, 650–658. https://doi.org/10.1016/j.chemosphere.2017.08.158

Kabata-pendias, A. (2004). Soil – plant transfer of trace elements: An environmental issue. *Geoderma*, *122*, 143–149. https://doi.org/10.1016/j.geoderma.2004.01.004

Kayode, A. A. A., & Kayode, O. T. (2011). Some medicinal values of telfairia occidentalis: A review. *American Journal of Biochemistry and Molecular Biology* 1(1), 30–38. https://doi.org/10.3923/ajbmb.2011.30.38

Kazadi, M., Pius, T. M., Bashwira, S., Archileo, N. K., Matthäus, B., & Justin, N. K. (2015). Composition of Fatty Acids and Tocopherols Content in Oilseeds of Six Wild Selected Plants from Kahuzi-Biega National Park / DR . Congo. *European Journal of Medicinal Plants, 8(3), 157-166.* https://doi.org/10.9734/EJMP/2015/17297

King, D. E., Mainous, A. G., Geesey, M. E., & Woolson, R. F. (2005). Dietary Magnesium and C-reactive Protein Levels. *Journal of the American College of Nutrition*, *24*(3), 166–171. https://doi.org/10.1080/07315724.2005.10719461

Łukaszewicz, M., Szopa, J., & Krasowska, A. (2004). Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. *Food Chemistry*, *88*(2), 225–231. https://doi.org/10.1016/j.foodchem.2003.12.042

Mannina, L., Patumi, M., Proietti, N., Bassi, D., & Segre, A. L. (2001). Geographical characterization of Italian extra virgin olive oils using high-field 1H NMR spectroscopy. *Journal of Agricultural and Food Chemistry*, *49*(6), 2687–2696. https://doi.org/ 10.1021/ jf001408i

Mbwana, H. A., Kinabo, J., Lambert, C., & Biesalski, H. K. (2017). Factors influencing stunting among children in rural Tanzania: an agro-climatic zone perspective. *Food Security*, *9*(6), 1157–1171. https://doi.org/10.1007/s12571-017-0672-4

Musalima, J. H., Ogwok, P., & Mugampoza, D. (2019). Anti-Oxidant Vitamins , Minerals and Tannins in Oil from Groundnuts and Oyster Nuts Grown in Uganda. *Food Science and*

Nutrition Research, *2*(2), 145-152.

Musila, G. A. (2018). Planrt genetic resources and food security in East and Central Africa. *Journal of Commonwealth Literature*, 53(4), 134-140. https: //doi.org/10.1177/ 0021989418801176

Nenadis, N., Llorens, L., Koufogianni, A., Díaz, L., Font, J., Abel, J., & Verdaguer, D. (2015). Biology Interactive effects of UV radiation and reduced precipitation on the seasonal leaf phenolic content / composition and the antioxidant activity of naturally growing Arbutus unedo plants. *Journal of Photochemistry & Photobiology*, *153*, 435–444. https:// doi.org/ 10. 1016/j. jphotobiol. 2015. 10.016

Nielsen, S. S. (2014). *Food Analysis Fourth Edition*. Springer new york, lafayyete USA. https://doi.org/10.1038/1841347a0

Okoli, B. E. (2007). *Telfairia pedata (Sm. ex Sims) Hook. Protabase Record.* http://database.prota.org/PROTAhtml/Telfairia pedata_En.htm

Okoli, B. E., & Nyananyo, B. L. (1988). Palynology of *Telfairia sp* (Cucurbitaceae). *Folia Geobotanica et Phytotaxonomica*, *23*(3), 281–283. https://doi.org/10.1007/BF02854822

Romero, N., Saavedra, J., Tapia, F., Sepúlveda, B., & Aparicio, R. (2016). Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage. *Journal of the Science of Food and Agriculture*, *96*(2), 583–592. https://doi.org/10.1002/jsfa.7127

Salter, A. M. (2013). Dietary fatty acids and cardiovascular disease. *Animal*, *7*(1), 163–171. https://doi.org/10.1017/S1751731111002023

Saracoglu, S., Saygi, K. O., Uluozlu, O. D., Tuzen, M., & Soylak, M. (2007). Determination of trace element contents of baby foods from Turkey. *Food Chemistry*, *105*(1), 280–285. https://doi.org/10.1016/j.foodchem.2006.11.022

Simopoulos, A. P. (2011). Evolutionary aspects of diet: The omega-6/omega-3 ratio and the brain. *Molecular Neurobiology*, *44*(2), 203–215. https://doi.org/10.1007/s12035-010-8162-0

Teh, S., & Birch, J. (2013). Journal of Food Composition and Analysis Physicochemical and quality characteristics of cold-pressed hemp , flax and canola seed oils. *Journal of Food*

Composition and Analysis, *30*(1), 26–31. https://doi.org/10.1016/j.jfca.2013.01.004

Thornton, P. K., Jones, P. G., Alagarswamy, G., & Andresen, J. (2009). Spatial variation of crop yield response to climate change in East Africa. *Global Environmental Change*, *19*, 54–65. https://doi.org/10.1016/j.gloenvcha.2008.08.005

USDA. (2015). *National Nutrient Database for Standard ReferenceRelease 28 Nutrients: Selenium, Se (µg)*. https://ods.od.nih.gov/pubs/usdandb/VitA-betaCarotene-Content.pdf

Velasco, J., & Dobarganes, C. (2002). Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, *104*(9–10), 661–676. https://doi.org/10.1002/1438-9312(200210)104:9/10<661::AID-EJLT661>3.0.CO;2-D

Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., & Wagentristl, H. (2007). Agronomic evaluation of camelina genotypes selected for seed quality characteristics. *Industrial Crops and Products*, *26*(3), 270–277. https://doi.org/10.1016/j.indcrop.2007.03.017

WHO. (2010). *WHO technical consultation on postpartum and postnatal care*. World Health Organization. https://www.google.com

Winiger, M. (2017). Karakorum-Hindukush-western Himalaya: Assessing high-altitude water Karakorum–Hindukush–western Himalaya. *Hydrology Process*, 19, 2329-2338. https://doi.org/10.1002/hyp.5887

Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., Höglinger, O., & Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, *42*(3), 46–55. https:// doi. org/10. 1016/j. jfca.2015.03.005

Xie, J. H., Shen, M. Y., Nie, S. P., Liu, X., Yin, J. Y., Huang, D. F., Zhang, H., & Xie, M. Y. (2013). Simultaneous analysis of 18 mineral elements in Cyclocarya paliurus polysaccharide by ICP-AES. *Carbohydrate Polymers*, *94*(1), 216–220. https:// doi. org/10. 1016/j.carbpol.2012.12.072

Yerlikaya, C., Yucel, S., Erturk, Ü., & Korukluoğlu, M. (2012). Proximate composition, minerals and fatty acid composition of *Juglans regia* L. genotypes and cultivars grown in Turkey. *Brazilian Archives of Biology and Technology*, *55*(5), 677–683. https:// doi.

org/10. 1590/S1516-89132012000500006

You, Q., Kang, S., Pepin, N., Flügel, W. A., Yan, Y., Behrawan, H., & Huang, J. (2010). Relationship between temperature trend magnitude, elevation and mean temperature in the Tibetan Plateau from homogenized surface stations and reanalysis data. *Global and Planetary Change*, *71*(1–2), 124–133. https://doi.org/10.1016/j.gloplacha.2010.01.020

Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. (2010). Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chemistry*, *118*(3), 656–662. https://doi. org/10. 1016/j. foodchem. 2009.05.038

APPENDICES

Appendix 1: Phytosanitary certificate used during transport of oyster nut samples to Austria

TRIPLICATE

PQS 4

00000513

THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF AGRICULTURE
Plant Protection Division Plant Quarantine and Phytosanitary Service

PHYTOSANITARY CERTIFICATE

(Under regulations 58) No. 323

TO: Plant Protection Organization of **Austria**

I. DESCRIPTION OF CONSIGNMENT

Name and Address of Exporter **Nelson Mandela African Institution of Science and Technology** **Nelson Mandela Institution of Science and**	Declared name and address of consignee **University of applied Science and Engineering (upper) Wels, Austria** **Upper Wels, Austria.**	
Declared means of conveyance **Aircraft**	Place of Origin **Tanzania**	Declared point of entry **Austria**
Distinguishing marks **Kweme**	Number and description of packages **01 , Plastic bag**	Name of the Produce **oysternuts**
Botanical name of plants ***Telfairia pedata***	Quantity and product declared **10.0 Kg oysternuts**	

This is to certify that the plants, parts of plants or plant products described above or representative samples of them were thoroughly examined on **10 September 2019** by **Alinanuswe A. Mwakijombo** authorized officer of the Ministry of Agriculture and were found to the best of his/her knowledge to be substantially free from injurious plant pests; *and that consignment is believed to conform with the current phytosanitary regulations of the importing country both as stated in the additional declaration and otherwise.

II. ADDITIONAL DECLARATION

NIL

III. DISINFESTATION AND/OR DISINFECTION TREATMENT

Date:	Treatment: **NIL**
Chemical and concetration: **NIL**	Duration of exposure: **NIL**
Additional Information:	

IV. AUTHORIZATION

Date: **10/09/2019**	Place of Issue: **Julius Nyerere International Airport**
Authorized officer **Mdili Sambayi Katemani** Signature **For Inspector In Charge**	10 SEP 2019 OFFICER IN CHARGE DAR-ES-SALAAM - HARBOURS Stamp of Organization:

"Note:"
1. To be filled in Quadruplicate
2. No liability attached to the Plant Health Services or any its officers in respects of this certificate.

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Appendix 2: Descriptions and coodinates for areas where the samples were collected in Tanga, Arusha, and Kilimanjaro in june and july 2019

region	coordinates	latitude	longitude	ELEVATIONS	name
kilimanjaro	3° 15' 47.90" S, 37° 23' 40.75" E	-3.263306	37.394667	1142 m	moshi-uru1
	3° 15' 32.54" S, 37° 23' 59.65" E	-3.259028	37.399917	1663 m	moshi-uru2
	3° 15' 31.26" S, 37° 24' 58.98" E	-3.25868333	37.41638889	1362 m	moshi-Materuni 2
	3° 16' 4.05" S, 37° 23' 24.85" E	-3.26779167	37.39027778	1501 m	moshi-Materuni kwa helman
	3° 16' 43.2" S, 37° 21' 57.85" E	-3.35657	37.57484	1798m	marangu-2
	3° 18' 09.1" S, 37° 21' 44.25" E	-3.329585	37.529958	1402m	marangu 1
tanga	5° 1' 11.92" S, 38° 26' 31.63" E	-5.01997778	38.44222222	906 m	east usambara-Fune kiwandani
	5° 1' 48.45" S, 38° 25' 45.82" E	-5.030125	38.42944444	1602 m	east usambara-Kwekibomi 1

	5° 1' 49.45" S, 38° 26' 2.43" E	-5.03040278	38.43416667	1063 m	east usambara-kwashemshi
	5° 1' 46.49" S, 38° 25' 58.25" E	-5.02958056	38.43277778	1166 m	usambara-kwamanoro
	5° 1' 44.38" S, 38° 25' 39.56" E	-5.02899444	38.42777778	1300 m	usambara-bungu
	5° 1' 55.46" S, 38° 27' 7.36" E	-5.03656567	38.44876643	1789m	usambara-msiga
arusha	3°20'12.69"s, 36°48'36.67"E	-3.336861	36.810194	962m	meru-nkoaranga (sumary)
	3°19'47.79"s, 36°48'53.34"E	-3.329944	36.814806	1562m	meru-nkoaranga 2
	3°18'52.28", 36°48'14.45"E	-3.314528	36.804028	1758m	meru-songolo
	3°19'32.67"S, 36°48'13.58"E	-3.32575	36.803778	1687m	meru-Nshing'a
	3°23'12.67"S, 36°49'13.58"E	-3.345777	36.896543	1174m	sura

	3°23'47.38"S, 36°47'18.63"E	-3.384646	36.836279	1298m	sura kibaoni

RESEARCH OUTPUTS

(i) Publication

Mwakasege, E., Treydte, A., Hoeglinger, O., Kassim, N., & Makule, E. (2021). Variations in nutrient composition of oyster nuts (*Telfairia pedata*) across different agro-climatic conditions. *Cogent Food and Agriculture*, 7, 1, 1-22, DOI: 10. 1080/ 23311932. 2021. 1913843

(ii) Poster presentation

Poster Presentations

Nutrient composition and oil stability of oyster nuts (*Telfairia pedata*) across different environmental conditions in northern, Tanzania

By

Emmanuel Mwakasege, Anna Treydte, Edna Makule, Otmar Hoeglinger and Neema Kassim

The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

BACKGROUND

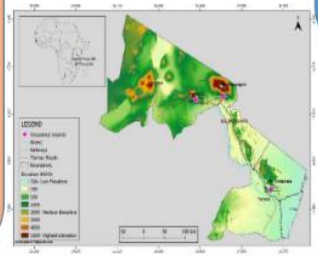
Oysternuts (*Telfairia pedata Hook*) are under-utilized oil seeds plant of the family Cucubitaceae, native to Africa. They are part of traditional and heritage food, grown particularly for their edible nuts which are source of protein and fats. The seeds are eaten fresh, roasted, ground or added as thickeners in soups, meat dishes or vegetables. The flour made from kernels is used to make breads and cookies, while the pressed oil is used for cooking, soap making and as skin and hair tonic. In addition, due to lactogenic and medicinal properties Oysternuts seeds have been used by pregnant and lactating mothers for milk production and fast healing.

However, nutritional composition of oysternut have not been properly quantified limiting their utilization and inclusion in modern diets. Therefore, we compared proximate, minerals and fatty acid compositions of oysternuts across different regions and elevation blocks. Furthermore, the influence of environmental conditions on nutrient composition was investigated

MATERIALS AND METHODS

Oyster nut samples were collected in May, June and July 2019 during harvest season for three growing regions, i.e., Arusha, Kilimanjaro and Tanga in northern Tanzania. Four different oysternut growing locations were purposefully selected from each of the regions, spanning different elevations, temperature and rainfall conditions, and total of 12 samples each of 1 kg of nuts of the individual plants were collected.

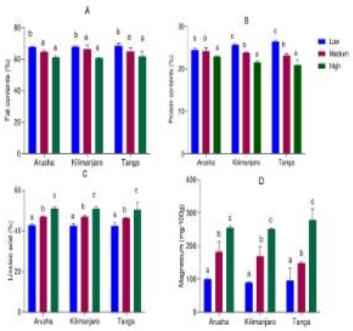
Samples were cleaned, sun-dried and transported to the University of Applied Sciences, Wels, Upper Austria, for analysis.

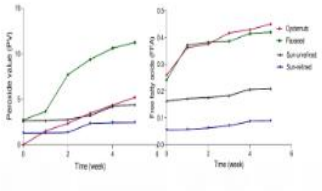


RESULTS & DISCUSSION

Descriptive statistics for proximate results for oysternuts indicated fat saturation as most abundant content in oysternuts seed kernels followed by protein, carbohydrates and ash contents in dry matter basis (as shown in table F1). Meanwhile, determination of quality of oysternut oil by fatty acid contents analysis found polyunsaturated fatty acid (PUFA) made of linoleic acid (C18:2 n-6) as the most abundant with amount equal to all other fatty acids contents combined.

Analysis of variance for nutrient contents of oysternuts across regions with environmental factors as covariate showed fat contents were slightly lower in Arusha compared to Kilimanjaro and Tanga ($F_{2,9}$ = 15.46, P=0.002; Table F1) while protein contents were slightly higher in Tanga





CONCLUSION

Our results highlight that oysternuts are nutrient-rich food and can act as additional income generation as well as for agricultural bio-diversification if the importance of optimizing growing conditions is considered.