COMPLIANCE OF FORTIFIED SUNFLOWER OIL AND PALM OLEIN WITH REQUIREMENTS FOR RETINYL PALMITATE CONTENT

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ABSTRACT

This study was conducted to assess compliance to fortification of sunflower oil and palm olein with retinyl palmitate. Fortified sunflower oil and palm olein samples were collected from large scale edible oil manufacturers and at selling outlets in Dar es Salaam region. Mean retinyl palmitate levels in fortified sunflower oil samples from manufacturer A collected at production site and at selling outlets was 12.42 and 10.19 mg/kg, respectively. In fortified palm olein samples levels were 21.58, 66.33 and 42.49 mg/kg at production site and 18.84, 46.54 and 28.40 mg/kg at selling outlets for manufacturer A, B and C, respectively. Based on East African Standard specifications for fortified edible oils 3 out of 36 samples (8.33%) collected at production site and 13 out of 39 samples (33.33%) collected at selling outlets were found to comply with the fortification requirements set for factory and regulatory limits, respectively. On storage, oils samples collected from production sites showed a decrease in retinyl palmitate with 11.09 and 10.87% after 3 and 6 months respectively in sunflower oil under normal condition. In palm olein samples retinyl palmitate concentrations percentage decrease were 21.22, 35.08 and 10.00% after 3 months and 22.61, 50.97 and 14.49% after 6 months for manufacturer A, B and C, respectively. Decrease of retinly palmitate in sunflower oil samples was associated with an increase of peroxide and acid value of 10.32 mEq/kg and 0.74 mgKOH/g after 3 months of storage and 31.57 mEq/kg and 0.96 mgKOH/g after 6 months respectively. In palm olein peroxide and acid value were 0.86, 14.88, 3.17 mEq/kg and 0.22, 0.71, 0.34 mgKOH/g after 3 months and 4.44, 15.08, 6.15 mEq/kg and 0.23, 0.79, 0.45 mgKOH/g after 6 months of storage for manufacturer A, B and C, respectively. Very low awareness was observed among edible oil vendors on handling of fortified edible oil. Authorities should ensure strict compliance by manufacturers and raise awareness to the vendors.

DECLARATION

I, **Rhoda Kidolezi** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

__________________________ ______________________

Rhoda Kidolezi **Date**

(Msc. Candidate)

The above declaration is confirmed;

______________________________ ______________________

Prof. Bendantunguka P. Tiisekwa **Date**

(Supervisor)

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DEDICATION

This work is dedicated to my beloved daughter Michelle I. Kasigwa, and my son Milan I. Kasigwa for their prayers, patience and inspiration during the period of my study of MSc. Food Quality and Safety Assurance.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
AV	Acid value
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CI	Confidence Interval
○C	Degree Celsius
FAO	Food and Agriculture Organization
FFA	Free Fatty Acid
g	Grams
ISO	International Standards Organization
mEq	Milliequivalent
mL	Millititres
N	Normality
NBS	National Bureau of Statistics
nm	Nanometer
PoV	Peroxide Value
ppb	Parts per billion
ppm	Parts per million
PUFAs	Polyunsaturated Fatty Acids
RBDPO	Refined, Bleached, Deodorized Palm Oil
RDI	Recommended Daily Intake
RP	Retinyl Palmitate
s	Seconds
SPSS	Statistical Package for Social Sciences

TBHQ	Tert-Butyl Hydroquinone
TFDA	Tanzania Food and Drugs Authority
TZS	Tanzania Standards
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
UV	Ultra Violet
VAD	Vitamin A Deficiency
VADD	Vitamin A Deficiency Disorders
WEOs	Ward Executive Officers
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Vitamin A is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function, and reproduction (UNICEF, 2009). This nutrient cannot be synthesized by the body and therefore must be provided through diet (FAO/WHO, 2001). The World Health Organization (WHO) has reported that Vitamin A deficiency (VAD) is one of the global micronutrient deficiency of public health importance and that infants, young children and pregnant women appear to be at greatest risk of vitamin A deficiencies disorders (WHO, 2009). The main underlying cause of VAD as a public health problem is a diet that is chronically insufficient in vitamin A that can lead to lower body stores and fail to meet physiologic needs (e.g. support tissue growth, normal metabolism, resistance to infection) (WHO, 2009). In most developing countries, diets are monotonous and mainly based on cereals and legumes that are poor sources of vitamin A (WHO/FAO, 2006).

Several interventions have been proposed worldwide to control and prevent vitamin A deficiencies disorders. These include food-based strategies such as dietary diversification, supplementation and food fortification (WHO/FAO, 2006). Of all the interventions, fortification of edible oil with vitamin A has been proven to be most cost effective and sustainable strategy for preventing and controlling VAD in developing countries (Klemm *et al.*, 2010; Pritwani and Mathur, 2015). This approach has also the advantage since it is more natural and sustainable. Considering this factor, introduction of vitamin A into oil is an appropriate strategy of fortification, considering the high lipophilicity of this compound. Oil and fat are important constituents of diet and contribute significantly to development and regulation of different functions of the body. Also, oils and margarine represent ideal vehicles for vitamin fortification because the vitamins can be uniformly distributed in the oil and lipidic medium allows for good bioavailability in humans (Pignitter and Somoza, 2012).

Almost everyone consumes some oil; thus, it is possible to improve consumer's access to fat soluble vitamins through oil fortification. Many countries around the world including Kenya, Uganda, Ethiopia and Tanzania have adopted the fortification strategy. Fortification of edible oils in Tanzania is currently mandatory, done by large scale oil manufactures most of which are located in Dar es Salaam region. Enforcement of mandatory food fortification in Tanzania is through the Tanzania Food, Drugs and Cosmetics (Food fortification), Regulations, 2011. This study therefore, was carried out to assess compliance of fortified sunflower oil and palm olein produced by large scale edible oil manufactures in Dar es Salaam region with requirements for retinyl palmitate (vitamin A precursor) content.

1.2 Problem Statement and Justification

Vitamin A deficiency is a serious problem especially in most developing countries, where diets are monotonous (Ruel, 2001) and mainly based on cereals and legumes that are poor sources of vitamin A (West *et al.*, 2002). Fortification of oils with vitamin A is intended to lower the prevalence of Vitamin A deficiency in population at risk (WHO/FAO, 2006). However, effectiveness of this strategy largely depends on the stability of vitamin A in the oil throughout the period of shelf life (Chakravarty, 2000). The actual availability of vitamin A in fortified edible oil at the consumer level can vary widely due to a number of factors: variability in the amount of vitamin A added in the oil during fortification, poor mixing resulting in uneven distribution within the batches and instability of vitamin A during storage, transportation and distribution. Various factors, such as exposure to light and oxygen, storage, cooking temperature, duration and method of cooking as well as type of packaging material affect vitamin A stability in fortified edible oil (Dary and Mora, 2002; Pignitter *et al.*, 2014; Hemery *et al.*, 2015). Luthringer *et al*. (2015) did a study on identifying barriers to compliance of fortified foods with set national standards across 17 countries in Africa and Asia and found that poor quality assurance measures, purposively under fortification and poor processing procedures among manufactures as well as political risk of enforcement and poorly resourced inspectorate capacity appear to adversely reinforce industry and government within an environment of unclear legislation to create a major hurdle for improving overall compliance of fortification programs against national standards. These factors are also well experienced in Tanzania. Post marketing surveillance of fortified edible oils conducted by Tanzania Food and Drugs Authority showed that, only 16% of fortified edible oils in the market contained required amounts of vitamin A (TFDA, 2015). With such very low level of compliance and the factors responsible for the observed non-compliance were not clear. To warrant adequate intake of vitamin A at the consumer level, assessment of compliance is an essential element of edible oil fortification program. Therefore, the present study was designed to investigate the compliance of fortified sunflower oil and palm olein produced by large scale edible oil manufacturers in Dar es Salaam region.

This study will provide evidence on how much fortification program is adhered to among the sampled edible oil manufacturers in Dar es Salaam region. Also, it will determine the factors influencing compliance of fortified oil during manufacturing and at selling outlets as well as vendor's awareness on oil fortification and handling of fortified edible oil.

The findings from this study will form a useful input for reviewing edible oil fortification standards and quality control guidelines in Tanzania in order to achieve the required compliance and intended nutritional and health benefits.

1.3 Objective of the Study

1.3.1 Overall objective

To assess compliance to fortification of sunflower oil and palm olein with retinyl palmitate for oil produced by large scale edible oil manufactures in Dar es Salaam region.

1.3.2 Specific objectives

i. To determine levels of retinyl palmitate in fortified sunflower oil and palm olein for samples collected at the production site and at selling outlets.

ii. To identify factors affecting compliance to fortification of edible oil with requirement of retinyl palmitate at production site and at selling outlets.

iii. To assess vendor’s awareness on oil fortification and handling of fortified edible oils.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Edible Oil

Oils and fats are major components of human diet, along with carbohydrates and proteins. They can be of animal or vegetable origin. Animal fats (lard, butter) contain vitamin A (FAO/WHO, 2001). There is no vitamin A naturally present in vegetable fats and oils (soy, canola, and corn) but unrefined oils may contain carotene, which is a vitamin A precursor. Red palm oil contains high levels of carotene but its intense red color makes it unacceptable for many applications (Loganathan and Tiu, 2014). Fats and oils are energy dense, useful in achieving a minimum, necessary level of caloric intake. They provide food with flavor, palatability and help reduce the feeling of hunger (Strayer, 2016). Fats and oils are necessary for the delivery and absorption of the fat-soluble vitamins (A, D, E and K) (Pignitter and Somoza, 2012).

Oils are composed of triglycerides containing polyunsaturated fatty acids (PUFA). PUFAs are long chain fatty acids with multiple double bonds, making them susceptible to oxidation (Abedi and Sahari, 2014). Oxidation occurs on exposure to air and heat resulting in a rancid odor. The presence of certain metals will accelerate the oxidation. The degree at which oil has been oxidized is indicated by its peroxide value (Pristouri *et al.*, 2010). Antioxidants, such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), tocopherol and Tert-Butyl Hydroquinone (TBHQ) are added to the oil to delay oxidation of oils and stabilize it during storage (Naz *et al*., 2004).

Oils are cheaper than solid (margarine) and semisolid (mayonnaise) fats and easier to handle in food aid programs (Dian *et al*., 2017). The higher heat achieved with oil frying helps break down vegetable matter, making it tenderer and easier to digest in a shorter cooking time than by boiling in water (Dawodu *et al*., 2015). Oil is also used in small amounts to prevent food from sticking to cooking surfaces. Because of these properties, vegetable oil is nearly always included in a food aid basket of rations along with cereal and legume (SUSTAIN, 1998).

2.2 Vitamin A

Vitamin A is a yellow, oil soluble crystal, which can be uniformly distributed in oil (WHO/FAO, 2006). The body easily absorbs added vitamin A in the presence of oil. Vitamin A is an essential nutrient required in trace amount for normal visual and immune function, maintenance, growth and development of body (WHO, 2009; UNICEF, 2009). Dietary requirements for Vitamin A are normally provided as a mixture of preformed vitamin A (retinol) present in animal foods and provitamin A carotenoids derived from foods of vegetable origin, which have to be converted into retinol by tissues such as the intestinal mucosa and the liver in order to be utilized by cells (WHO /FAO, 2004).

2.3 Vitamin A Deficiency Disorders

Increased morbidity and mortality, poor reproductive health, increased risk of anaemia and slowed growth and development have been reported as the consequences of vitamin A deficiency disorders (VADD) particularly during periods of high nutritional demand such as infancy, childhood, pregnancy and lactation (FAO/WHO, 2006; WHO, 2009). The World Health Organization (2009) has shown that Xerophthalmia is the most specific VADD, and is the leading preventable cause of blindness in children. It is estimated that 250 000 to 500 000 vitamin A-deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 1996).

Vitamin A Deficiency (VAD) is a serious public health problem in developing countries that affects mainly children and women of reproductive age (Christian, 2002; WHO, 2009; Akhtar *et al*., 2013). According to the recent World Health Organization (WHO) estimates, VAD has moderate and severe public health significance in 45 and 122 countries in the world respectively, with Africa and southern Asia demonstrating the highest burden of VAD (WHO, 2009). It has been reported that Africa alone contributes more than one-third of the global burden of childhood Xerophthalmia whereby 44% and 2% of the pre-school children are affected by sub-clinical VAD and night blindness, respectively, West (2002). In Ethiopia VAD is a severe problem with the overall 8.6% prevalence of Xerophthalmia among preschool children (Tariku *et al*., 2016; Tariku and Woldie *et al*., 2016). Aguayo *et al*., (2005) found that that the prevalence of VAD among children below the age of 5 years in Mozambique is estimated to be 2.3 million that is estimated to contribute 34.8% of all-cause mortality in this group. The survey conducted by the Tanzanian National Bureau of Statistics (NBS) revealed that the situation in Tanzania is not different from other parts of Africa since the prevalence of VAD in under-five children and women of reproductive age is 33% and 37%, respectively (NBS, 2011).

2.4 Sunflower Oil

Sunflower oil is the non-volatile oil compressed from the seeds of sunflower (*Helianthus annuus*) (Basak *et al*., 2017). Sunflower oil is high in the essential vitamin E and low in saturated fat. The two most common types of sunflower oil are linoleic and high oleic (Skorić *et al*., 2008). Linoleic sunflower oil is common cooking oil that has high levels of polyunsaturated fat (Orsavova *et al.*, 2015). It is also known for having a clean taste and low levels of trans-fat. High oleic sunflower oils are classified as having monounsaturated levels of 80% and above (Arshad and Amjad, 2012).

Sunflower oil is light amber in colour with a mild and pleasant flavor; refined oil is pale yellow (Pal *et al*., 2015). Presence of compounds such as phosphatides, free fatty acids, odiferous volatiles, colourant, waxes and metal compounds in oil negatively affect taste, smell, appearance and storage stability of the refined oil and hence must be removed to yield a stable product with a bland or pleasant taste (Aluyor *et al*., 2009). Refining losses are low and the oil has good keeping qualities with light tendency for flavor reversion (Verhe *et al*., 2006). The oil contains appreciable quantities of vitamin E, sterols, squalene, and other aliphatic hydrocarbons (Madhavi *et al*., 2010). Since sunflower oil is primarily composed of healthier-but-less-stable polyunsaturated and monounsaturated fatty acids, it can be particularly susceptible to degradation by heat, air, and light, which trigger and accelerate oxidation (Brevedan *et al*., 2000). So, keeping sunflower oil at low temperatures during manufacture and storage in bottles that are made of either darkly-colored glass or, plastic that has been treated with an ultraviolet light protectant can help to minimize rancidity and nutrient loss (Abdelmonem *et al.*, 2012).

2.4.1 Sunflower oil refining

Refined sunflower oil simply refers to oil that has been obtained by expression and or extraction and in addition it has been neutralized with alkali, washed with water , dried, bleached with bleaching earth or activated carbon and deodorized with steam (Tasan and Demicri, 2005). In refining process no other chemical agent is allowed except citric acid (Kovari *et al*., 2000). There are two principal of refining processes: physical refining (Figure 1) and chemical refining (Figure 2). The crude oil contains several impurities that must be reduced in order to make the oil suitable for food application. There are two groups of impurities present in the crude sunflower oil which are macro impurities that can be measured in percentage of the crude oil and micro impurities that are present in small amounts, generally at parts per million (ppm) or even parts per billion (ppb) levels.

Although they are present in minute quantities, the presence or absence of some of the micro impurities can have very significant effect on the stability of the oil. Refining sunflower oil through solvent extraction, de-gumming, neutralization, and bleaching can make it more stable and suitable for high-temperature cooking (Pal *et al*., 2015). Refining also removes some of the oil's nutrients, flavour, colour (resulting in a pale-yellow), free fatty acid, phospholipids; polyphenols and phytosterols.

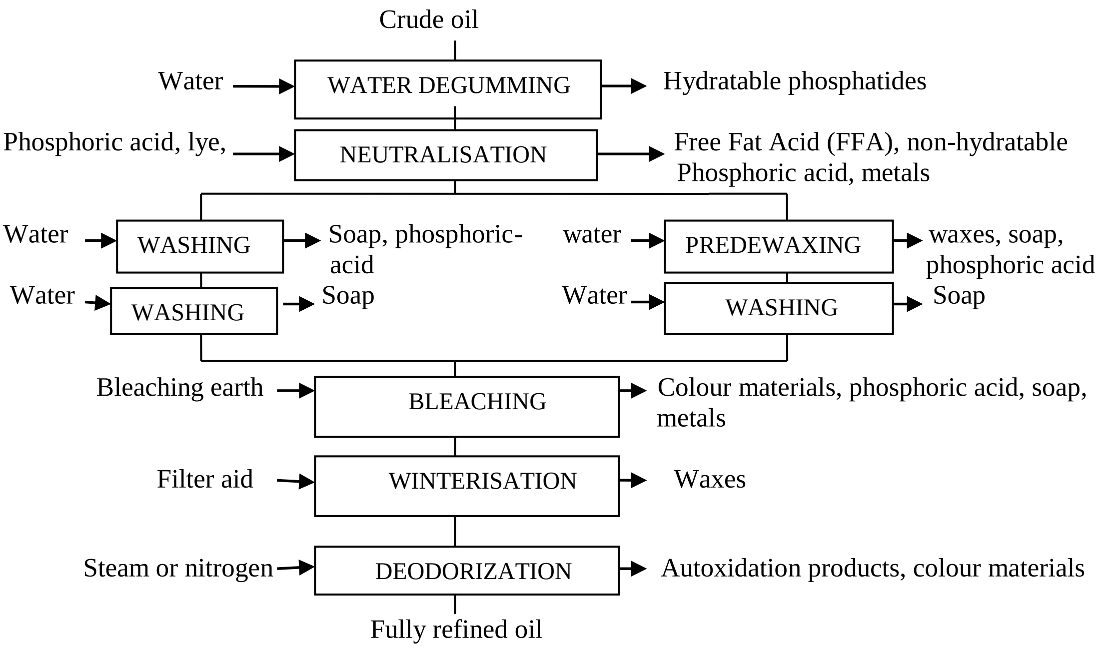


Figure 1: Chemical refining of sunflower oil

Source: Kovari *et al*. (2000)

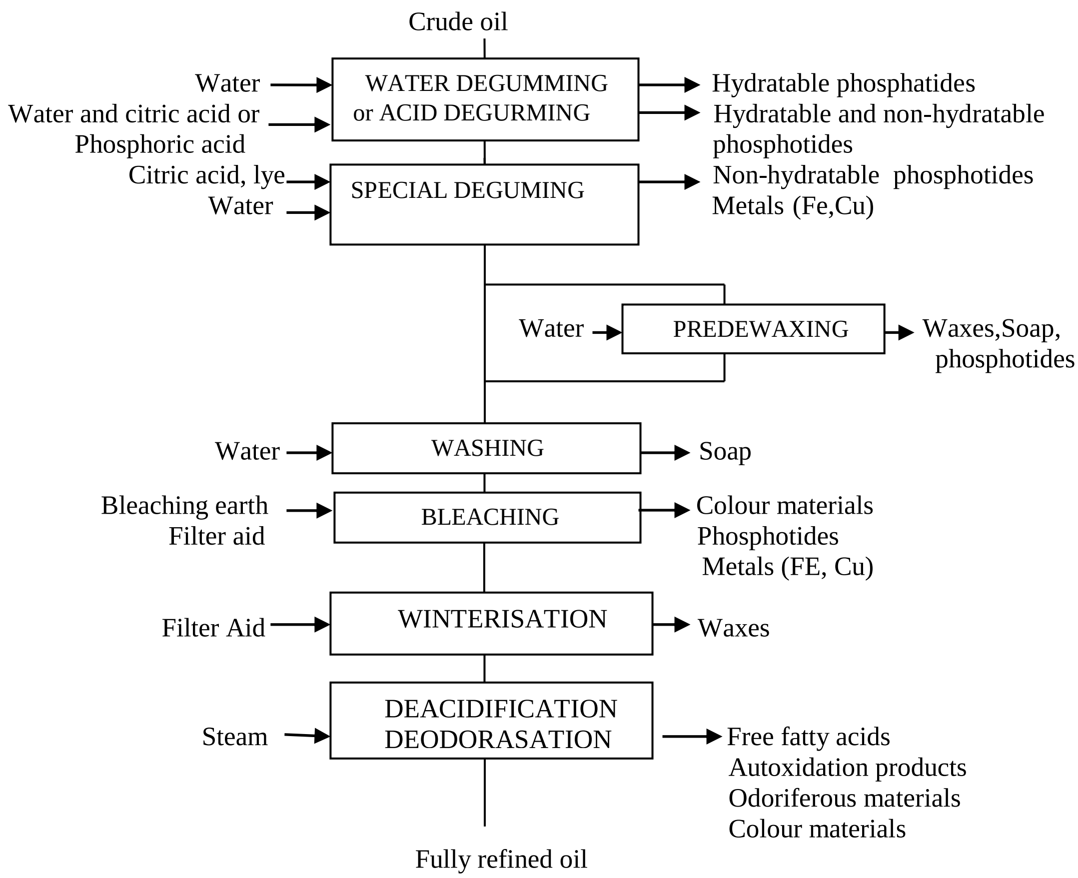


Figure 2: Physical refining of sunflower oil

Source: Kovari *et al*. (2000)

2.5 Palm Olein

Palm olein is the liquid component of palm oil obtained when the oil is separated by a process called fractionation (Latip *et al*., 2013). Palm oil (also known as dendê oil, from Portuguese [ˈdɛnde]) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms, primarily the African oil palm *Elaeis guineensis*, (Reeves *et al*., 1979). Palm oil is naturally reddish in colour and is rich in carotene, vitamin A precursor and vitamin E (antioxidant) (Murthy *et al*., 1996; Ahmand and Idris, 2005).

The carotenoids (vitamin A), tocopherols (vitamin E), sterols, phosphatides, triterpenic, and aliphatic alcohols form the minor constituents of palm oil. Though present in less than 1% altogether in palm oil, nevertheless they play a significant role in the stability and refinability of the oil, in addition to increasing the nutritive value of the oil (Sambanthamurthi *et al*., 2000).

Palm oil is one of the few highly saturated vegetable fats and is semisolid at room temperature (Behrman *et al*., 2005*)*. Palm oil is a common cooking ingredient in the tropical belt of Africa, Southeast Asia and parts of Brazil (Nnadozie *et al*., 1990). Its use in the commercial food industry in other parts of the world is widespread because of low cost and the high oxidative stability (saturation) of the refined product when used for frying (Che Man *et al*., 1999; Matthäus, 2007).

2.5.1 Palm oil refining

Palm oil refining is a process to make the palm oil edible (Nagendran *et al*., 2000). The crude oil cannot be directly consumed. Refining of palm oil is carried out step by step and at each step, quality of oil is improved. The step per step method of palm oil refining improves credibility and acceptance of oil (Gibon *et al*., 2007).

Conversion of crude palm oil to refined oil involves removal of the products of hydrolysis and oxidation, colour and flavour (Gibon *et al.*, 2007). After refining, the oil may be separated (fractionated) into liquid and solid phases by thermo-mechanical means (controlled cooling, crystallization and filtering) and the liquid fraction (olein) is used extensively as liquid cooking oil.

In refining process the first step is fractionation with crystallization and separation processes to obtain solid (stearin) and liquid (olein) fractions. This is followed by melting and degumming to remove impurities such as trace minerals, copper and iron by the application of phosphoric acid. Then the oil is filtered and bleached. In bleaching, the oil is mixed with bleaching earth (bentonite calcium) in a vacuum room to remove impurities and colour pigments in the palm oil. Then the oil is deodorised, the odour and taste of the oil are removed when the oil is steamed at high temperatures of between 240 °C to 260 °C and then cooled to room temperature (Gibon *et al*., 2007).

Refining can be operated according to two main routes: chemical refining or physical refining (Greyt *et al*., 2000). The principal difference between the two routes is how the free fatty acids (FFAs) are removed. In a physical operation, most FFAs are removed in the deodorizing unit while in chemical refining the oil is cleared from gums and FFA during the alkali neutralization step and soap stocks are produced (Pal *et al*., 2015).

The physical refining process offers important advantages than chemical refining such as higher oil yield, reduction of the use of chemicals (like phosphoric acid, sulphuric acid and caustic soda), reduction of water and effluent, and hence considerable reduction of the environmental impact (Kellens *et al*., 2000). Physical refining process removes smells and colour to produce "refined, bleached and deodorized palm oil" (RBDPO) and free fatty acids which are used in the manufacture of soaps, washing powder and other products (Udeh and Obibuzor, 2017). RBDPO is the basic palm oil product sold in the world's commodity markets. The diagram below shows the sequence of the palm oil refining process.

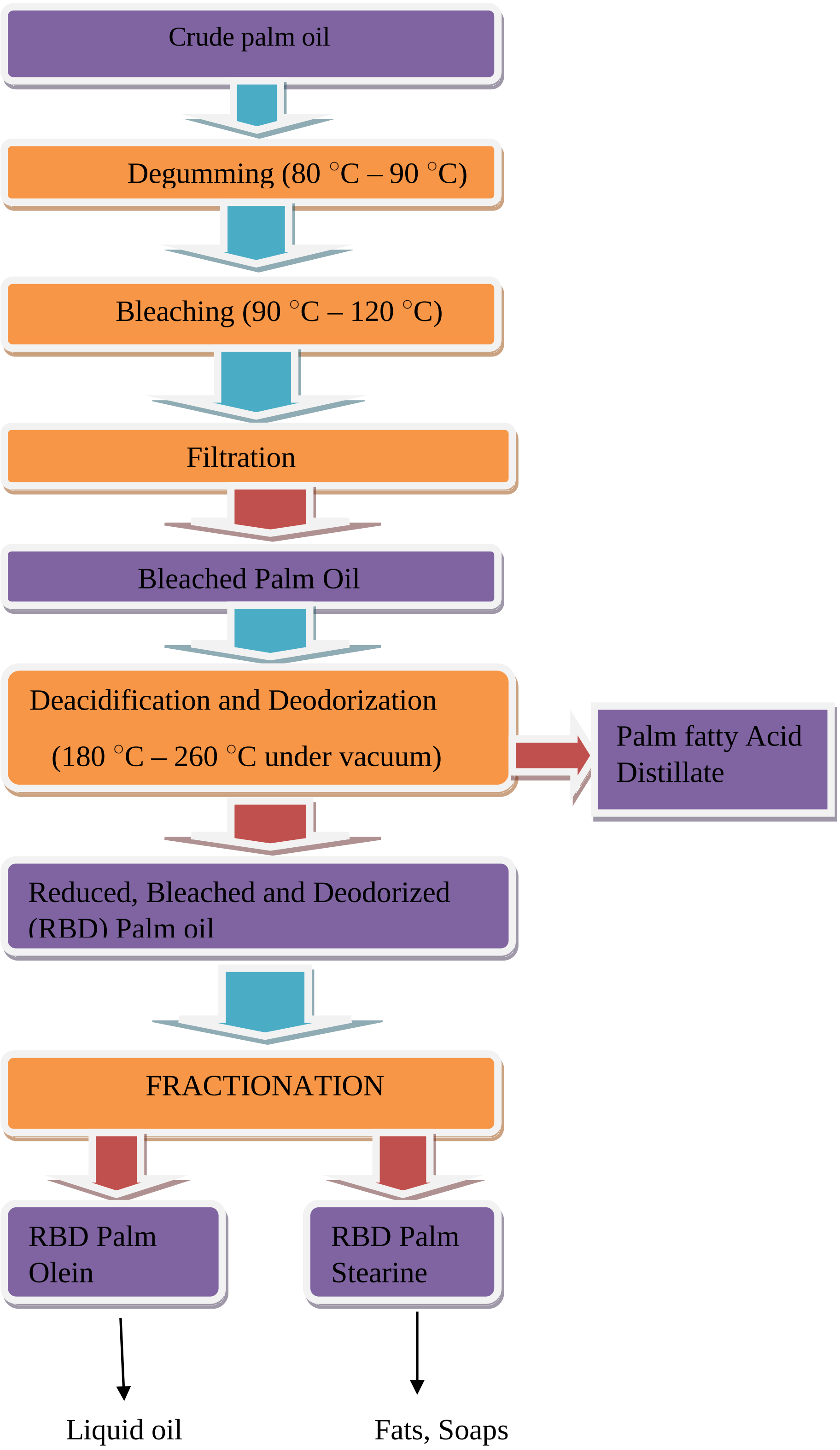


Figure 3: Schematic diagram for palm olein refining process

Source: Survey (2017)

2.6 Fortification of Edible Oil with Vitamin A

Food fortification is defined as the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) into a food to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk to health (WHO/FAO, 2006). Fortification of staple food with vitamin A is well accepted and promising strategy to reduce vitamin A malnutrition (Akhtar *et al.*, 2011). Among food vehicles ideal for fortification, edible oil has been proven to be among the ideal matrix for addition of vitamin A, which is fat soluble vitamin since it stabilizes retinol and delays oxidation of the vitamin (Sommer and Davidson, 2002). Furthermore, fortification of edible oil with vitamin A is considered a cost-effective and simple strategy for preventing and controlling VADD (WHO/FAO, 2006; West *et al.*, 2008).

Different strategies are used to fortify food products with vitamin A. The choice of fortificant is mainly governed by the type of food vehicle, but also by technological, religious and regulatory considerations (WHO/FAO, 2016). Since the active form of vitamin A (retinol) is unstable, commercial preparations are proposed in the more stable esterified form, usually with palmitic or acetic acid (WHO/FAO, 2006; O'Byrne and Blaner, 2013). Two main forms of fortificant are used depending on the final food vehicle selected for fortification; oily forms which will be incorporated in fat-based products or dry forms that can be dry mixed into foods (e.g. cereal flours) or dispersed in water (Pritwani and Mathur, 2015).

Although certain food vehicles are promising due to their properties or high penetration to reach low income people, vitamin A degradation is observed during processing, shipping, storage and/or cooking of the final product (Silalahi *et al*., 2017). Therefore, food manufacturers need to increase the quantity of vitamin A in fortified products to compensate for possible losses occurring at different phases of the product's life (WHO/FAO, 2006). This higher dosage may be problematic if the fortified food is directly consumed after production, where almost no vitamin A is degraded, since adverse physiological effects have been associated with vitamin A chronic high intake and hypervitaminosis, especially for young children and pregnant women (Penniston and Tanumihardjo, 2006).

2.7 Requirements for Fortification of Edible Oil

Dary and Mora (2002), reported that the amount of vitamin A to be added in oil mainly depends on the amount of vitamin A intended to be supplied by the fortification program to the population and the amount of edible oil consumed by the that population. Internationally, it is recommended that the amount of vitamin A should meet at least 15% of the recommended daily intake (RDI) for the target population (Dary and Mora, 2002; WHO/FAO, 2006).

Also the quality of edible oil (assessed by peroxide value) prior to fortification is among the factors important to be taken into consideration (Andarwulan *et al.,* 2014). A recent study by Laillou *et al.* (2012) also highlighted peroxide values as a potential barrier to ensuring the stability of retinyl palmitate. The oxidation level greatly interacts with the stability of vitamin A added to the oil. Vitamin A oxidizes faster and loses its activity in the presence of highly-oxidized oils (with a high level of peroxide) (Viana *et al.*, 2007). Several governments have set national standards for fortification practices of edible oils (Sablah *et al.*, 2012; Laillou, Wieringa *et al.*, 2013; Laillou, Panagides *et al.*, 2013). United Republic of Tanzania has passed a legislation that requires edible oil, wheat flour and maize flour produced by large scale manufacturer to be fortified with micronutrients of public health importance (URT, 2011). According to these regulations the edible oil manufactured by large scale processors or any imported oil shall be fortified with vitamin A in the form of retinly palmitate in at levels of 35±5 mg/kg (factory level) and 20-40 mg/kg (regulatory level) (EAS 769: 2013). Also from the same guidelines chemical requirements for fortified edible fats and oils shall conform to maximum values of 10 mEq/kg of peroxide, 0.6 KOH/gm of Acid, 1.5 % m/m unisaponifiable matter and 0.05% m/m insoluble impurities.

2.8 Stability of Vitamin A in the Oil

Vitamin A is unstable when exposed to light particularly ultra violet (UV) light, air, oxidizing agents and heat, due to its high number of unsaturated bonds, which renders it prone to oxidation (Laddawan *et al.*, 2007; Laillou *et al.*, 2012; Andarwulan *et al.*, 2014; Rajaram and Manimehalai 2015; Hemery *et al.*, 2015).

Storage of fortified oils can result in a 30% loss of vitamin A (Atwood *et al*., 1995), and the fluorescent light commonly used in retail stores and households can also induce a vitamin A loss of more than 80% (Pignitter, Dumhart *et al*., 2014; Pignitter, Stolze *et al*., 2014).

In food products, vitamin A is highly unstable especially in medium to high moisture environment and in the presence of pro-oxidant compounds such as iron, heavy metals and acids (even in trace quantities) can accelerate decomposition (Loveday and Singh, 2008). Thus, it is challenging to fortify vitamin A in food matrices and in particular in products containing high amounts of water (e.g. dairy) or in products that are processed or cooked.

It can be assumed that there is a direct relationship between the stability of vitamin A and the oxidation status or peroxide value of the oil. The higher the peroxide values the greater the loss of vitamin A (Viana *et al*., 2007). Using high quality oil and protecting the oil from oxidization and rancidity are therefore basic requirement for preserving the vitamin A. Moreover, on a percentage basis stability of vitamin A depends on the level of fortification. Since there are a given amount of oxidative components in the oil with which the vitamin A can react, the percentage of retention will be greater when higher levels of vitamin A are added.

A study conducted by Habibullah *et al.* (2007) revealed that acid value and iodine value of the oil have significant relationship with vitamin A stability whereby higher acid and iodine values decrease vitamin A retention during storage. Also vitamin A is unstable in thermo processing like cooking, frying, where food materials such as vegetables, meat, seafood etc., are brought into direct contact with the vegetable oil (Orthoefer and List, 2007; Farmer, 2014). Thermal temperatures are usually within the range of 175–195 °C (Banks, 2007). The thermal treatment of the food material is often accompanied with the presence of hydrolysing conditions namely water and steam, causing hydrolysis and triacylglycerol breakdown (free fatty acids) (Gupta, 2005). Thermal treatment (180 °C) associated with a typical domestic application could further accelerate the onset of peroxides and the degradation of vitamin A in vegetable oil (Pignitter *et al*., 2014). Silalahi *et al*. (2017), did a study on stability of vitamin A in fortified palm olein during thermal treatment and the result indicated that vitamin A reduction was between 33% and 49% after sustained thermal treatment of 180 °C.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Research Area

The study was conducted in Dar es Salaam region where data were collected at production sites and selling outlets. Three large scale manufactures of fortified sunflower oil and palm olein which implement fortification of oil were purposively selected and coded as A, B and C for the purpose of confidentiality. Selling outlets from 12 wards of Kinondoni and Ilala Municipalities were also selected.

3.2 Materials

Samples of fortified sunflower oil and palm olein were collected from three large scale fortified edible oil manufacturing plants and other samples were purchased from selling outlets in Kinondoni and Ilala Municipalities. The samples collected at production sites were packed in high-density polyethylene (HDPE) yellow containers and samples at selling outlets were purchased in their original containers (HDPE). Analytical grade reagents, chemicals and equipment for analysis were obtained from Tanzania Food and Drugs Authority (TFDA) chemistry laboratory.

Figure 4: Samples of fortified oil collected at production site packed into high-density polyethylene (HDPE) yellow containers

3.3 Methods

3.3.1 Research design

A cross sectional study was conducted between October 2016 and February 2017. Data collections were based on actual sample collection from manufacturing plants and selling outlets. Checklists as well as face to face administration of semi-structured questionnaire with individual vendors at selling outlets was conducted using a checklist and structured questionnaire (Appendices 1, 2 and 3).

3.3.2 Sampling design and sampling

Stratified sampling design was employed in this study and multi-stage sampling technique (Kothari, 2004). In the first stage, 12 wards namely Upanga West, Mchikichini, Kipawa, Vingunguti, Ukonga, Kinyerezi, Kinondoni, Hananasif, Magomeni, Makumbusho, Kawe and Msasani were randomly selected from the list of 49 wards in Kinondoni and Ilala municipalities obtained from the National Bureau of statistics (NBS, 2013) and Municipal offices. In the second stage, two sub wards were purposively selected from each ward based on the volume of trade experience as informed by Ward Executive Officers (WEOs). Lastly, food selling outlets were randomly selected based on geographical representations in respective sub ward. Three large scale oil manufacturers were purposively selected since are among large oil manufacturers currently implementing fortification in Tanzania.

3.3.2.1 Collection of edible oils samples at production sites

A total of 36 samples were collected from three large scale edible oil manufacturing plants. In every manufacturing plant, samples were collected from three different batches produced on different days. At each batch, a total of three samples were collected at an interval of 20 minutes of production time. One sample of unfortified oil from each of the same batches used for fortification was also collected and used as blank in the vitamin A analysis. For each sample of edible oil, one litre was collected, coded and transported to the TFDA laboratory on the same day. The samples were stored at normal storage conditions at TFDA laboratory and analysis was done on the following day. Checklist (Appendix 1) was used for recording samples information's at production site.

3.3.2.2 Collection of oils samples at the selling outlets

A total of 39 samples were purchased from selling outlets located in the research areas. At each stage the smallest retail pack size (one litre) were purchased and coded. All collected samples were stored in their original container under the normal storage conditions at TFDA laboratory until the day of analysis. Oils samples were collected once and analysed. All information regarding the collected samples at selling outlets was recorded in the checklist (Appendix 2).

3.3.3 Laboratory analysis of retinyl palmitate (RP)

To monitor stability of retinyl palmitate (RP) concentration, samples from same containers collected at production sites were analysed at interval of three and six months on storage. All samples of edible oil (from manufacturing plants and vending outlets) were analysed for retinyl palmitate, acid and peroxide values at TFDA Chemistry Laboratory. Each sample was analysed in duplicate and averages values for each analysed parameters were calculated.

3.3.3.1 Determination of retinyl palmitate (precursor of vitamin A)

Retinyl palmitate content was determined quantitatively by spectrophotometric method (TZS 1313:2010). In a 25 mL amber flask, 0.5 g of oil sample was weighed and then solvent (Dichloromethane) was added up to the mark to dissolve the oil.

The same procedure was repeated using blank oil (unfortified oil from the same batch). Thereafter the absorbance of the samples and controls was read at 325 nm. The amount of retinyl palmitate was calculated using the following equation;

$$\text{Retinyl palmitate (mg/kg)} = \frac{\text{Absorbance}_{\text{corrected}} * VF * CF_{\text{spectrophotometer}}}{A * W}$$

Where;

$\text{Absorbance}_{\text{corrected}} = \text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{unfortified oil}}$

$\text{Absorbance}_{\text{unfortified oil}}$ is the average absorbance of the unfortified oil treated in similar manner as the samples.

A = Retinyl palmitate absorption coefficient in dichloromethane (i.e. 0.094)

VF = Final volume in mls (i.e. 25)

W = Weight of samples in gram

$CF_{\text{spectrophotometer}}$ = Correction factor of the spectrophotometer (i.e. 1)

Figure 6: Preparation of samples for vitamin A analysis

3.3.3.2 Determination of peroxide value

To measure peroxide value (PoV), ISO 3960:2007(E) method was used: Oil (5 ± 0.5 g) was dissolved in 50 mL of glacial acetic acid/isooctane solution. After the addition of 0.5 mL of saturated potassium iodide with gentle swirl for exactly 60 s (timer was used accurate to +/-1s) and 100 mL of distilled water, the solution was immediately titrated with the 0.01N Na_2SO_3 until the yellow color faded. Starch indicator (0.5 mL) was added, and the titration was continued until the blue color disappeared. A blank determination was conducted and the PoV (mEq/kg) was calculated using the following equation:

$$PoV = \frac{(V - V_0) * C_{thio}.F.1000}{m}$$

where

V = the volume of sodium thiosulfate solution used for the determination, in ml

V_0 = the volume of sodium thiosulfate solution used for the blank test, in ml

F = the factor of the 0.01N sodium thiosulfate solution

C_{thio} = the concentration of the sodium thiosulfate solution, in moles per litre;

m = the mass of the test portion, in grams.

3.3.3.3 Determination of acid value (AV)

Acid value was determined according to the ISO 660:2009(E) method. Hot ethanol method was used using indicator. 10 g of sample was weighed into a flask. In a second flask, 50 mL of ethanol containing 0.5 mL of the phenolphthalein indicator was boiled. While the temperature of the ethanol was still above 70 ^oC, the ethanol was carefully neutralized with a solution of sodium hydroxide (0.1N). Then neutralized ethanol was added to the test portion in the first flask and mixed thoroughly. Thereafter, the final solution was boiled and titrated against sodium hydroxide solution (0.01N) until a permanent pink colour persisted for at least 30 s.

The acid value was calculated using the formula as follows;

$$\text{Acid value} = \frac{56.1 * c,V,}{m}$$

Where

c = the exact concentration, in moles per litre, of the standard sodium hydroxide solution used.

V = the volume, in milliliters, of standard sodium hydroxide solution used.

m = the mass, in grams, of the test portion.

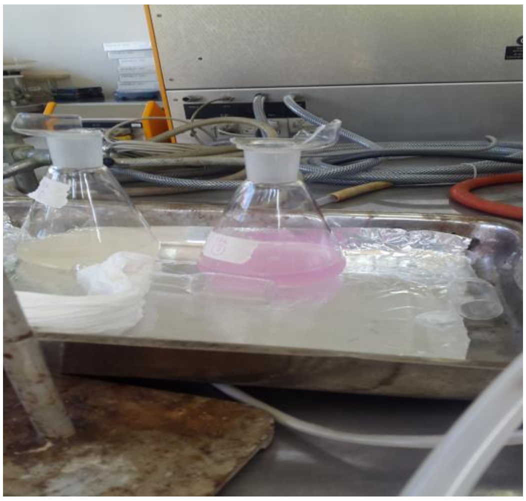


Figure 7: Colour change at the end point of titration during acid value determination.

3.3.4 Administration of questionnaire

Survey semi- structured questionnaire (Appendix 3) was administered to 39 vendors of edible oils at the selling outlets where the samples were purchased. The information collected included demographic information and information on their awareness on handling of fortified edible oil.

3.3.5 Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) software version 20. Descriptive statistics (means and standard deviations) were computed at 95% confidence interval (CI) to determine the levels of retinly palmitate in the oil while frequencies and proportions were computed to assess the extent to which the oil complied with the requirements as well as vendors' awareness on oil fortification and handling of fortified edible oil. A chi-square test of association was computed to assess if there is any association between compliance of the oil and source of sample. Correlation test was computed to check the association between vitamin A, acid value and peroxide value. Analysis of variance (ANOVA) was used to compute the means. Means separation was done by Turkey's Honest**.** All statistical tests were two tailed and p value was considered significant at 0.05.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Distribution of Samples by Source

The distribution of samples across three manufacturers and at selling outlets is shown in Tables 1. Table 2 shows number of tones of edible oils produced per month by each manufacturer.

Table 1: Distribution of collected oil samples by source

Manufacturer	Oil type	Number of samples at production site (n)	Number of samples at selling outlets (n)	Total n (%)
A	Sunflower	9	11	20(26.67)
A	Palm	9	10	19(25.33)
B	Palm	9	5	14(18.67)
C	Palm	9	13	22(29.33)
Total		**36**	**39**	**75(100)**

Table 2: Monthly production of fortified sunflower oil and palm olein for manufacturer A, B and C

Manufacturer	Average number of tones
A	13,500
B	1,000
C	11,000 – 12,000

Source: Survey data (2017)

Sunflower oil samples were collected from manufacturer A only since this was the only one who was found to produce sunflower oil during the survey. With regards to palm olein, more samples were collected at selling outlets and few samples from manufacturer B due to low production volume as compared to others and that large part of it was sold outside the research area (Dar es Salaam region).

4.2 Retinyl Palmitate (PR) Levels in Fortified Sunflower Oil and Palm Olein

Table 3 and 4 presents mean retinyl palmitate (RP) concentration in fortified sunflower oil and palm olein, respectively collected at production sites and selling outlets.

Table 3: Mean retinyl palmitate (RP) levels in fortified sunflower oil

Source of samples	Number of samples (n)	RP concentrations (mg/kg)	RP concentration (mg/kg) range
Production site	9	12.42 ± 1.18	11.24-14.78
Selling outlets	11	10.19 ± 2.14	8.05-12.33
All	**20**	**11.20 ± 2.07**	**8.05-14.78**

Data are expressed as the mean and standard deviation (SD).

Table 4: Mean retinly palmitate (RP) levels in fortified palm olein across manufactures

Manufacturer	Production sites				Selling outlets			
	Number of samples (n)	Mean ± SD	Range	P – value (ANOVA)	Number of samples (n)	Mean ± SD	Range	P – value (ANOVA)
A	9	21.58^{a} ±2.41	19.6-26.2	0.000	10	18.84^{a} ±9.25	10.9-42.3	0.003
B	9	66.33^{c} ±17.55	50.3-91.0		5	46.54^{c} ±18.36	27.9-74.7	
C	9	42.49^{b} ±6.92	31-48		13	28.40^{b} ±13.58	14.1-60.8	
All samples	**27**	**43.47 ±21.41**	**19.6-91.0**		**28**	**28.79 ± 15.99**	**10.9-74.7**	

Means values with different superscripts in a column are significantly difference at $p < 0.05$.

From the results in Table 3, the concentration of retinyl palmitate (RP) in fortified sunflower oil samples collected at production site had mean of 12.42 mg/kg (range 11.13 - 14.02 mg/kg) which was below the requirement of 35±5 mg/kg as set out under EAS 769: 2013. Whereas concentration of retinyl palmitate (RP) in fortified sunflower oil samples collected from selling outlets ranged from 7.32 - 13.85 mg/kg with a mean of 10.19 mg/kg. This was also below the regulatory levels of 35±5 mg/kg.

Considering retinyl palmitate (RP) concentration in fortified palm olein collected at production site (Table 4), for manufacture A ranged from 19.57 to 26.20 mg/kg with the mean of 21.58 mg/kg which was below the requirement of 35±5 mg/kg as per EAS 769: 2013. However, the results showed high concentration of retinyl palmitate in fortified palm olein samples for manufacturer B (66.33 mg/kg) and C (42.49 mg/kg) collected at production site. The results also indicate significant differences in mean concentrations of retinyl palmitate (RP) among manufactures ($p < 0.05$). The variation could probably be due to the disparity in method of fortification used or due to poor quality assurance and quality control measures that can result to incorrect amount of retinyl palmitate dosage during production (WHO, 2006). A storage condition like high temperature might also have effect on the strength of the fortificant (retinyl palmitate) since it is unstable when stored under high temperatures (Kuong *et al*., 2016).

At the selling outlets (Table 4), results indicated that, the mean concentration of retinly palmitate in fortified palm olein collected from manufacturer C was within recommended factory levels (i.e. 35±5 mg/kg), while the oils from manufacturer A had low concentration (18.84 mg/kg) while from manufacturer B high concentration of retinyl palmitate (46.54 mg/kg) was recorded indicating inconsistence in compliance with the fortification requirements. Analysis of variance (ANOVA) showed significant differences in retinly palmitate concentration in palm olein samples collected at selling outlet for manufacturers A, B and C. The study observations showed that mean levels of retinly palmitate (RP) at the production site 21.58, 66.33 and 42.49 mg/kg for manufacturer A, B and C, respectively, were higher than the mean levels of retinly palmitate (RP) at selling outlets i.e. 18.84, 46.54 and 28.40 mg/kg for manufacturer A, B and C, respectively.

Several studies indicated loss of vitamin A concentration during storage due to various factors including fluorescent light commonly used in retail stores (Pignitter and Dumhart *et al*., 2014) and chemical reactions (oxidation) that can take place even when the oil is stored under normal condition (Laillou *et al.,* 2012) or under elevated temperatures (Dewi *et al.,* 2017). Oxidation reactions can continue during storage of the packaged product, as the oil in the product continues to be broken down via autoxidation and develops oxidized or rancid flavor in the product (Gupta, 2005).

4.3 Compliance of Fortified Oil with Standard

Table 5 shows compliance of fortified sunflower oil and palm olein collected at production site and at selling outlets compared with the East African Standard specification (EAS 769: 2013). Requirements for vitamin A levels in fortified edible oil are shown in Table 6.

Table 5: Compliance of fortified sunflower oil and palm olein as compared to the mandatory regulation requirements grouped by sources of sample

Source of sample	No. of samples assessed	No. of samples complied	% Compliance	No. of samples not complied	% Not complied	$\chi 2$	P-value
Production site	36	3	8.33	33	91.67	0.982	0.412
Selling outlets	39	13	33.33	26	66.67		
All samples	**75**	**16**	**21.33**	**59**	**78.67**		

The results indicate no statistically significant association between compliance and source of samples ($p > 0.05$)

Table 6: Requirements for vitamin A in fortified edible oil or fat

Nutrient	Fortification compound	Recommended factory level, mg/kg	Regulatory levels, mg/kg	
			Minimum	**Maximum**
Vitamin A	Retinyl palmitate	35±5	20	40

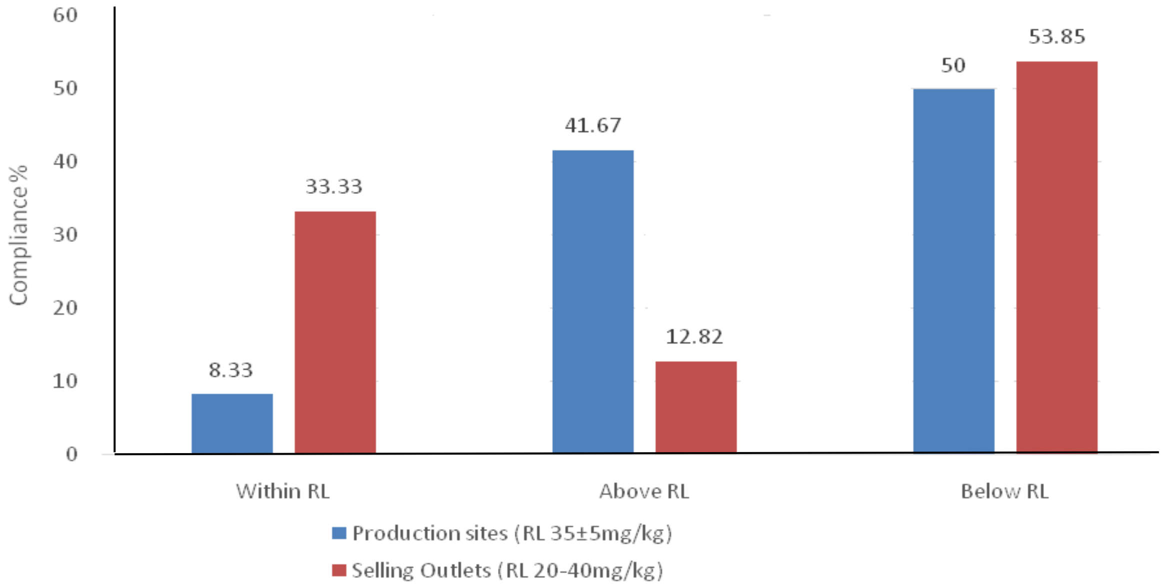
Source: EAS 769:2013

Based on the requirements for vitamin A (retinyl palmitate) in fortified edible oil at factory level (35 ± 5 mg/kg) and regulatory levels (20 - 40 mg/kg), the results obtained showed that, 3 (8.33%) out of the 36 samples of fortified oils collected at production sites complied with the fortification specification while 13 (33.33%) out of 39 samples collected at selling outlets complied with the fortification requirement. However, more than 41.67% of the oils collected at production sites had retinly palmitate at levels above the required limit while 50% had retinly palmitate levels below the required limit (Figure 8). From the same figure, it shows that the oils collected from selling outlets (12.82%) had retinly palmitate at levels above the regulatory levels and 53.85% of the samples had retinly palmitate below the regulatory limit.

Compliance at retail rather than at production site seems to be unusual. High level of compliance at selling outlets was because of the exceeding high levels of retinly palmitate content at factory which become lowered during storage. Storage conditions like temperature can cause vitamin A degradation in the oil (Kuong *et al*., 2016). Also physical chemical changes of the oil during storage cause degradation of Vitamin A even in the normal storage condition (Andarwulan *et al.*, 2014). Low level of compliance at production was brought by 41.67% of collected oil samples being having higher content of Vitamin A and 50% had levels below the required standard (EAS, 2013). A chi-square test for association shows that there was no significant association between compliance and source of samples ($p > 0.05$) (Table 5).

The low level of compliance was also observed in the study done in Nigeria by Ogunmoyela *et al*. (2013) on the critical evaluation of survey results of vitamin A and Fe levels in the mandatory fortified food vehicles whereby 24.2% of the vegetables oil samples complied based on the requirements of 20 000 – 14 000 IU/kg. These authors suggested that high levels of specified standards might be one of the factors encouraging low compliance as most of samples were found with low level of vitamin A. However, from the present study, it was observed that there was a great variation in compliance between oils samples collected from manufactures as well as those from selling outlets since some of the samples contained high and some had low levels of retinly palmitate which could be the results of over dosage or under dosage of fortification premix during processing. The observed low level of compliance could probably be due to the results of poor quality assurance and quality control measures regarding vitamin A fortification. During the survey, poor storage of fortificant was observed among some of the manufacturers since fortificant were stored just close to production area at higher temperature. Also, it was observed that analysis of vitamin A at the factories was not done as per guidelines (WHO, 2006) since there were no records of the amount of vitamin A added at the start up or even at the end of production that would allow to check the correctness of premix dosage (flow rate). Also it was observed that semi-quantitative analysis was mostly done rather that quantitative analysis contrary to the WHO guideline for food fortification. Also according WHO guideline for food fortification, (2006) and in order to ensure compliance at production level, issue of internal monitoring which refers to quality control and quality assurance practices, is required to be taken into much consideration by producers.

The study conducted by Luthringer *et al*. (2015), also indicated that poor regulatory monitoring of food fortification including internal quality assurance and control measures as well as external monitoring by government are the barriers of food fortification compliant with relevant standards. Therefore, improving internal quality assurance and control measures at factory level as well as external monitoring by government can improve compliance in food fortification.



RL=Recommended levels

Figure 8: Percentage compliance to EAS 769:2013 specifications for retinyl palmitate content in fortified sunflower oil and palm olein samples collected from production sites and selling outlets

4.4 Concentrations of Retinyl Palmitate (RP) in Fortified Sunflower Oil and Palm Olein during Storage

The results of the concentration of retinyl palmitate in fortified sunflower oil and palm olein samples collected from production sites that were stored and analysed after three and six months are shown in Table 7 and 8, respectively.

Table 7: Concentration of retinly palmitate (RP) in fortified sunflower oil samples stored for three and six months under normal storage conditions

Manufacturer	Mean concentration of retinyl palmitate (RP) (mg/kg)				
	RP at time of sample collection	**RP conc. after 3 months**	**%decrease after 3 months**	**RP conc. after 6 months**	**%decrease after 6 months**
A	$12.42^{b} \pm 1.18$	$11.09^{a} \pm 1.07$	10.87	$10.95^{a} \pm 1.07$	11.84

Data were expressed as the mean ± SD (n = 3). The means values with different superscripts in a row are significantly difference at $p < 0.05$.

Table 8: Concentration of retinly palmitate (RP) in fortified palm olein samples stored for three and six months under normal storage conditions

Manufacturer	Mean concentration of retinyl palmitate (RP) (mg/kg)				
	RP at time of sample collection	**RP conc. after 3 months**	**% decrease after 3 months**	**RP conc. after 6 months**	**% decrease after 6 months**
A	$21.5^{b} \pm 2.41$	$17.0^{a} \pm 4.29$	21.22	$16.70^{a} \pm 4.10$	22.61
B	$66.33^{b} \pm 17.55$	$43.06^{a} \pm 11.24$	35.08	$32.52^{a} \pm 4.57$	50.97
C	$42.49^{b} \pm 6.92$	$38.24^{a} \pm 6.23$	10.00	$36.33^{a} \pm 5.92$	14.49
All	**43.47 ± 21.41**	**32.80 ± 13.79**	**24.55**	**28.51 ± 9.87**	**34.41**

Data were expressed as the mean ± SD (n = 3). The means values with different superscripts in a row are significantly difference at $p < 0.05$.

The results showed a significant decrease of retinyl palmitate (RP) concentration in fortified sunflower oil by a maximum of 11.84% (Table 7), 22.61, 50.97 and 14.49% in palm olein for manufacturer A, B and C, respectively (Table 8) after six months of storage under normal conditions. A significant decrease of retinyl palmitate (RP) concentration was also observed even after three months of storage for both oil types from manufacturers.

The implication is that, an accelerated degradation of retinyl palmitate (RP) occurs during storage of fortified oils under normal storage and handling conditions, which could be due to the effect of exposure of light during storage. Since vitamin A is relatively unstable under normal storage conditions, light can destabilize the vitamin particularly on longer storage time. The instability is mostly due to its chemical structure, which contain many double bonds susceptible to degradation (Andarwulan *et al.,* 2014). An even higher loss of vitamin A was observed by Hemery *et al*. (2015) who detected a vitamin A loss of 60 - 68% in fortified soybean oil exposed to natural light after two months of storage under normal condition.

Vitamin A losses in oils could be also attributed to the nature of the packaging material used by the vendors (Chakravarty, 2000). Storing fortified oil in opaque containers or plastic material of Polymerizing Vinyl Chloride (PVC) or acrinytrile and away from open, direct sunlight can minimise the hydrolytic and oxidative deterioration of the oil rather than clear plastic bottles (WHO, 2006; Oluwalana *et al*., 2015). The fortified edible oil samples in this study were all packaged in a high-density polyethylene (HDPE) yellow containers and almost 99% of the samples were sold under shade so the loss of vitamin A could be attributed to oxidative deterioration oil during storage.

4.4.1 Chemical characteristics in fortified sunflower oil and palm olein during storage

The results of peroxide value (PoV) in fortified sunflower oil and palm olein are presented in Table 9 and 10, respectively.

Table 9: Peroxide value (PoV) in fortified sunflower oil during storage

Manufacturer	Mean peroxide value (PoV) (mEq/kg)			p-Value
	PoV at time of sample collection	**PoV after 3 months**	**PoV after 6 months**	
A	$1.67^a \pm 0.27$	$10.32^b \pm 2.25$	$31.57^c \pm 3.87$	0.00

Data are expressed as the mean ± SD (n=3). The means values with different superscripts in a row are significantly difference ($p < 0.05$).

Table 10: Peroxide value (PoV) in palm olein during storage

Manufacturer	Mean peroxide value (PoV) (mEq/kg)			p-Value
	PoV at time of sample collection	**PoV after 3 months**	**PoV after 6 months**	
A	$0.72^a \pm 0.13$	$0.86^a \pm 0.36$	$4.44^b \pm 0.29$	0.00
B	$14.77^a \pm 4.92$	$14.88^a \pm 0.29$	$15.08^a \pm 0.315$	0.106
C	$1.58^a \pm 0.06$	$3.17^b \pm 0.47$	$6.15^c \pm 0.67$	0.00
All samples	$\mathbf{5.69^a \pm 6.54}$	$\mathbf{6.3^b \pm 6.23}$	$\mathbf{8.56^c \pm 4.78}$	**0.00**

Data are expressed as the mean ± SD (n=3). The means values with different superscripts in a row are significantly difference ($p < 0.05$).

From the results, it showed that, significant increase in PoV was observed after 3 months of storage in sunflower oil samples from1.67 to 10.32 mEq/kg (Table 9) and palm olein samples increased from 1.58 to 3.17 mEq/kg for manufacturer C (Table 10). PoV for palm olein samples from manufacturer B was higher than the prescribed standard value for fortification which is 10 mEq/kg (TZS: 1313, 2010).

The results of palm olein for manufacturer A and B showed no significant increase of PoV after 3 months of storage but a significant increase was observed after six months of storage for both samples. The increase of peroxide value with storage time was also observed by Laillou *et al*. (2012) and Pignitter *et al*. (2014) in fortified soya been stored under normal temperature. Table 11 and 12 shows the results of acid values in fortified sunflower oil (for manufacturer A) and palm olein (for manufacturer A, B and C), respectively.

Table 11: Acid value (AV) in fortified sunflower oil during storage

Manufacturer	**Mean Acid value (AV) (mgKOH/g of oil)**			**p-value**
	AV at time of sample collection	**AV after 3 months**	**AV after 6 months**	
A	$0.61^{a} \pm 0.17$	$0.74^{b} \pm 0.15$	$0.96^{c} \pm 0.24$	0.00

Data are expressed as the mean ± SD (n = 3). The means values with different superscripts in a row are significantly difference ($p < 0.05$).

Table 12: Acid values (AV) in fortified palm olein during storage

Manufacturer	**Mean Acid value (AV) (mgKOH/g of oil)**			**p-value**
	AV at time of sample collection	**AV after 3 months**	**AV after 6 months**	
A	$0.18^{a} \pm 0.11$	$0.22^{a} \pm 0.18$	$0.23^{a} \pm 0.02$	0.467
B	$0.36^{a} \pm 0.10$	$0.71^{b} \pm 0.08$	$0.78^{b} \pm 0.07$	0.00
C	$0.18^{a} \pm 0.19$	$0.34^{b} \pm 0.03$	$0.45^{c} \pm 0.04$	0.00
All samples	$\mathbf{0.2^{a}4 \pm 0.13}$	$\mathbf{0.42^{b} \pm 0.22}$	$\mathbf{0.49^{b} \pm 0.42}$	**0.00**

Data are expressed as the mean ± SD (n = 3). The means values with different superscripts in a row are significantly difference ($p < 0.05$).

The results indicate a significant increase of acid value in fortified sunflower oil and palm olein samples (for all manufacturers) after three months of storage. This means that after three months of storage the oils became rancid probably due to exposure to light and air. Also the results showed a higher increase of acid value in sunflower oil after six months of storage. However, it was noticed that acid value was not too high in palm olein samples after six months of storage probably due to less rancidity rate. This finding goes hand in hand with observations made by Abdelmonem *et al.* (2012) that there was a relative small change observed in acidity values of stored soybean oil samples.

Therefore in this study, using correlation test, it was observed that a significant ($p = 0.01$) decrease in retinyl palmitate concentration was associated with an increase in peroxide and acid value during storage. The peroxide value (POV) which is influenced by temperature, time and light measures the extent to which primary oxidation of oils (acidification) may have occurred. The oxidation level greatly interacts with the stability of vitamin A added to the oil. Vitamin A oxidizes faster and loses its activity in the presence of highly-oxidized oils (with a high level of peroxide) (Viana, 2002). The same observations was seen this study where a high loss of retinyl palmitate concentration by 50.97% in palm olein samples for manufacturer B was observed after six months of storage, which corresponded with high peroxide value (15.08 mEq/kg).

A loss of vitamin A associated with an increase in the peroxide value with storage time has also been reported by Laillou *et al*. (2012). Also Pignitter *et al*. (2014) reported loss of retinyl palmitate in fortified soybean oil by a maximum of 84.8% after 56 days of storage under normal condition (22 - 32 °C). Instability of retinyl palmitate can also be associated with other factors like amount of antioxidant added in the oil during production and traces of metals (Pignitter, 2014; Laillou, 2012; Loveday and Singh, 2008) which was not observed in this study.

Therefore, it is essential to improve peroxide and acid values of the oil in order to make vitamin A more efficient and with longer shelf life (Laillou *et al*., 2012).

4.5 Vendor's Awareness of Oil Fortification and Handling of Fortified Edible Oil

4.5.1 Demographic characteristics of vendors of edible oil

Demographic characteristics of vendors of edible oil from selling outlets in Ilala and Kinondoni municipalities are presented in Table 13. Out of 39 interviewed edible oil vendors 27(69.2%) were male and 12(30.8%) were females. More male than females responded because individuals in the study areas were randomly selected based on where samples were purchased. Respondents' average age was 34.4 years (ranges from 18-47 years). Majority (46.4%) of respondents had primary education while 21.4 % had no formal education.

Table 13: Demographic characteristics of vendors of edible oil

Variable	Ilala (n= 20)	Kinondoni (n=19)	Pooled (n=39)
Average age of respondents	32 .6	34	34.4
Level of education (%)			
Secondary school	3 (15)	3(15.8)	6(15.4)
Primary school	12(60)	11 (57.9)	23(59.0)
No formal education	5(25)	5 (26.3)	10(25.6)
Gender of respondent (%)			
Female	5(25)	7(36.8)	12 (30.8)
Male	15 (75)	12(63.2)	27 (69.2)

4.5.2 Awareness of respondents

Table 14 shows survey findings on vendor's awareness regarding edible oil fortification and handling.

Table 14: Awareness of vendors on edible oil fortification

Variable	Ilala (n= 20)	Kinondoni (n=19)	Pooled (n=39)
Awareness on fortification (%)			
Yes	2 (10.0)	1 (5.3)	3 (7.7)
No	18 (90.0)	18 (94.7)	36 (92.3)
Awareness on VA fortified edible oil (%)			
Yes	0 (0.0)	0 (0.0)	0 (0)
No	20 (100.0)	20 (100.0)	39 (100)
Product displaying during selling (%)			
Under shade	19 (95)	17 (89.5)	36 (92.3)
Under direct sunlight	1 (5)	2 (10.5)	3 (7.7)
Why displaying fortified edible oil under shade (%)			
Aware	3 (15.0)	3 (15.8)	6 (15.4)
Not aware	17 (85.0)	16 (84.2)	33 (84.6)

From the results, majority (92.3%) of edible oil vendors had never heard about food fortification. However, only 7.7% of vendors were aware of adding micronutrient in wheat flour and salt. Regarding edible oil fortification, it was seen that 100% of edible oil vendors were not aware of edible oils fortification with retinyl palmitate. The implication is that they might not handle the fortified oils correctly leading to deterioration of the retinyl palmitate contained in the oil.

Low awareness on food fortification and edible oil fortification in specific might be due to the fact that majority of respondents had low level of education (Büyükkaragöz *et al.*, 2014). Another reason could be linked with the dominance of male among respondents who in most cases are not targeted for nutritional education. The present study observed low awareness on edible oil fortification among all interviewed males and females.

With regard to handling of fortified edible oil, it was observed that, 36 (92.3%) of the vendors store edible oil under shade during displaying for sale. However 6 (15.4%) were able to associate storage of the oil and preservation of the quality of the oil while 33 (84.6%) were not. Among the vendors 3 (10.7%) was found displaying the oil under direct sunlight for the reasons that customer can easily see the type (brand) of oil they sell. Vitamin A is affected by sunlight (Laillou *et al.*, 2012), displaying oils under direct sunlight accerelate vitamin A degradation as the result consumers may end up consuming oil which are inadequately fortified. WHO recommend that at all processes, from the factory to the retail stores, and right up until the time of consumption by targeted individuals, it is vital that the product maintains its expected quality (WHO, 2006). Vitamin A loss occurs during processing, distribution and storage, therefore special attention should be paid during handling, such as limiting exposure to light and temperatures to minimise excessive oxidation of vitamin A during distribution and storage (Andarwulan *et al*., 2014). The technique of handling and storing fortified food before consumption can positively influence the content of vitamin A (Dwyer *et al*., 2015).

Therefore, an effective fortification practices should be coupled with clear and measurable retailers' best handling practices. This can be successfully done by raising awareness among handlers through education programmes, providing simple information about storage and handling (Dwyer *et al*., 2015; Nair *et al*., 2015). The advantage of raising awareness was observed by edible oil vendors who previously displayed the oils under the sun on the roadsides from Singida all the way to Dodoma but nowadays they are displaying under constructed shade.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study showed that current levels of retinyl palmitate in fortified edible oils are unsatisfactory across almost all manufacturing plants. Only 33 % and 8.33% edible oil samples collected at production site and selling outlets respectively, complied with the standards recommended level for retinyl palmitate concentration which is 35±5 mg/kg at factory level and 20-40 mg/kg at selling outlets. Non compliance was very likely due to under-addition/over-addition of fortification premix at the industry level due to technological or other constraints and due to poor internal monitoring of products during processing that lead to some samples being having low and some had high concentration of retinyl palmitate. Although fortification is mandatory for all large scale edible oil manufacturers, still there is lack of commitment among manufacturers which is indicative of inadequate or ineffective monitoring of compliance by the regulators, possibly due to capacity or other constraints and the absence of self-regulation. Also this study found that awareness regarding edible oils fortification and handling of fortified edible oils was very low among vendors in the prescribed study area. Ultimately, the effect is that there would be improper handling of the product during storage and this potentially reduced the expected amount of vitamin A targeted to final consumers.

5.2 Recommendations

Based on the findings of this study it is recommended that, food quality regulators and other stakeholders should strengthen the external monitoring systems towards fortified edible oil manufacturers to ensure adherence to fortification standards since mandatory legislation alone will not automatically lead to increased coverage of complied fortified products without strengthened enforcement and adequate capacity.

Also training/education programmes which are designed to create and maintain awareness among edible oil vendors regarding edible oil fortification and handling of fortified edible oils need to be developed. Also further investigation /study need to be conducted to explore what are the other reasons that are linked with compliance that contribute to having greater variations of retinyl palmitate concentration in fortified edible oil especially at production sites.

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APPENDICES

Appendix 1: Checklist for Collection of Fortified Edible Oil Samples at Production Site

COMPLIANCE OF FORTIFIED SUNFLOWER AND PALM OLEIN OILS WITH REQUIREMENTS FOR RETINLY PALMITATE CONTENT

1.0 General information

1.1 Municipality: (Please tick one appropriate)

(i) [Ilala] (ii) [Kinondoni]

1.2 Ward: ______________________ Street: ____________________

1.3 Name of the premises (Manufacturing plant):

____

1.4 Date of sample collection: [dd | mm | yyyy]

2.0 Information regarding fortification process

2.1 System used for addition of vitamin A in the oil (Please tick one)

(i) [Continuous] (If the answer is (i) go to 2.2)

(ii) [Batch] (If the answer is (ii) go to 2.3)

2.2 (i) Amount of vitamin A (gm) added in the oil per minute [ ]

(ii) Oil flow rate (L/min)

2.3 (i) Actual amount of vitamin A added into the oil ☐

(ii) Theoretical amount based on calculations ☐

2.4 Mechanism used for homogenizing/mixing vitamin A in the oil (Please tick)

(a) Agitation (b) Circulation

(c) Both agitation and circulation

3.0 Information about edible oil sample

3.1 Sample identification number:

3.2 Batch number

3.3 Type of edible oil (Please tick one)

(i) Sunflower oil (ii) Palm olein

3.4 Type of packaging material (Please tick where appropriate)

(i) HDPE (ii) PET

4.0 Information related to quality assurance/quality control

4.1 Is the mill supervisor trained on QA/QC with regards to fortification?

(i) Yes (If the answer is (i) go to 4.2)

(ii) No (If the answer is (ii) go to 4.3)

4.2 For the last time when did the supervisor received fortification QA/QC training?

(i) Less than a year (ii) Two years past

(iii)	Three years past	(iv)	Over three years back

4.3 Does the mill have laboratory for testing of vitamin A content?

(i)	Yes	(ii)	No

5.0 Laboratory result for key parameters (To be filled after laboratory analysis)

(i)	Retinly palmitate (Vitamin A) in g/kg	
(ii)	Peroxide value in Meq/Kg	
(iii)	Acid value in mgKOH/gm	

Appendix 2: Checklist for Collection of Fortified Edible Oil Samples at Food Selling Outlet

COMPLIANCE OF FORTIFIED SUNFLOWER AND PALM OLEIN OILS WITH REQUIREMENTS FOR RETINLY PALMITATE CONTENT

1.0 General information

1.1 Municipality:

(i) [Ilala] (ii) [Kinondoni]

1.2 Ward: ________________________ Street: ______________________

1.3 Name of the premises (selling outlet): ______________________________________

1.4 Date of sample collection:

dd	mm	yyyy

2.0 Information about edible oil sample

2.1 Sample identification number: [ ]

2.2 Batch number [ ]

2.3 Type of edible oil (Please tick one)

(i) [Sunflower oil] (ii) [Palm olein]

2.4 Type of packaging material (Please tick where appropriate)

(i) [HDPE] (ii) [PET]

5.0 Laboratory result for key parameters (To be filled after laboratory analysis)

(i)	Retinly palmitate (Vitamin A) in g/kg	
(ii)	Peroxide value in Meq/Kg	
(iii)	Acid value in mgKOH/gm	

Appendix 3: Questionnaire for Edible Oil Vendors

Compliance of Fortified Sunflower and Palm Olein Oils with Requirements for Retinly Palmitate Content

My name is Rhoda Nathaniel Kidolezi, a student from Sokoine University of Agriculture. I am currently doing my research on compliance of fortified sunflower and palm olein oils with requirements for retinly palmitate content which requires to be completed for me to be able to graduate for Msc. Food Safety and Quality Assurance. The purpose of this interview is to collect information on handling of fortified edible oil. This information will be useful for assessment of vendors' awareness on handling of fortified edible oil. I am requesting you to take part in this research project. You will be interviewed on your awareness on handling of fortified edible oil. The interview will be recorded in a questionnaire. No one else but the interviewer will be present unless you would like someone else to be there. The information recorded will be confidential, and no one else except the researchers will be able to access it. The findings of this will have no direct effect to you instead they will help fortified edible oil manufacturers and food safety regulators in designing intervention strategies to ensure minimization of the loss of vitamin A from fortified edible oil. If you have any question regarding this research please ask to the interviewer and he/she will explain to you.

1.0 General information

1.1 Municipality: (Please tick one)

(a) Ilala (b) Kinondoni

1.2 Ward: ______________________ Street: ____________________

1.3 Name of the premises (selling outlet):

____

1.4 Date of sample collection:

Dd	mm	yyyy

2.0 Personal information of the respondent

2.1 Sex of respondent (Please tick one) (a) Male (b) Female

2.2 Age of the respondent (in years)

2.3 Level of education

3.0 General information regarding food fortification

3.1 Have you ever heard about food fortification? (circle one)

(a) Yes

(b) No (If no skip to question No. 4)

3.2 Do you know that edible oil is fortified with vitamin A?

(a) Yes

(b) No

4.0 Information regarding handling of edible oil

4.1 Where do you place edible oil when displaying for sale?

(a) Under shade (If the answer is (a), go to question No. 4.2)

(b) under direct sunlight (If the answer is (b), go to question No. 4.3)

4.2 Why do you place edible oil under shade when displaying for sale?

……………………………………………………………………………………

……………………………………………………………………………………

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4.3 Why do you place edible oil under direct sunlight when displaying for sale?

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4.4 How do you sell the edible oil to your customers?

(a)	Sell them as they are (in their original containers)	If the answer is b or c, go to 4.5
(b)	Open the containers and sell them in small amount	
(c)	Both a and b	

4.5 How long does it take for edible oil to finish sold in small quantities since the container was first opened?

(a)	Less than a week
(b)	A week
(c)	More than two weeks