STUDY ON PHYSICOCHEMICAL PROPERTIES OF BLENDS OF FLAX SEED, NIGER SEED AND SESAME SEED OILS

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BY

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STUDY ON PHYSICOCHEMICAL PROPERTIES OF BLENDS OF FLAX SEED, NIGER SEED AND SESAME SEED OILS

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Declaration

I hereby declare that this thesis entitled "**PHYSICOCHEMICAL PROPERTIES OF BLENDS OF FLAXSEED, NIGER AND SESAME SEED OILS**" was prepared by me, with the assistance of my advisor. The work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted, in whole or in part, for any other degree or professional qualification. Parts of this work have been published in [state previous publication].

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List of abbreviations and acronyms

ALA	Alpha Linolenic Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemistry
AOAS	America Oil Analytical Society
AOS	Acceleration Oxidative Stability
BHT	Butylated Hydroxytoluene
BHA	Butylated Hydroxy Anisole
CVD	Cardiovascular disease
FAMEs	Fatty Acid Methyl Esters
FFA	Free Fatty Acid
GMOs	Genetically Modified Organisms
IT	Induction Time
IV	Iodine Value
MUFA	Mono unsaturation Fatty Acid
OSI	Oxidative Stability Index
PFA	Prevention of Food and Adulteration
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
RI	Refractive Index
SFA	Saturated Fatty Acid
SFG	Soluble Flaxseed Gum
SPSS	Statistical Package for the Social Sciences
SSO	Sesame Seed Oil
SVs	Saponification Values
TBHQ	Tertiary Butyl Hydro Quinone
TGs	Triglycerides
TPC	Total Polar Compound
WHO	World Health Organization

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Abstract

Today, a wide range of edible oils are available in the market but no single edible oil is able to meet recommendations given for healthy oil. So, blending of different edible oils is an emerging mechanism to develop a healthier and stable edible oil so as to satisfy consumer needs. Blending is an economical way of modifying the physicochemical characteristics besides enhancement of oxidative stability. Thus, in this study blends of sesame, niger and flax seed oil in different proportions were evaluated for various physicochemical parameters: density, viscosity, refractive index, accelerated oxidative stability, saponification value and peroxide value were determined using standard method using digital analytical balance, Brookfield RHEO meter DV3T instrument, Krusi Optronic, 892 professional Rancimate apparatus, AOAC official Method no. 920.160 and titration with $Na_2S_2O_3$ solution, respectively. The density, viscosity, refractive index, saponification value and accelerated oxidative stability value ranged: 0.921($N_{12}F_{81}S_7$)-0.957($N_8F_{20}S_{72}$) g/mL; 46.2(S_{100})-63.6(F_{100}) centipoise; 1.473(F_{100})-1.482(S_{100}); 171($N_{34}S_{66}$)-177($N_{47}F_{53}$) mg of KOH/g of oil; 0.874(F_{100})-4.697(S_{100}) hours, respectively. Similarly, the peroxide value after 15, 45 and 75days of storage time were found in the range: 1.493($N_{34}S_{66}$)-3.186 (F_{100}), 2.469 (S_{100})-5.01($N_{12}F_{81}S_7$) and 3.493(S_{100})-7.778(F_{100}) meq O_2/kg, respectively. It was concluded that the flax seed oil has low stability was enhanced by addition of either of niger or sesame oil separately and by addition of sesame and niger oil at the same time. The best stability was seen at $N_8F_{20}S_{72}$ with the mixing ratio of niger, flaxseed and sesame seed oil. The sensory evaluation based on the 9-hedonic scale were done and the results showed that wthe overall acceptance of the tested parameters was better in using tertiary ($N_8F_{20}S_{72}$) blended oils followed by the binary blended ($N_{34}S_{66}$) and single flax seed oil (F_{100}). Further study in chemical composition analysis for blended and none-blended oils (niger, sesame and flaxseed oil) and other types of oil which are not included in this study are highly recommended.

Key words: Viscosity, Refractive Index, Saponification Value, Accelerated Oxidative Stability, Blending of oil, Edible oil.

CHAPTER 1. INTRODUCTION

1.1. Background

Oils and fats are used for cooking and frying as well as in food formulations (Hashempour-Baltork *et al*., 2016; Dimberu *et al*., 2015). The widely consumed edible oils in worldwide are sourced from either oilseeds or vegetables such as sunflower oil, rice bran oil, palm olein oil, soybean oil, mustard oil, coconut oil, groundnut oil, sesame oil, flax oil and niger oil. Among these sources of oils, oilseeds are leading suppliers of superior quality and specialty vegetable oils to nutritional products, natural food and premium snack foods worldwide. They are primarily used for the extraction of edible oils. In addition to the oils extracted from oilseeds, oilseed cake or the meal obtained after extraction of oil is largely used as cattle feed or fertilizer. The defatted meal is a rich source of protein and, if suitably processed, has a great potential as food for human consumption (Deme *et al*., 2017; Goyal *et al*., 2014).

Some oilseeds are consumed as such and, though in small amounts, contribute to the daily intake of several nutrients (Deme *et al.*, 2017; Bekhit *et al.*, 2018). Niger seed (*Guizotia abyssinica L.f.*), flax (*Linum usitatissimum*), sunflower (*Helianthus annuus L.)* and sesame (*Sesamum indicum L.*) are major oil seed crops cultivated in the world and used for edible oil sources, although other oilseed are used as sources of oil (Deme *et al*., 2017; Goyal *et al*., 2014; Peiretti and Tassone, 2015).

Different types of oils extracted from different oilseeds can have different chemical composition that can determine their quality. The quality of the oil that comes from different sources depends on different physical properties (such as odor, color, density and viscosity), chemical properties (such as oxidative stability, shelf life and ability to produce toxic chemicals during frying) and nutritional values (such as fatty acid and antioxidants amounts) of oils. Among these properties, oxidative stability of the oil and nutritional values are the prominent properties of the oil under consideration while taking about the oil quality, since they have a direct impact on the health of the consumer (Hashempour-Baltork *et al.*, 2018; Hashempour-Baltork *et al.*, 2016).

Oxidative stability of edible oils can be improved by addition of antioxidants and

helpful in retarding the oxidation of lipids. However, synthetic antioxidants that are commonly used in the food industry such as butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), tertiary butyl hydroquinone (TBHQ), and propyl gallate may have toxic effects. Therefore, the consumers prefer the least-processed foods and avoid synthetic

vegetable oils to obtain oils/fats with improved texture and oxidative stability has been used for a long time. Hydrogen gas and nickel as a catalyst saturate some double bonds in the unsaturated fatty acids. Techniques such as hydrogenation can the cause for the transformation of cis fatty acids to trans fatty acids which cause health impact to the consumers, since trans fatty acids can cause different diseases. A
prevents isomerism, it needs sophisticated instrument that cost the techniques. World Health Organization (WHO) stated that genetic modification organisms(GMOs) have potential risks for human health and growth and have no history of being consumed as a secure food and also that replacing a new gene to the genome of the food modified can cause undesirable developmental and physiological effects (Algan Ozkok, 2015). Among these techniques, oil blending or extract addition have emerge as an economical way of modifying the physicochemical characteristics besides improving the oxidative stability without any chemical or biological process (Kavuncuoglu *et al*., 2016; Hashempour-Baltork *et al.*, 2018; Hashempour-Baltork *et al*., 2016).

In addition to improving of oxidative stability, blending of different fats/oils with different

fats/oils leads to change in the physical properties of oils (such as, odors, color, density and viscosity) and give higher levels of natural antioxidants (Hashempour-Baltork *et al*., 2016; Fm Ali, 2012; Li *et al.*, 2014). As a result, several research studies have demonstrated the quality and properties of blended oils like sunflower and rice bran oil, sunflower and palm olein oil, soybean and palm olein oil, rice bran and mustard oil, coconut and groundnut oil, sesame and coconut oil and other (Choudhary and Grover, 2013; Hashempour-Baltork *et al*., 2018; Li *et al*., 2014). Despite these facts, studies in blending of different varieties of oils originated and consumed widely in Ethiopia were very few.

1.2. Statement of the problem

There is no pure oil with an ideal nutritional content, fatty acid composition and oxidative stability. Furthermore, using unmixed single seed oil can have low physical, chemical and nutritional properties and also poor oxidative stability as compared with the blended oils. For example, using pure flaxseed oil only has some drawbacks such as being low in linoleic -6 essential fatty a -3), but low oxidative stability and poor flavor. Using sesame oil alone has also some drawbacks such as their low amount of 3 (linolenic acid) essential fatty acids and high price. Sesame oil in spite of containing 85% unsaturated fatty acids, is one of the most stable vegetable oils to oxidation because of the presences of sesamin and sesamolin lignans antioxidants (Hashempour-Baltork *et al*., 2016; Ramadan, 2012; Guimarães *et al*., 2013).

On the other hand, niger oil has high linoleic acid contents (68.9-73.0%) but has high oxidative stability. In addition, diets with a polyunsaturated fatty acid (PUFA):single saturated fatty acid (SFA) ratio below 0.45 are considered inadequate because of their potential to increase blood cholesterol levels (Hashempour-Baltork *et al*., 2016; Ramadan, 2012; Guimarães *et al*., 2013; Hashempour-Baltork *et al*., 2017; Kavuncuoglu *et al*., 2016). Hence, suitable ratio of essential fatty acids has a vital role in maintaining good health. Studies have shown the ratio obtained for flaxseed was 0.31 which is below the found in niger oils is 7.7:1 which is fitted to the recommendation given by WHO (Escudero *et* al., 2006; Guimarães *et al*., 2013). Consequently, blending niger seed oils (high stability), sesame oils (rich in natural antioxidants and high cost and good nutritional properties) with flax seed oils (low oxidative properties) is a good choice to produce oils with good oxidation stability and good nutritional value oil with low cost. However, to our knowledge no study has been reported to document the physical and chemical improvement of blended flax seed, sesame seed and niger seed oil in Ethiopia.

Therefore, the main goal of the present study was to evaluate the physicochemical properties of oil obtained by blending niger seed, sesame and flax seed oils through assessing peroxide value (PV), saponification value (SV), sensory analysis, refractive index, density, viscosity and accelerate oxidative stability.

1.3 Significance of the study

Generally, this study is used to see the synergetic effect on the magnitude of peroxide value, saponification value, density, viscosity, accelerated oxidative stability and refractive index of the flax seed oil, niger seed oil and sesame seed oil during their blending. The result of the study can help to improve the physicochemical properties and nutritional values of the blended oil in addition to identifying good stability oils. In addition, it will also provide information to governmental stakeholders to conduct further studies on blending potential of the niger oil with other edible oils sourced from vegetable, since Ethiopia is the largest production site of niger seed in the world. The study will also be used to provide information to the public about the stability of blended oils of niger, sesame and flax seed oil. Moreover, since Ethiopia is the world known producer of niger seed, the government will use the result of the study to promote the niger seed oil.

1.4 Objectives of the study

1.4.1 General objective

The main objective of the present study was to determine the physicochemical properties of pure oils of niger, sesame and flax seed oil compared to oils obtained by their blends at different levels.

1.4.2 Specific objectives

The specific objective of the study was to:

To evaluate total density, peroxide value, refractive index, viscosity, sensory analysis and saponification value of flax seed oil, sesame oil, niger seed oil and their blends.

To evaluate the oxidative stability (shelf life some blended and unblended) of the flax seed oil, niger seed oil, sesame oil and their blends.

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CHAPTER 2. LITERATURE REVIEW

2.1 Ethiopian largely consumed edible oils

edible oil. According to the Central Statistics Agency of Ethiopia 2016, the country has annual potential of producing more than 784,809 tons of oil seeds. However, Ethiopia is importing more than 350 million metric tons of subsidized palm oil per annum mainly due to challenges in the oil value chain in the country (Muhammed, 2017).

There are different types of edible oils consumed in Ethiopia including niger oil, sunflower oil, sesame oil, soya bean oil, palm oil, rapeseed oil and olive oil which were produced either locally and imported from other countries. Among the locally produced one, the widely consumed edible oils are sesame and niger seed oils which are extracted from respective seed (Atinafu, 2011). However, the consumption level soya and palm oils are the leading source of the world supply of oils and fats, despite of this palm oil has a saturated fatty acid content of 45% and partially hydrogenated soybean oil, although much lower in saturated fat, contains trans fatty acids introduced as a byproduct of hydrogenation (Kebede, 2015).

2.1.1 Flaxseed oil

Flaxseed or linseed (*Linum usitatisimum*), popularly known as Telba in Amharic languages, is a blue flowering rabi crop and a member of family *Linaceae* (Ganorkar and Jain, 2013).There is a small difference in using the terms flaxseed and linseed. Flaxseed is used to describe flax when consumed as food by humans while linseed is used to describe flax when it is used in the industry and feed purpose (Ayelign and Alemu, 2016; Bekhit *et al.*, 2018).

Flaxseed is one of the oldest crops, having been cultivated since the beginning of civilization. It was used for medical purposes in ancient Egypt and Greece, mainly to relieve abdominal pains. Flax was first introduced in United States by colonists, primarily to produce fiber for clothing. Currently, it is cultivated in more than 50 countries, the majority of them in the northern hemisphere. Canada is the main flaxseed producer, followed by China, United States, India and Ethiopia. Its percentage of production by the aforementioned countries in 2004 was 26%, 24%, 14%, 11% and 4% respectively. Ethiopia is the fifth largest producer worldwide and flaxseed is the second oil crop next to Niger seed (*Guizotia abyssinica*) (Ayelign and Alemu, 2016a; Ganorkar and and Jain, 2013).

Globally, flaxseed is grown as either oil crop or a fiber crop with fiber linen derived from the stem of fiber varieties and oil from the seed of linseed varieties (Ganorkar and Jain, 2013). Every part of the flaxseed plant is utilized commercially, either directly or after processing. The stem yields good quality fibers having high strength and durability. Flax has been used until 1990s principally for the fabrication of cloths (linen) and papers, while flaxseed oil and its sub-products are used in animal feed formulation (Goyal *et* al., 2014). On the other hand, it is used as food due to its good nutritional composition. Hence, flaxseed is a flat, oval-shaped seed which contains a protein (20%), total carbohydrate (29%), 7.7% moisture and 3.4% ash, 236 mg/100 g calcium, 622 mg/100 g phosphorus,

3.21 mg/100 g niacin. But also, its oil contains 53% alpha-linolenic acid (ALA), dietary fiber (20-25%) and lignans (> 500 µg/g). Oil extraction yield and fatty acid content vary slightly between authors, and both depend on oil extraction technology (Guimarães *et al*., 2013; Goyal *et al.*, 2014; Ganorkar and Jain, 2013; Bekhit *et al.*, 2018).

Furthermore, in the last two decades, flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with

-linolenic acid ALA), omega-3 fatty acid), lignans, and fiber). For instance, the omega-3 fatty acids found in flaxseed oil contribute to increase cholesterol excretion via bile, thus depleting the liver cholesterol pool and increasing the synthesis of free cholesterol. In addition, diets containing alpha linolenic acid (ALA) decrease fat accumulation in the liver because the acid stimulates the

-oxidation of fatty acids and inhibits their synthesis. Flaxseed oil, fibers and flax lignans have potential health benefits such as in reduction of cardiovascular disease, atherosclerosis (fatty deposits inside arterial wa[redacted]diabetes, cancer, arthritis(inflammation of a joint or joints), osteoporosis (abnormal loss of bony tissue resulting in fragile porous bones attributable to a lack of calcium), autoimmune(relating to the immune response of the body against substance normally present in the body) and neurological disorders. In addition, traditionally humans have been used flaxseed for the prevention of constipation (abdominal pain) (Goyal *et al*., 2014; Ayelign and Alemu, 2016; Guimaraes Drummond *et al*., 2017).

Recently, researches indicated that flaxseed fiber has an effect on blood glucose metabolism and hyperlipidemia (presence of excess lipids in the blood). The insoluble flaxseed fiber, due to its water binding ability, increases the intestinal bulk which is helpful in the treatment of constipation, irritable bowel syndrome and diverticular disease. Soluble flaxseed gum (SFG) delays gastric emptying, improves glycemic control, prevents constipation and reduces serum cholesterol. Flax protein helps in the prevention and treatment of heart disease and in supporting the immune system. As a functional food ingredient, flax or flaxseed oil has been incorporated into baked foods, juices, milk and dairy products, muffins, dry pasta products, macaroni and meat products (Goyal *et al*., 2014; Ayelign and Alemu, 2016; Guimaraes Drummond *et al*., 2017; Basch *et al*., 2007; Chishty and Bissu, 2016).

2.1.2 Niger seed oil

Niger is an oil seed (*Nug*, in Amharic) that has been cultivated for approximately 5000 years. It is a new source of vegetable oils; a crop that is produce from *Guizotia Abyssinica*. Niger seed is the most important oil crop in Ethiopia and a minor crop in India but it is not involved in the world-

crop in some other African countries (Ramadan, 2012; Dimberu *et al*., 2015; Deme *et al*., 2017; Adarsh *et al*., 2014). There are six species of *Guizotia* with *G. abyssinica* being the only cultivated species. It is a dicotyledonous herb, moderately to well branched, and grows up to 2 m in height. The crop grows best on poorly drained, heavy clay soils. Niger is cultivated in both temperate and tropical climates at elevations between 1,800 and 2,500 m, being considered a temperate-region plant that has adapted to a semi-tropical environment. It prefers moderate temperatures for growth, from about 19 C to 30 C (Ramadan, 2012; Peiretti and Tassone, 2015).

Since niger seed have a full of different nutritional composition, it is use as food in different forms (either in extracted oil form or directly mix with other food type). Reports showed that niger seeds contain 483 calories, 2.8-7.8% moisture, 17-30% protein, 34-39% total carbohydrate, 9- -9.9 g/ ash, 50-587 mg/100 g calcium, 180-800 mg/100 g phosphorus, 0.43 mg/100 g thiamine, 0.22-
niacin (Ramadan, 2012; Syume and Chandravanshi, 2015). Furthermore, the literature showed the oil content in niger seed to be in the range 30-50% and there was a large variation in oil content (28.5-38.8%) of different samples collected from Ethiopia depends up the species of niger. Finding showed that niger seed oil has linoleic acid (C18:2) as the principal fatty acid (65.7-68.5 %, weight percent of total lipid). Oleic acid (C18:1) was the second major unsaturated fatty acid (5.4-7.5 %). Niger contains two major saturated fatty acids [palmitic (9.6-10 %) and stearic (7.6-8.1 %)] (Bhavsar *et al*., 2017; Bhagya and Shamanthaka Sastry, 2003; Dutta *et al*., 1994).

Niger (*Guizotia abyssinica Cass*.) seed oil is one of the sources of edible oils which is the best interest and may play an important role in human nutrition and health, because of its fatty acid composition and its high levels of fat-soluble bioactive components (Ramadan, 2012; Dimberu *et* al., 2015). For instance, the presence of high content of omega-6 PUFA i.e. linoleic acid (63-75%), dietary fats and oils, have been used to prevent cardiovascular disorders such as coronary heart disease, atherosclerosis, as well as high blood pressure. Also linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (Ramadan, 2012; Yen, 1991; Bhavsar *et al*., 2017). It is also used in human food mainly as a spice in the frying of vegetables. Seeds pressed with honey are made into cakes in Ethiopia. It can also be used in the manufacture of soap and as a lubricant or lighting fuel, used in paints (being slow-drying), used in perfumes as a carrier of the scents and fragrances (Ramadan, 2012; Solomon and Zewdu, 2009).

2.1.3 Sesame seed oil

Sesame (Sesamum indicum L.)

oldest spices and oilseed crop grown mainly for its small pearly-white seeds (approximately 50% oil and 25% protein). It contains and it ranks sixth in the world among vegetable oilseeds. It is cropped in both tropical and subtropical countries. Sesamum indicum, L. is believed to originate in the Savannas of Central Africa. From there, it spread to Egypt, India and China. The world production was estimated at 3.26 million tones with African countries producing 15.2%. Global imports of sesame increased by 550% between 1960 and 2000 following the increase in land area under its cultivation and production rose by 149% and 207%, respectively. The major producers and exporters of sesame in Africa are Sudan, Uganda and Nigeria. Unfortunately, average world yield of sesame is still low at 0.46 t ha^{-1}. On the other hand, India and China are the major producers accounting for 70% of world production. In Brazil, 13,000 tons of sesame seeds are produced over nearly 20,000 ha, yielding approximately 650 kg/ha (Olowe and Adeoniregun, 2010; Guimarães *et al*., 2013).

Researcher at different parts of the world have reported that sesame oil has different nutritional compositions. Thus, the moisture (wb), crude protein, ash, fat, fiber, total carbohydrate, Ca, Zn and Fe (db) were found in the range between: 3.17% - 3.96%, 22.58% - 24.27%, 4.46% - 6.19%, 50.88% - 52.67%, 5.60% - 6.26%, 8.3% - 11.69%, 1172.08 - 1225.71 mg/100g, 4.23 - 4.45 mg/100g and 10.2 - 10.75 mg/100g, respectively. Phytic acid contents range from 307.61 to 324.91 mg/100g, total phenolics from (23.16 - 25.69 mg GAE/g) and ferric ion reducing power value from (32.33 - 34.53 µmol/g) (db) (Zebib *et al*., 2015; Mbaebie *et al*., 2010; Nzikou *et al*., 2009).

Sesame seed oil (SSO) has been used as natural salad oil, cooking oil, shortening and margarine, a synergist for insecticides and considered as a healthy food. Sesame oil, due to its high level of sesamolin and sesamin lignans, sesangolin, 2-episalatin and others has a good health promoting effects, such as anti-inflammatory effects (medicine intended to reduce inflammation) and anti-proliferative activity on cancer cells, antioxidant activity, levels lowering cholesterol, and showing antihypertensive effects and neuro protective effects against hypoxia or brain damage. Apart from sesame lignans, sesame seed and oil also contain other important biologically active compounds, such as vitamin E (tocopherol homologues). The tocopherol found in sesame oil has many beneficial properties, such as antiproliferative effects in human cancer cells, anti-inflammatory activity and partial prevention of age-associated transcriptional changes in heart and brain of mice (Hashempour-Baltork *et al*., 2018; Olowe and Adeoniregun, 2010; Guimarães *et al.*, 2013; Hassanei and Abdel-razek, 2012; Kavuncuoglu *et al*., 2016)

Seed oils are important sources of nutritional oils, industrial raw materials and nutraceuticals. The characteristics of oils from different sources depend mainly on their compositions. No oil from a single source can be suitable for all purposes that the study of their constituents is important. Many consumers are looking for variety in their diets and aware of the health benefits of fresh fruits, vegetables and of special interest are food sources rich in antioxidants (Bello *et al.*, 2011).

A good quality characteristic of edible oils is depending on their physio-chemical properties. For instance, low density oils are highly appreciable to consumers. Oils having higher value of the moisture content can be used for food texturing, baking, frying and industrially in the manufacture of soaps, detergents, cosmetics and oil paints. Increase in peroxide value indicates the rancidity of oils due to relative higher oxidation. Higher acid value indicates that triglycerides of oil are converted into fatty acids and glycerol which cause rancidity of the oil. So, the cooking oils must have lower acid value and peroxide value otherwise the oil can damage human health. The higher the unsaturation, the greater the possibility of the oils to go to rancid. Rancidity is a term generally used to denote unpleasant odors and flavors in foods resulting from deterioration in the fat or oil portion of a food (Hasan *et al.*, 2016; Dimberu *et al.*, 2015). Rancid oil forms harmful free radicals in the body. Free radical is forms as oxidative stress occurs when an oxygen molecule splits into single atoms with unpaired electrons, which are called free radicals. Electrons like to be in pairs, so these atoms, called free radicals, scavenge the body to seek out other electrons so they can become a pair, which are known to cause cellular damage and have been associated with diabetes, Alzheimer's disease and other conditions. Rancid oils can also cause digestive distress and deplete the body of vitamins B and E. Rancid oil can also cause to damage DNA, accelerate aging, promote tissue degeneration and foster cancer development (Okparanta, 2018; Dimberu *et al.*, 2015).

Three different mechanisms of rancidity may occur. Hydrolytic, ketonic and oxidative. Hydrolytic the cause of rancidity is by a combination of enzymes and moisture. Enzymes such as lipases liberates fatty acids from the triglyceride to form di and/or monoglycerides and free fatty acids and such liberation of free fatty acids is called hydrolysis, hydrolysis is also caused by chemical action that is prompted by factors such as heat or presence of water. Rancidity caused by hydrolysis is called hydrolytic rancidity. The hydrolytic rancidity depends on moisture in the oil, elevated temperature (above room temperature) and most important of all, lipases (enzyme) coming from the source or contaminating microorganisms. Rancidity could also be as a result of microbial decomposition of fats, oils and other lipids that undesirable odors and flavors of food (Okparanta, 2018; Dimberu *et al*., 2015).

Ketonic rancidity some moulds (*penicillium &Aspergillus spp*) attach fats containing short chain fatty acid and produce ketone with a characteristic odor and taste. Butter, coconut and palm kernel oils are the most susceptible. Oxidative rancidity changes can cause rancidity such as off flavors, loss of color, altered nutrient value, and may produce toxic compounds, which can be detrimental to the health of consumers. Oxidative rancidity arises from the decomposition of peroxides (Ahmed *et al.*, 2016).

Peroxides are the result of the oxidation of unsaturated fats. These help to produce the flavors and odors associated with oxidative rancidity. The abnormal characteristics of a product that has undergone oxidative rancidity are paint like or acrid (burning) odor and an abnormal (rancid) taste (Okparanta, 2018; Dimberu *et al*., 2015).
Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage it could be used as an indication of the quality and stability of fats and oils. The peroxide value was also found to increase with the storage time, temperature

and contact with air of the oil samples. The peroxide value determines the extent to which the oil has undergone

cation values suggests that the mean molecular weight of fatty acids is higher (Okparanta, 2018).The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (Okparanta, 2018; Dimberu *et al*., 2015).

All these physicochemical parameters are qualitative properties of oils and do not indicate

status of unsaturation of the oils, so it is not possible to point out the position of double bonds which are more susceptible to oxidation. The primary oxidation products that develop in triacylglycerol are hydroperoxides or peroxols (are compounds containing the hydroperoxide functional group (ROOH)), which later break down to produce lower molecular weight compounds, such as free fatty acids, alcohols, aldehydes, and ketones, leading to a rancid product (Okparanta, 2018).

Oils containing higher amount of Mono-Unsaturated Fatty Acids (MUFA) and Poly-

cholesterol, whereas oils with high saturated fatty acids are considered bad since they are increase LDL (bad) cholesterol. Mustard oil, canola (rapeseed) oil, olive oil and groundnut oil have the best combination of good and bad fatty acids. Meanwhile, gingelly oil is another excellent option. Soybean, corn, sunflower and safflower oils have low saturated fatty acid contents, but their MUFA content is lower than the PUFA content. This is because the amount of both MUFA and PUFA vary with varying the species of seed (Ivanova *et al*., 2016; Sekhar and Bhaskara Rao, 2011).

2.2 Physicochemical properties of edible oils

The knowing of the physio-chemical properties (such as oxidative stability**,** viscosity and density, saponification value, peroxide value and sensory analysis) of edible oils (such as sesame. niger, flax seed oils) is essential in the design of unit processes such as distillation, heat exchangers, reactors and piping. It has also used to monitor the compositional quality of oils and overall quality and stability of a food system. Physical properties of vegetable oils depend primarily on composition (and hence on biological origin) and temperature (Neagu *et al*., 2013; Zahir *et al*., 2017). Seasonal conditions, harvesting, duration of storage, moisture, sunlight, soil fertility, nutrients and processing technology also factors that affect the quality of edible oils (Tesfaye and Abebaw, 2016).

Temperature is one of the main factors affecting different properties of oils including the stability, viscosity, peroxide value during cooking food. Deep frying is one of the most common methods used for the preparation of food. Repeated frying causes several oxidative and thermal reactions which results in change in the physicochemical, nutritional and sensory properties of the oil. During frying, due to hydrolysis, oxidation and polymerization processes the comp
and stability. During deep frying different reactions depend on some factors such as frying condition, original quality of frying oil, food materials, type of fryer, type and concentration of antioxidants and oxygen concentration (Zahir *et al*., 2017; Hasan *et al*., 2016; Ivanova *et al*., 2016).

Other factors such as frying temperature, quantity of frying, type of food material, design and maintenance of fryer, light, use of filters and unsaturated fatty acid content of the oil also affect the oxidative stability and overall quality of oil during the frying process. Atmospheric oxygen reacts instantly with lipid and other organic compounds of the oil to cause structural degradation in the oil which leads to loss of quality of food and is harmful to human health. Generally, deep frying is doing in the presence of oxygen, moisture, physiochemical reactions such as the oxidation, hydrolysis, polymerization, isomerization or cyclization take place at high temperatures of the frying process, thus leading to the decomposition of frying oil and formation of monomeric, polymeric, primary and secondary oxidative compounds (Simon *et al*., 2017; Andrikopoulos *et al*., 2002; Bhattacharya *et al*., 2008).

Therefore, it is essential to monitor the quality of oil to avoid the use of abused oil due to the health consequences of consuming foods fried in degraded oil, to maintain the quality of fried foods and to minimize the production costs associated with early disposal of the frying medium (Zahir *et al*., 2017; Hasan *et al*., 2016; Ivanova *et al*., 2016; Simon *et al*., 2017). During frying thermo-oxidative or lipid oxidation and hydrolytic reactions take place that results in deterioration in quality of the edible oil. The primary oxidation products that develop in triacylglycerol are hydroperoxides, which later break down to produce lower molecular weight compounds, such as free fatty acids, alcohols, aldehydes, and ketones, leading to a rancid product (Okparanta, 2018).

2.2.1 Oxidative stability

One of the most chemical properties which affect the quality of oil and its health impact is oxidative stability. Thus, oxidative stability of oils is the resistance to oxidation during processing and storage. Resistance to oxidation can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process. Oxidative stability is an important indicator to determine oil quality and shelf life. The off-flavor compounds make oil less acceptable or unacceptable to consumers or for industrial use as a food ingredient. Oxidation of oil also destroys essential fatty acids and produces toxic compounds and oxidized polymers. Different chemical mechanisms, autoxidation and photosensitized oxidation, are responsible for the oxidation of edible oils during processing and storage depending upon the types of oxygen (Choe and Min, 2006; Dimberu *et al.*, 2015; Rahman *et al*., 2017).

The oxidation of oil is influenced by the fatty acid composition of the oil, oil processing, energy of heat or light, type of oxygen, thermally oxidized compounds and antioxidants. These factors interactively affect the oxidation of oil and it is not easy to differentiate the individual effect of the factors (Choe and Min, 2006). Thus, oils that are more unsaturated are oxidized more quickly than less unsaturated oils, oil-processing method such as roasting of safflower and sesame seeds before oil extraction improved the oxidative stability of their oils, partly due to the Maillard products produced during roasting. Some

Maillard reaction products were reported to be antioxidants. Maillard reaction defined as nonenzymatic browning reaction. While foods are processed or cooked at high temperature, a chemical reaction occurs between amino acids and reducing sugars which generate different flavors and brown color. So, it is often used in food industry for giving food different taste, color, and aroma. Oxidative stability increased as the roasting and expelling temperatures of the seeds increased (Choe and Min, 2006).

Peoples use various method to improve oxidative stability of oil has been developed and studied, for example, partial hydrogenation, fatty acid modification and blending with more saturated or monosaturated oils to reduce the amount of polyunsaturated fatty acids (Naghshineh *et al*, 2010; Ivanova *et al*., 2016). Partial hydrogenation decreases polyunsaturated fatty acid but increases saturated fatty acid and trans-fatty acid to produce more stable frying oil. However, trans fatty acid may have adverse effects on cardiac health. Blending has long been used to modify oils and fats to improve the fat functionalities and thus optimize their application in food products. It modifies the physicochemical properties of oils without changing their chemical composition (Ivanova *et al*., 2016).

2.2.2 Viscosity and density of oil

Viscosity is used to characterize the fluid texture. Oils are mixtures of triglycerides (TGs) and their viscosity depends on the nature of the TGs present in the oil. The viscosity changes due to the different arrangement of the fatty acids on the glycerol backbone of the triglyceride molecule. Therefore, viscosity is related to the chemical properties of the oils such as chain length and saturation/unsaturation. Thus, viscosity increases with chain lengths of triglyceride fatty acids and decreases with unsaturation. So, viscosity is a

on its temperature and composition as well as on the previous treatment applied to the samples (Severa *et al*., 2006; Okparanta, 2018).

rature

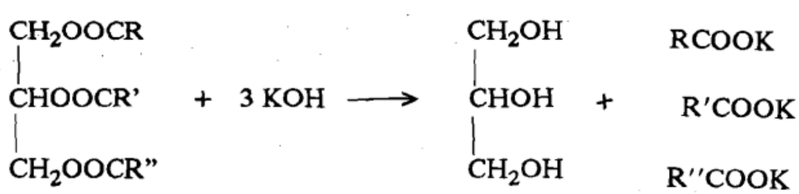
that it decreases (thin) while temperature increase and vice versa. Kinematic viscosity is the most commonly used method for measurement of the oil viscosity. It is measured in the time take for a specific volume of oil to flow through a special device called capillary tube that different oils have different ability to resist the change in viscosity at a given temperature. Such properties is referred to as the oil viscosity index or VI that the higher the value of VI, the less change in viscosity change by temperature change (Hasan *et al*., 2016). Density is an important factor which influences oil absorption as it affects the drainage rate after frying and also the mass transfer rate during the cooling stage of frying. Density has been experimentally shown to be linearly dependent on temperature. However, there is no mathematical equation to predict the effect of temperature on density, especially at high temperatures at which frying is conducted (Sahasrabudhe *et al.*, 2017).

2.2.3 Saponification value (average molecular weight) of oil

Saponification is the process of breaking down or degrading a neutral fat into glycerol and fatty acids by treating the fat with alkali potassium hydroxide or sodium hydroxide. The saponification number (value) is defined as the milligrams of potassium hydroxide (KOH) required to saponify 1g of fat or oil. It is an index of average molecular weight of the triacylglycerols in the sample. The molecular weight of the triacylglycerols may be divided by three (3) to give an approximate average molecular weight of the fatty acid present. The high saponification values of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids (Dimberu *et al.*, 2015).

According to Nielson, that the smaller the saponification values the longer the average fatty acid chain. If the fatty acids present in the glycerides are low molecular weight (short-chain acids), there will be more glycerides molecules per gram of fat than if the acids are high in molecular weight (long-chain acids). Thus, since each glyceride molecule requires three potassium hydroxide molecules for saponification, fats containing glycerides of low molecular weight correspondingly have higher saponification values. Saponification values have been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions. In combination with acid values; saponification values are useful in providing information as to the quantity, type of glycerides and mean weight of the acid in a given sample (Odoom and Edusei, 2015).

The saponification value of oils is interest if the oil is going to be used for industrial purposes. Saponification value is also used in checking adulteration. The larger the saponification number, the better the soap making ability of the oil. Higher saponification value for triglyceride indicates higher medium chain fatty acids. Saponification value for unrefined vegetable oils may also be affected by the compounds in the non-saponifiable fraction. For example, compounds such as phenolic acids that can react with KOH may also contribute to higher saponification value of coconut oils (Odoom and Edusei, 2015; Dimberu *et al.*, 2015).



2.2.4 Peroxide value oils

Peroxide serves as a useful indicator of the extent of oxidation of lipids, fats, and oil. The quality of the oils is dependent on their chemical compositions, like the percentage of the degree of unsaturation. The peroxide value shows the degree of peroxidation and measures the amount of total peroxides in the substance. It has been associated with the rancidity in lipid-containing food products. It is one of the main causes of generation of off-flavors, deterioration, and production of toxins in oils. Therefore, it negatively affects the quality and storage life of oils. The radical species formed in the peroxidation process degrade fatty acids and other components of the lipids. The peroxide value is widely used as a measurement of these unwanted reactions in food stuffs and oils, as well as in biological samples where such reactions are implicated in physiological processes related to the modification of macromolecules causing the initiation of degenerative diseases, cancer and aging ( ).

Peroxide value is an indicator for the measurement of the initial stages of oxidation in oils. The unsaturated fatty acids present in the oils easily react with atmospheric oxygen and form hydroperoxides. Oils with a high degree of unsaturation are highly susceptible to oxidation as compared to saturated oils. Variations of PV can arise from different factors such as the degree of unsaturation of the fatty acids present in the particular oil, storage, exposure to light, and the content of metals or other compounds that may catalyze the oxidation processes *et al.*, 2012; Kaleem *et al*., 2015; Popa *et al*., 2017).

The peroxide value (PV), which depends on temperature, time and light, measures the extent of primary oxidation of oils (rancidification). Rancidity of oils can produce potentially toxic compounds associated with long-term health effects such as neurological disorders, heart and cancer (*et al*., 2012; Kaleem *et al*., 2015; Popa *et al*., 2017).

2.2.5 Sensory analysis

The most important parameter for the assessment of edible oils is the sensory evaluation, because the product has to fit the consumer likes, otherwise the product has no chance on the market. The result of the sensory evaluation can be objectified by different chemical parameters, but the sensory impression is the decisive factor for the evaluation of the oil as edible provided that no toxic contaminants are against this assessment. One reason for the importance of the sensory quality of the oil is that more than any other parameter the appearance and the taste deeply influence the buying decision of the consumer (Matthäus and Brühl, 2019; Velickovska *et al.*, 2015).

The sensory assessment can be done by a consumer panel with a huge number of untrained consumers which assess the product regarding likes and dislikes. The result says nothing about sensory defects of the oil resulting from deterioration but reflects acceptances and preferences of consumers for the product. On the other hand, the sensory evaluation of edible oils is possible by a trained group of tasters which are familiar with the product and possible perceptions resulting from sound or defective products. In the case of this analytical sensory evaluation the available methods range from simple triangle testing via recognizing differences between two samples to the more complex descriptive analyses of edible oils (Matthäus and Brühl, 2019; Velickovska *et al*., 2015).

2.3 Blending of edible oils

Blending is one of the methods used to modify oils and fats for specific applications. Blending helps extend the range of applications of oils and fats. In their original form, the use of oils and fats is very limited. Blending oils with high stability and good nutritional properties is a good choice to decrease the rate of oxidation. Blending oils with different properties can produce oil product with good stability at frying temperatures without hydrogenation and formation of trans fatty acids. By blending different types of oils, the consumer can be offered a better quality product with respect to flavor, frying quality and nutritive value (Simon *et al.*, 2017).

2.3.1 Effect of blending on physicochemical properties of oil

Blending different fats/oils with various properties gives a new oil with improved functional character

tend to crystallize and change their clarity when cooled. Studies show that mixing these oils with higher and more unsaturated oils gives a more stable and clear mixture which remains stable during storage. Also blending fats/oils leads to changes in triacylglycerol

ch as, sensory quality,

density and viscosity. Studies also shows that the color of the blended oils is different from the individual oils. For instance, when the soybean oil blended with sunflower oil it shows pale yellow color, soybean oil with rice bran oil shows yellow color, soybean oil with palm oil gives golden yellow color. Sunflower oil with rice bran oil gives light yellow color, sunflower oil with palm oil gives golden yellow color and the palm oil combines with the rice bran oil shows yellow color. These are the different variations of color in the blended oils (Peeter Simon *et al*., 2017; Hashempour-Baltork *et al.*, 2017).

Furthermore, mixing different vegetable oils can change fatty acid composition and give higher levels of natural antioxidants and bioactive lipids in the blends and, therefore, can improve the stability of oils. For example, sesame oil alone has some drawbacks such as their low amount of omega-3 essential fatty acids. Instead, flaxseed oil is a rich source of omega-3 and other bioactive compounds, but in pure form it is very unstable and oxidizes quickly (Hashempour-Baltork *et al*., 2017; Winkler-Moser and Mehta, 2015).

2.3.2 Effect of blending on nutritional properties

The World Health Organization (WHO) introduced three important factors for the nutritional evaluation of oils: 1) presence of antioxidants, 2) ratio of saturated, mono- and poly-unsaturated fatty acids and 3) essential fatty acid ratio. The WHO suggested a ratio of 1:1.5:1 for saturated: mono-unsaturated: poly-unsaturated fatty acids and 1:5 to10 alpha linolenic acid (omega 3): linoleic acid (omega 6) in the food intake. Omega 3 and omega 6 fatty acids are essential fatty acids required in our diet for the prevention or treatment of some diseases (Hashempour-Baltork *et* al., 2018).

Omega 3 fatty acids have important roles in normal growth and prevention of cancer, cardiovascular diseases and the improvement of immune function. Blending of oils and

acid composition.

Blending proper oils can result in a functional oil with a healthy omega-6/ omega-3 ratio. Sesame oil has anti-

cells due to its vitamin E (tocopherol homologues). However, this oil, which has many nutritious and healthy effects, is expensive and has limited application in food industry. Thus, blending sesame oil with a less stable one such as niger seed or flaxseed oil can improve their stability and shelf-life at an affordable price (Gunstone, 2013; Winkler-Moser and Mehta, 2015).

2.4 Health benefits of edible oils

Edible oils are vital constituents of our daily diet, which provide energy, precursor for steroid hormones and prostaglandins synthesis, essential fatty acids and serve as a carrier of fat soluble vitamins (Zahir *et al.*, 2017; Diosady and Krishnaswamy, 2018; Dorni *et al.*, 2018). They can increase digestibility and control diabetics (improve insulin serration and utilization of blood glucose), helps to reduce weight, body lotion /cosmetics and improve cardio vascular health (by lowering the cholesterol level). Similarly, polyunsaturated oils put a heavy strain on the immune system. They cause the immune system to shift into feverish activity while at the same time interfering with its ability to form protective compounds. In addition, oils rich in omega-3 fatty acid gives a positive effect on reducing the cardi -

3 have also the potential to prevent cancer and are important for the proper functioning of the vital organs such as brain and eyes, in combination with vitamins and carotenoids, they protect human skin from sun damage (Rethinam, 2019; Ivanova *et al.*, 2016; Dorni *et al.*, 2018).

Studies showed that consumption of non-hydrogenated unsaturated oils like soybean and sunflower are preferable to the consumption of palm oil for lowering the risk of heart disease (Hasan *et al.*, 2016). Oil contains the least amount of saturated fatty acids, making it safe for heart patients (Okparanta, 2018; Sekhar and Bhaskara Rao, 2011).

2.5 Oxidative stability problem

On the contrary of oils health benefits, rancid oil may taste bad, it doesn't normally make sick, at least not in the short term. Taking of rancid oil can cause for developing diseases such as cancer or heart disease down the road, since it does contain free radicals. In addition, rancid oils may produce damaging chemicals and substances that can cause different health problems over time. For instance, chemicals such as peroxides and aldehydes can damage cells and contribute to atherosclerosis. The free radicals produced by rancid oil can also damage DNA and can cause damage to arteries as well act as carcinogens, substances that can cause cancer in cells. Thus, in general, a potential health hazard may exist, if oxidative rancid oil is present in the body at severe quantities (Okparanta, 2018).

Malonaldehyde (decomposition product of polyunsaturated fatty acids) compounds found in rancid oils can act as carcinogenic and a potential health hazard if it does exist in high amount. Eating rancid oil will expose to accelerated aging, raised cholesterol levels, obesity and weight gain. Daily consumption increases the risk of degenerating diseases such as cancer; diabetes; Alzheimer's disease; and atherosclerosis, a condition in which artery walls thicken due to a buildup of fatty materials. According to a study from the University of Basque Country, the breakdown rate and total formation of toxic compounds depends on the type of oil and temperature. Initially, the oil decomposes into hydroperoxides, then into aldehydes (Okparanta, 2018). In addition, excess fat intake (> 30 %), particularly of saturated fats, is also known to be a health hazard, since it is predisposing factor in the causation of obesity and cardiovascular disease (Rao, 2001). Studies shows that sulfur compounds found in edible oils such as Isothiocyanates, Oxazolidinthiones and Glucosinolate has Goitrogen toxic effect. In addition, alkaloid compounds including Sanguinarine, gossypols and saponins compounds has also cause for the health impacts (Gunstone, 2013; Rao, 2001; Winkler-Moser and Mehta, 2015).

CHAPTER 3. MATERIALS AND METHODS

3.1 Study area

Some parts of the work were done in Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia. Whereas the remaining work was done at Bahir Dar, capital town of Amhara regional state.

3.2. Chemical and reagent

Acetic acid, chloroform, a saturated potassium iodide solution, distilled water, $Na_2S_2O_3$ (Sigma Aldrich, Germany), 1% starch indicator, KOH (Sigma Aldrich, Germany), ethanol (95 % Sigma Aldrich, Germany), hydrochloric acid (37%, Sigma Aldrich, Germany) and phenolphthalein indicator, oil solvent (95% ethanol) chemical reagents were used in this study.

3.3 Apparatus and instrument

A Krusi Optronic (model GmbH-DR6200-T, Germany) was employed for the determination of refractive index for both oils (either pure or blended once). Professional Rancimate (Metrohm, model 8.892.8001 EN/2014-05-09, Switzerland) was used for the measurement of accelerated oxidative stability of oils. A digital analytical balance with ±0.001 g precision was used to weight the oil samples for the determination of density, saponification analysis and peroxide value. Monterts Oekotec oil press (GmbH and Co. KG, model 203445, Germany) machine was used for oil extraction. The viscosity of oil samples was measured using Brookfield RHEOmeter DV3T, USA.

A 250 mL conical flask (Pyrex, grade A) was used to put the oils for the titration.10 and 5 mL graduated pipettes (Grade A, USA), 5, 10, 15, 25, 50 and 250 measuring cylinders (Grade A, Germany) were used to measure volumes during sample preparations and analysis. Plastic bottles were used to store both the pure and blended oils. Apparatus such as volumetric flasks, measuring cylinder, plastic beaker, plastic volumetric flask, plastic containers and polyethylene bottles were washed with detergents and tap water, rinsed with deionized water. Reflux condenser was used to reflux the solution during saponification value determination.

3.4 Methods

3.4.1 Sample collection description

The oilseeds (niger, flax and sesame) samples were collected from Holeta agricultural research institute. For each sample, about 20 kilograms of the oilseed were collected in the three different pre-cleaned sacks and brought to Bahir Dar University laboratory for further cleaning of the dusts present in it. After cleaning of the foreign matters and dust, the sample was extracted to oil.

3.4.2 Oil extraction using mechanical expression method

Mechanical expression was used to extract oil from seed of flax, sesame and niger in this study. Thus, the oils from the niger seeds, sesame seed or flaxseed were extracted using mechanical extraction machine, Monterts Oekotec oil press (GmbH and Co. KG, model 203445, Germany). 1 kg cleaned oilseed was put on the hopper and then the switch of the power was on followed by the speed adjustment. Furthermore, the extract of the second oil was done in similar manner to the first oil, after cleaning of machine using hot water. Finally, after the extraction, the extracted oil was wrapped with aluminum foil and brought to Addis Ababa science and technology laboratory for analysis. It was stored in dark place at room temperature until blending and analysis was done. The blends of oils were formulated by mixing niger seed, sesame and flaxseed oils in different proportions. The details of the proportion was given at Table 1.

Table 1. The Mixing ratio patterns of oils (in mL)

Run	Niger	Flax	Sesame
1	0.00	312	358
2	397	0.00	273
3	397	0.00	273
4	0.00	0.00	670
5	0.00	670	0.00
6	318	352	0.00
7	0.00	330	340
8	318	352	0.00
9	226	0.00	444
10	670	0.00	0.00
11	670	0.00	0.00
12	0.00	0.00	670
13	79.0	544	47.0
14	670	0.00	0.00
15	0.00	0.00	670
16	51.0	135	484
17	0.00	670	0.00
18	0.00	670	0.00
19	491	158	21.0

3.5 Determination of properties of oils

3.5.1 Determination of density

The density of both oil type (the blend oil and the pure oil) were analyzed at room temperature. Thus, 3 mL of the oil was transferred to the beaker and its weight was measured by a digital analytical balance with ±0.001 g precision, and then its density was calculated by a formula of mass to volume ratio (Ahmad *et al.*, 2010).

(1).

3.5.2 Determination of viscosity

The viscosity of the oil sample was measured using the method of (Ahmad *et al.*, 2010) by Brookfield RHEO meter DV3T, USA instrument. After adjusting the parameter such as speed, temperature, shearing stress, shearing rate, time, running step and spindle of Brookfield RHEO meter DV3T, 200 mL of the oil sample was transferred to a beaker and put it in RHEO meter then results were recorded after 5 minutes .

3.5.3 Determination of peroxide value (PV)

Like other methods used in this study peroxide values were determined by the developed procedure elsewhere (Ahmad *et al.*, 2010). Hence, the sample oil (0.5g) was taken into a conical flask ((Pyrex, grade A), 30 mL of the acetic acid and chloroform (mixture 2:3 respectively) was added, followed by 0.5 mL of saturated potassium iodide solution. After 1 minute of occasional shaking, 30 mL of distilled water was added. It was titrated slowly with a 0.1 M $Na_2S_2O_3$ solution with vigorous shaking until the disappearance of the yellow color. Then 0.5 mL of 1% starch indicator was added and titration continued until the blue color disappeared. The PV of the individual oils and their blends were determined in 4 weeks intervals while all the oil samples were kept in uncovered beakers at room temperature during this time. The peroxide value was calculated using equation 2.

——————— ... (2)

where S = volume (ml) of sodium thiosulphate solution used in the titration after correction for the blank titration and M = molar concentration of the sodium thiosulphate solution.

3.5.4 Determination of saponification values (SVs)

SVs was determined by the AOAC official Method no. 920.160. Thus, firstly, 0.1 N KOH solution was prepared with 95% ethanol. Secondly, 4.5 g of oil sample and 50 mL of alcoholic potassium hydroxide was transferred to a conical flask (Pyrex, grade A), and the flask was connected to an air condenser followed by heating of the content up to $60^{o}C$ for 30 minutes. Finally, after cooling the solution to room temperature, 4 drops of phenolphthalein indicator were added and titrated by a titrant hydrochloric acid having a concentration of 0.5 M until the pink color disappeared. The blank analysis was also carried out, and the saponification value was calculated using the following equation as mentioned in the method of (Ahmad *et al.*, 2010).

(3)

where saponification value is the mass of KOH in milligram (mg) that required to saponified 1 gram of fat; B is volume (mL) of 0.5 mol/L HCl consumed in the blank test; S volume (mL) of 0.5 mol/L HCl consumed in the sample test; Mwt is molecular weight of KOH which is 56.1 g/mol; M is molar concentration of KOH which is 0.5 mol/L.

3.5.5 Determination of refractive index

A refractometer (Krusi Optronic model GmbH-DR6200-T, Germany) was used for the refractive index determination. Thus, 3 drops of the oil sample was transfer into the glass slide of the refractometer, and then the prism box was open and three drops of the oils were placed on the ground surface of the lower prism. The prism box was then closed and the box flattened again. Finally, the refractometer was turned on for two minutes and the refractive index of the oils were recorded.

3.5.6 Determination of accelerated oxidative stability

The susceptibility of all oil samples to oxidation was studied using professional Rancimate (Metrohm, model 8.892.8001 EN/2014-05-09, Switzerland) apparatus. The induction period (IP) of the individual oils and their blends were performed on an automated Metrohm Rancimat at 120±0.1ºC and an air flow of 20 L/hr. Thus, approximately, 3 mL of individual or its mixtures oils were transferred to the sample vessel (test tube) and 60 mL deionized water was added to the reaction vessel, and then both vessels were connected to 892 professional rancimat. Finally, after the required temperature was reached (120.1 ºC) the conductivity of the distilled water started to increase. Thus, the volatile oxidized compounds coming out of the sample during heating is transport to a reaction vessel containing 60 mL of distilled water, where the cause for change in conductivity of distilled water. The time at which the sharp increase in conductivity of water was recorded as the oxidative stability index (OSI).

3.5.7 Sensory Analysis

Sensory analysis of blended $N_8F_{20}S_{72}$, $N_{34}S_{66}$ and F_{100} from each treatment was evaluated by 70 untrained sensory panelists (AASTU second, third- and fourth-year Food Science and nutrition students). The sensory evaluators were from both sexes, and from different ages, they were requested to evaluate each sample separately without comparing it with other samples. Panelists were first familiarized with the questionnaire form. The samples were evaluated for desirability in odor, color, taste, flavor, mouthfeel and overall acceptability using a 9 hedonic scale which ranged from 9 to 1, where 9, 8, 7, 6, 5, 4, 3, 2 and 1 means like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely, respectively. Oils of three treatments were given random three-digit numbers and served, at room temperature. Sensory evaluation was carried out in a well lightened room. Each evaluator sat in a separate cabinet for around 7 min to evaluate each of the 3 samples. Salad was prepared by oils (bread) and water was used to taste between samples (Ismail *et a*l., 2015).

3.6 Statistical package used in data analysis

The obtained results of all analyses performed in triplicate samples were analyzed and the mean values calculated for each physicochemical parameter determined. Mean and standard deviations of saponification value (SV), sensory analysis, peroxide value (PV), density, viscosity and refractive index (RI) were calculated using MS excel. The statistical data analysis was carried out using IBM SPSS version 20.0 and Microsoft Excel 2013. Analysis of Variance (ANOVA) was used for multiple comparison to establish the significant difference within one type of oil, across various oil types and their blends as compared to the pure oils ($p < 0.05$).

CHAPTER 4. RESULT AND DISCUSSION

4.1 Physicochemical analysis

In this study, the quality of individual oils (sesame, niger, flax seed oils) and blended oils of sesame, niger and flax seed with different proportion were analyzed by evaluating physicochemical properties such as density, viscosity, refractive index, accelerated oxidative stability, peroxide value 2 showed that results of their physicochemical properties for all mixing ratio, for the individual oils and their blends.

Table 2. The physicochemical results for individual and blended oils at different mixing ratio.

Run	Density (g/mL)	RI	SV (mg of KOH/g)	Viscosity (centipoise)	AOS (hr)	Peroxide Value (meq O_2/kg)		
						Day 15	Day 45	Day 75
1	0.9467	1.476	175	52.00	2.457	1.60	2.80	4.46
2	0.9187	1.474	174	61.10	2.913	1.80	2.50	6.20
3	0.9267	1.474	176	60.29	3.213	1.74	2.54	5.00
4	0.9286	1.463	175	47.00	4.790	1.31	2.44	3.58
5	0.9846	1.474	174	62.80	1.787	2.80	3.40	5.00
6	0.9256	1.477	174	60.00	4.513	2.00	3.24	6.60
7	0.9267	1.476	176	57.30	0.937	2.40	3.00	6.06
8	0.9270	1.482	180	32.90	0.907	2.14	3.26	5.00
9	0.9261	1.473	173	45.30	0.780	1.50	2.60	4.04
10	0.9370	1.478	174	53.30	0.987	1.80	2.60	3.78
11	0.8993	1.472	178	48.10	0.880	1.74	2.66	3.70
12	0.9284	1.474	171	48.00	2.307	1.56	2.60	3.40
13	0.9281	1.480	175	56.00	4.520	3.00	5.00	7.20
14	0.9687	1.477	176	60.00	2.683	1.76	2.60	3.74
15	0.9270	1.466	175	43.70	2.440	1.46	2.50	3.50
16	0.9207	1.474	173	48.00	2.433	1.60	3.00	3.80
17	0.9260	1.488	176	65.00	1.017	3.00	5.22	8.80
18	0.9297	1.486	178	63.00	1.377	3.80	5.24	8.40
19	0.9282	1.473	177	54.70	1.320	2.00	4.00	6.20

Note: RI means refractive index; SV means Saponification value and AOS means accelerated oxidative stability

4.1.1 Density and refractive index

Table 3 showed the density and refractive index of pure/unblended and blended oils. The density of the blended and unblended oils showed no significant (*p* between oil varieties, whereas for refractive index of the blended and unblended oil varieties significance (*p* ce was observed. The density and the refractive index of pure oils were found 0.9280-0.9467 g/mL and 1.467-1.482, respectively. The density and the refractive index for the binary blends were found 0.9227-0.9283 and 1.473-1.479, respectively, whereas the density and the refractive index of the mixture of three oils (tertiary) were found 0.9207-0.9282 and 1.473-1.480 respectively. The lowest value of density was recorded in $N_8F_{20}S_{72}$ oil where the highest value was measured at unblended oil which is pure flax (F_{100}). Hence the variation in densities might be due to the variation of minor components (Neagu *et al.*, 2013). Studies have showed that oils with lower values of viscosity and density are highly appreciable to consumers, that can provide good stability during frying (Okparanta, 2018).

The refractive index of fats and oils is sensitive to their composition. In fats RI increases with increasing chain length of fatty acids in the triglycerides or with increasing unsaturation. This makes it an excellent spot test for uniformity of compositions of oils and fats (AL Majidi and Bader, 2015).

The molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation have direct relation to the refractive index of oil. The RI obtained in this study for all types of oils were appeared to be approximately similar (Table 3). The minimum and the maximum refractive index value was measured at S_{100} and F_{100} oil, respectively. Such variation in refractive index might be due to the variation in the acidic composition of oils (free fatty acid, degree of unsaturation, length of hydrocarbon chains). For instance, the RI of the oil increase with increasing of number of double bond (Borchani *et al*., 2010; Mohdaly *et al.*, 2017). The density of niger seed oils of the present study was found in comparable to other studies reported in literature (Getachew *et al*., 2016). The patterns followed for density and refractive index for both blended and unblended oils were:

$N_8F_{20}S_{72} < N_{59}S_{41} < N_{34}S_{66} < N_{47}F_{53} < F_{49}S_{51} < S_{100} < N_{12}F_{81}S_7 < N_{74}F_{23}S_3 < F_{47}S_{53} < N_{100} < F_{100}$ and $S_{100} < N_{34}S_{66} = N_{74}F_{23}S_3 < N_{59}S_{41} = N_8F_{20}S_{72} < N_{100} < F_{47}S_{53} = F_{49}S_{51} < N_{47}F_{53} < N_{12}F_{81}S_7 < F_{100}$. This result indicates that $N_{12}F_{81}S_7$ and F_{100} has rich in double bond as compare with other studied oils.

ANOVA test was applied to see the significance difference among the oil type and the result has showed no significant difference in density and significant difference in refractive index. The details are given at Appendix I.

Table 3. Physicochemical characteristics of individual oils and their blends.

Pure and their blend oils	Mixing ratio (%) (ml)	Density (g/mL)	Ref. index*	AOS (hrs)	SV (mg of KOH/g of oil)	Viscosity (centipoise)	Peroxide value (in meq O_2/kg ± SD)		
							15 (day)	45(day)	75 (day)
Sesame (S_{100})	100	0.9280±0.001	1.467	4.69±0.05	174±0.11	46.2+0.77	1.56±0.70	2.47±0.16	3.49±0.52
Niger (N_{100})	100	0.9350±0.001	1.475	2.52±0.05	176±0.13	53.8+1.54	1.76±0.18	2.63±0.02	3.74±0.02
Flax (F_{100})	100	0.9467±0.001	1.482	0.87±0.05	176±0.60	63.6+0.77	3.19±0.31	4.62±0.61	7.78±0.90
$F_{47}S_{53}$	47:53	0.9283±0.001	1.476	2.45±0.05	175±0.61	52.0+0.00	1.59±0.12	2.81±0.01	4.53±0.18
$N_{59}S_{41}$	59:41	0.9227±0.001	1.474	3.06±0.04	175±0.61	60.7+1.15	1.77±0.01	2.50±0.02	6.07±0.04
$N_{47}F_{53}$	47:53	0.9263±0.001	1.479	0.93±0.05	177±0.04	46.7+1.15	2.05±0.08	3.25±0.01	6.33±0.15
$F_{49}S_{51}$	49:51	0.9267±0.001	1.476	2.31±0.03	176±0.61	57.3+1.15	2.39±0.01	3.01±0.01	6.06±0.12
$N_{34}S_{66}$	34:66	0.9261±0.001	1.473	4.52±0.03	171±0.23	49.7+2.08	1.49±0.02	2.61±0.01	4.04±0.01
$N_{12}F_{81}S_7$	12:81:7	0.9281±0.001	1.480	1.02±0.06	175±0.20	56.0+0.00	2.99±0.01	5.01±0.01	7.20±0.12
$N_8F_{20}S_{72}$	8:20:72	0.9207±0.001	1.474	3.38±0.05	173±0.25	48.0+0.00	1.59±0.01	3.26±0.01	3.87±0.18
$N_{74}F_{23}S_3$	74:23:3	0.9282±0.001	1.473	1.32±0.02	177±0.40	54.7+2.31	1.99±0.12	4.00±0.12	6.14±0.13

Note: *the Standard deviations of Refractive index values are below 0.0000; $F_{47}S_{53}$ is blending ratio of flax to sesame which is 47 to 53; $F_{49}S_{51}$ is blending ratio of flax to sesame which is 49 to 51; $N_{59}S_{41}$ is blending ratio of niger to sesame which is 59 to 41; $N_{34}S_{66}$ is blending ratio of niger to sesame which is 34 to 66; $N_{47}F_{53}$ is the blending ratio of niger to flax which is 47 to 53; $N_{12}F_{81}S_7$ is the blend ratio of niger to flax to sesame which is 12 to 81 to 7; $N_8S_{20}F_{72}$ is the blend ratio of niger to flax to sesame which is 8 to 20 to 72; $N_{74}S_{23}F_3$ is the blend ratio of niger to flax to sesame which is 74 to 23 to 3.

4.1.2 Saponification of oil

Saponification value is an indication of the size or nature of fatty acid chains esterified to glycerol, and gives a measure of the average length of the fatty acid chain that makes up a fat. In combination with acid values, saponification values are useful in providing information to the quantity, type of glycerides and mean weight of the acids in a given sample of oil. In addition to this saponification value is a measure of oxidation during storage, and also indicates deterioration of the oils (AL Majidi and Bader, 2015; Neagu *et al.*, 2013).

The saponification value for the pure and blended oils in this study are given in Table 3. Results showed no significant (*p* en varieties of blended and unblended oils. The SV of pure oils were found to be 174-176 mg of KOH/g of oil. The SV for the binary blended oils were found in the 171-177mg of KOH/g of oil and mixture of three oils were found in 173-177 mg of KOH/g of oil. The highest SV value was recorded in both binary and tertiary blends oil ($N_{47}F_{53}$ and $N_{74}F_{23}S_3$), where the lowest value was measured at unblended and blended oil ($N_8F_{20}S_{72}$, $N_{34}S_{66}$ & S_{100}). The lower SV for sesame oils indicates that it contains high amount of short chain fatty acids (Dimberu *et al.*, 2015; Odoom and Edusei ,2015). Studies have showed that high molecular weight fatty acids are good for human health. In that sense fats or oils having low saponification number (value) good for human health (Paula *et al.*, 2011). The different plantation area, environments and chemical composition are the factor for variation in SV of the oils (Dimberu *et* al., 2015). The SV of the niger oil found in this study was found lower as compared to other study carried out by (Bhavsar *et al.*, 2017) which was 191 mg of KOH/g.

The patterns of saponification values for the studied oils were in increasing order of $N_{34}S_{66} < N_8F_{20}S_{72} < S_{100} < F_{47}S_{53} = N_{59}S_{41} = N_{12}F_{81}S_7 = F_{49}S_{51} = N_{100} = F_{100} < N_{47}F_{53} = N_{74}F_{23}S_3$. Fat molecules did not interact with each other. Hence, $N_{74}F_{23}S_3$ has the higher the saponification value, that it contains (lower) shorter average chain length of the fatty acids and the lower the average molecular weight of the fatty acids (Okparanta, 2018; Ngassapaa *et al.*, 2012).The saponification value of sesame, flaxseed and niger seed oil of the present study was found in comparable to other studies reported in literature. Saponification value of most edible oils were found in 175 to 251mgKOH/g (Belsare and Badne, 2017).

4.1.3 Viscosity of oils

The viscosity of the oil is a very important factor. In food it draws the interest of the customer, and oils with lower values of viscosity, density are highly desirable to consumers. Viscosity depends on shear stress and temperature. Shear stress does not have much effect on the storage of the oils which are used for edible purposes but the temperature does affect it. Viscosity decreased with increase in temperature. An increase in temperature enhances the movements of the molecules and reduces intermolecular forces so the layers of the liquid easily pass over one another and thus contribute to the reduction of viscosity oils. High level of double bonds in their fatty acid chains show lower viscosity levels because of their weak structures. Double bonds, due to their space requirements do not allow molecules to be stacked close to each other. Therefore, oils with high amount of unsaturated oils cannot have a rigid and fixed structure and so behave in a more fluid-like way (Ahmad *et al.*, 2010; Hashempour-Baltork *et al.*, 2018).

The viscosity value for the pure and blended oils studied was given in Table 3. The difference in the viscosity of oils were tested by ANOVA, and the result showed that unblended oils were should significance (*p* nce to each other. In addition, the binary blended oils were not showing significance (*p* ce, except $F_{47}S_{53}$ and $N_{59}S_{41}$ which showed a significance (*p* ce. Moreover, the tertiary blended oils also showed a significant (*p* n each of them, except $N_8F_{20}S_{72}$ and $N_{74}F_{23}S_3$ where significant (*p* viscosity of pure oils was found in 46.2-63.6 cp. The viscosity for the binary and tertiary blends oils were found of 46.7-60.7 and 48.0-56.0 cp, respectively. The highest value of viscosity was recorded at pure flaxseed oil, while the lowest value was measured at pure oil of sesame seed oil. Viscosity of the oil decrease with increasing unsaturation and increase with increasing of saturation and polymerization (Okparanta, 2018).

Unsaturated fatty acid rich oils are good for human health as compared to saturated fatty acids, but they are highly unstable at high temperature and easily undergo oxidation. Saturated oils are less prone to oxidation as compared to unsaturated oils and thus more stable, but consumption of these oils has disadvantage related to CVDs. Hence it is required to encourage the consumption of oils that have low saturated fatty acid content, but are stable under frying conditions (Dhyani *et al*., 2018). Moreover, the viscosity of the oils also depends on the nature of the triglycerides (TGs) present in the oil, that the viscosity changed due to the different arrangement of the fatty acids on the glycerol backbone of the triglyceride molecule (Okparanta, 2018).

The patterns of viscosity values for the studied oils were in increasing order of, $S_{100} < N_{47}F_{53} < N_{8}F_{20}S_{72} < N_{34}S_{66} < F_{47}S_{53} < N_{100} < N_{74}F_{23}S_{3} < N_{12}F_{81}S_{7} < F_{49}S_{51} < N_{59}S_{41} < F_{100}$. (Nzikou *et al.*, 2010). The variation might be due to the molecular structure of oil and

make the bonding more rigid and rotation between C-C bonds becomes more strenuous. Also, the extended chain makes the flow easier and reduces viscosity (Ahmad *et al*., 2010; Mohdaly *et al*., 2017). Studies have showed that oils with high amount of polyunsaturated fatty acid have low viscosity that in this study it was observed that sesame seed oils viscosity was the lowest from both blended and unblended oils which it might rich in polyunsaturated fatty acid (Hashempour-Baltork *et al.*, 2016).

4.1.4 Accelerated oxidative stability of oils

The susceptibility of oils to oxidation can be determined by a method called Rancimat (it is a method used an accelerated aging test and a commonly used procedure in the food industry for the examination of the oxidative stability of edible oils and prediction of their shelf life) (Mohdaly *et al.*, 2017). Oxidation imparts undesirable flavors and aromas, and leads to the induction of toxic compounds. The fatty acid (FA) composition of the oil, oxygen, light and storage temperature are the factors affecting the oxidative stability of oil (Hamed and Abo-Elwafa, 2012).

The Rancimat test for the studied oils showed that the most oxidative stable oil was sesame seed oil temperature at 120 C (the induction time equal to 4.69 hr, while the lowest stability showed flax seed oil (the induction time equal to 0.87 hr). The level of AOS of niger seed oil has lower than sesame and higher than flax seed oil, this could be due to the fact that it has higher antioxidant activity as it contains tocopherols in it (Bhavsar *et al*., 2017). The highest AOS of sesame oils might be due to the presence of natural ant-oxidants including sesamol, sesamolin and sesamin (Gharby *et al*., 2017). The highest AOS for binary oil is $N_{34}S_{66}$ with induction time 4.52 hr, whereas the lowest was obtained at $N_{47}F_{53}$ binary oil with induction time 0.93hr. Furthermore, the highest and the lowest AOS for tertiary blend oils were found in $N_8F_{20}S_{72}$ and $N_{12}F_{81}S_7$ with induction time 3.38 hr and 1.02 hr, respectively. The details of the result are given in Table 3. ANOVA test was applied to the AOS of blended and unblended oils, that both blended and unblended showed a significance difference in their AOS (*p* $_{12}F_{81}S_7$ vs F_{100}; $N_{12}F_{81}S_7$ vs $N_{47}F_{53}$; $N_{34}S_{66}$ vs S_{100}; $F_{49}S_{51}$ vs $F_{47}S_{53}$; $F_{49}S_{51}$ vs N_{100}; $N_{47}F_{53}$ vs F_{100} and $F_{47}S_{53}$ vs N_{100} which did not show a significancy (*p*

The patterns of accelerated oxidative stability (AOS) values for the studied oils were in increasing order of $F_{100} < N_{47}F_{53} < N_{12}F_{81}S_7 < N_{74}F_{23}S_3 < F_{49}S_{51} < F_{47}S_{53} < N_{100} < N_{59}S_{41} < N_8F_{20}S_{72} < N_{34}S_{66} < S_{100}$. The AOS of the blended oils increase with increasing the proportion of sesame oils in blending ratio. Generally, the higher level of natural antioxidants, phenolic compounds and phosphatides can have synergistic effects on the oxidative stability of a given oil, which is known by higher induction time (Mohdaly *et al.*, 2017 , Hamed and Abo-Elwafa, 2012).

In this study the shelf life of selected mono (F_{100}), di ($N_{34}S_{66}$) and tertiary ($N_8F_{20}S_{72}$) blended oils at 25 ^{o}C were estimated using linear relationship between the natural logarithm of the induction time and the temperature. The selection of these oils were based on their stability. The 100 C, 110 C, 120 C, 130 C, and 140 C) and the induction time were recorded. The results are given in Table 4.

Hence, the linear relationship between the natural logarithm of the induction time (IT) and the temperature for F_{100}, $N_{34}S_{66}$ and $N_8F_{20}S_{72}$ oils are given in equation 4, 5 and 6.

F_{100}, log (IT) = -0.0358x+ 4.2938 (4)

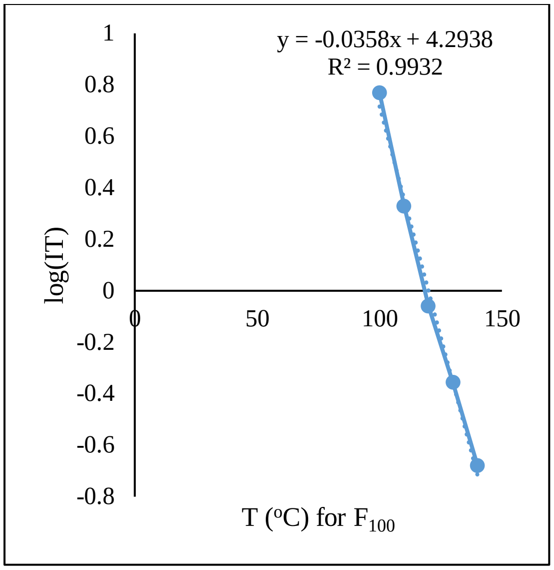
$N_{34}S_{66}$, log (IT) = -0.0345x+ 4.787 5)

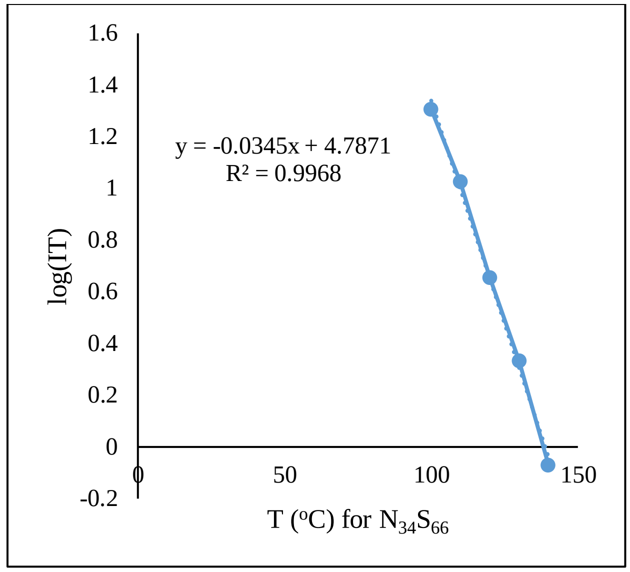
$N_8F_{20}S_{72}$, log (IT) = -0.0356x+ 4.4838 (6)

Hence, by substituting 25 o was obtained. Accordingly, 104, 350 and 374 days are the expected shelf life for F_{100}, $N_{34}S_{66}$ and $N_8F_{20}S_{72}$ oils, respectively. The maximum shelf life was observed at $N_8F_{20}S_{72}$ oil, whereas the lowest value was F_{100} oil. This might be due to the variation in amount of antioxidant and rate of oxidation. The graphs of the linear relationship between the natural logarithm of the IT assessed by the Rancimat test and the temperature are given at Figure 1.

Table 4. Induction time (IT) for different oils at different temperature.

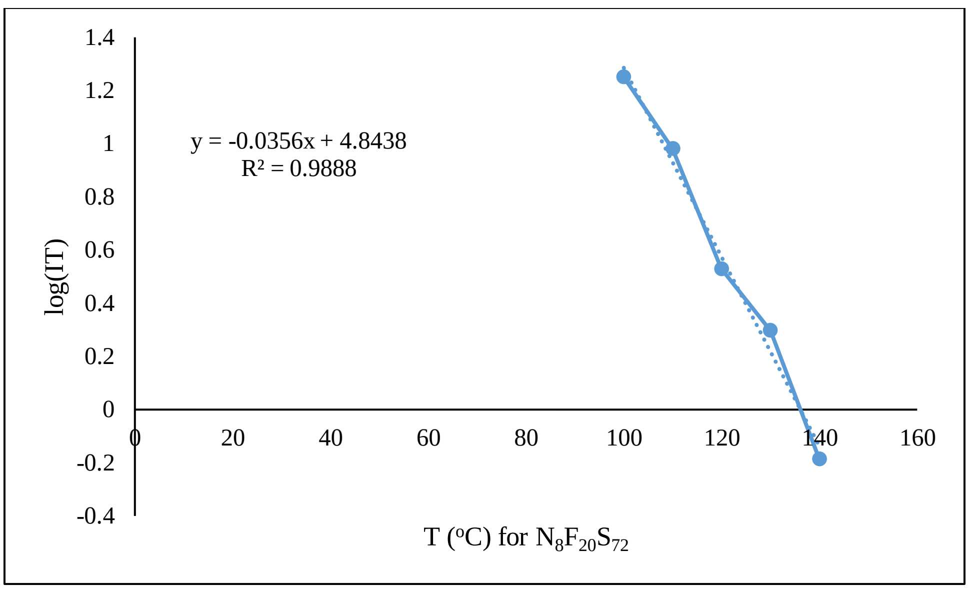
	Induction time (hr)		
100	5.87	20.25	17.8
110	2.13	10.62	9.55
120	0.87	4.52	3.38
130	0.44	2.15	1.98
140	0.21	0.85	0.65





A

B



C

Figure 1. The linear relationship between the natural logarithm of the oxidation induction time (IT) and the temperature for F_{100} (A), $N_{34}S_{66}$ (B) and $N_8F_{20}S_{72}$ (C) oils.

4.1.5 Peroxide value of oils

Peroxide value (PV) is used as indicator of the quality and stability of oils by measuring of the extent to which rancidity reactions have occurred during storage (Okparanta, 2018; Fm Ali, 2012). In addition to this peroxide value of oils also will definitely increase after exposure to light and air at room temperature. The trace amount of heavy metals can also positively affect enhancement of PV. Peroxide value is an indication of the extent of oxidation suffered by oil. High peroxide value indicates high degree of unsaturation, which in turn responsible for oxidative rancidity (AL Majidi and Bader, 2015).The peroxide value for the studied oils at different storage time are given in Table 3. The result showed that the PV for pure oils after 15 days of storage, 45 day of storage (PV at day 45) and 75 day of storage (PV at day 75) were found in 1.56-3.19, 2.47-5.01 and 3.49-7.78 meq O_2/kg, respectively. Thus, the lowest value for pure oil at all storage time was observed in sesame oil, while the highest value was seen at flax seed oil extract. This might be due to the fact that sesame oils have natural antioxidants (such as sesamol, sesamolin and sesamin together with other phenolic compounds, and tocopherols) in it which hinder the oxidation process, whereas flax seed oil has no such antioxidants (Ahmad *et al*., 2010; Gharby *et al*., 2017; Hashempour-Baltork *et al*., 2016b). The PV for niger seed oils found in this study is lower than from the study conducted by (Bhavsar *et al*., 2017) which was 2.6 meq O_2/kg. This might be due to the difference in geographical and environmental factors.

The PV for binary oils after 15 days of storage, 45 day of storage (PV at day 45) and 75 day of storage (PV at day 75) were found in 1.49-2.39, 2.50-3.01 and 4.04-6.33 meq O_2/kg, respectively. Furthermore, the lowest peroxide value for binary oil at all storage time was observed in $N_{34}S_{66}$, where the highest peroxide value was seen at $F_{49}S_{51}$ blends, except at day 75 which is $N_{47}F_{53}$.

The PV for tertiary oils after 15 days of storage, 45 day of storage (PV at day 45) and 75 day of storage (PV at day 75) were found in 1.59-2.99, 3.26-5.01 and 3.87-7.20 meq O_2/kg, respectively. The highest value for tertiary oil at all storage time was observed in $N_{12}F_{81}S_7$, where the lowest value was seen at $N_8F_{20}S_{72}$ blends. Generally, the variation in the peroxide value might be due to variation in chemical composition, the storage condition and contact with air of the oil samples. Thus, studies showed that the PV of each oils increases with increasing of the storage time (Okparanta, 2018). The overall peroxide value obtained at day15, at day 45 and at day 75 were found in 1.49-3.19; 2.47-5.01 and 3.49-7.78 meq O_2/kg, respectively.

Generally, the peroxide value of blended oils was varied according to their corresponding constituents of individual oils. For instance, the peroxide value of both niger and flax seed oil was reduced while increasing with mixing ratio of sesame oils in their blends. The peroxide values of oils in this studies were found in acceptable range as given by Preservation of Food and Adulteration Act (PFA) and codex standard for vegetable oils (CODEX-STAN210-1999) which is 10 meq/kg, and according to WHO and FAO virgin oils and cold pressed fats and oil which are up to 15 meq/kg oil (CODEX STAN 19-1981). Furthermore, when PV is greater than 20 meq/kg noticeable rancidity of oils will be occurred that all the studied oils were under 20 meq/kg (Gouveia Lde *et al*., 2016; Dimberu *et al*., 2015). Moreover, the PV of the flax seed oils has been improved by both sesame and niger seed oils.

The patterns of the PV at 15, 45 and 75 incubation days were found in decreasing order of $N_{34}F_{66} < S_{100} < F_{47}S_{53} = N_8F_{20}S_{72} < N_{100} < N_{59}S_{41} < N_{74}F_{23}S_3 < N_{47}F_{53} < F_{49}S_{51} < N_{12}F_{81}S_7 < F_{100}$; $S_{100} < N_{59}S_{41} < N_{34}S_{66} < N_{100} < F_{47}S_{53} < F_{49}S_{51} < N_{47}F_{53} < N_8F_{20}S_{72} < N_{74}F_{23}S_3 < F_{100} < N_{12}F_{81}S_7$ and $S_{100} < N_{100} < N_8F_{20}S_{72} < N_{34}S_{66} < F_{47}S_{53} < N_{59}S_{41} < F_{49}S_{51} < < N_{74}F_{23}S_3 < N_{47}F_{53} < N_{12}F_{81}S_7 < F_{100}$, respectively. The peroxide value of each oil was increased upon the increasing of storage time or incubation time. The ANOVA test showed that the individual sesame and niger oils had a significantly different in PV from individual F_{100} oil, binary blend ($F_{49}S_{51}$) and tertiary blend oil ($N_{12}F_{81}S_7$) during the fifteen day of incubation time. Similarly, F_{100} seed oil showed a significant difference in PV to all of the three type of oil (individual, binary and tertiary blend oils), except $N_{12}F_{81}S_7$ did not show a significant difference. $F_{49}S_{51}$ and $N_{12}F_{81}S_7$ also showed a significant difference in their PV from all the three types of oils, except $N_{47}F_{53}$ and $N_{74}F_{23}S_3$ has no significant difference with $F_{49}S_{51}$. Furthermore, after 45 day of incubation the ANOVA test showed that the individual oil F_{100} (except, F_{100} between $N_{12}F_{81}S_7$ and $N_{74}F_{23}S_3$), the tertiary oil $N_{12}F_{81}S_7$ and $N_{74}F_{23}S_3$ (except, $N_{74}F_{23}S_3$ between $N_8F_{20}S_{72}$ and $N_{47}F_{53}$) had a significant different in their PV from all the three type oils.

According to WHO the nutritional value of a given oil can be influenced by three major things including the availability of antioxidant, the ratio of saturated: monounsaturated : polyunsaturated fatty acid (1:1.5:1) and essential fatty acids (omenga-3: omega-6; 1:5-10)). The oil with higher amount of polyunsaturated fatty acid possess highly susceptibility to oxidation. On the other hand, consumption of high level of saturated fatty acids promoting vascular smooth muscle proliferation due to increasing the concentration of low density lipoproteins (LDL) (Kostik *et al*., 2012). Hence, balancing of these fatty acids is highly important for health. Thus, this study has done blending of flax seed having higher amount of polyunsaturated fatty acids with niger or sesame having low amount of polyunsaturated fatty acid separately or their blends to increase the oxidative stability of flax seed oil and nutritional value of both oils (Hamed and Abo-Elwafa, 2012).

4.1.6 Sensory evaluation

One of the most important parameters for the assessment of the quality of edible oils is the sensory evaluation. Sensory evaluation is a scientific method used to evoke, measure, analyses and interpret those responses to products as perceived through the senses of sight, hearing, touch, smell and taste. The result of the sensory evaluation can be objectified by different chemical parameters, but the sensory impression is the decisive factor for the evaluation of the oil as edible provided that no toxic contaminants are against this assessment. One reason for the importance of the sensory quality of the oil is that more than any other parameter the appearance and the taste deeply influence the buying decision of the consumer**.** The sensory parameters such as color, flavor, mouth feel, taste, and overall acceptability of any food product depends on the extent of oxidation of fats and oils in the food due to the formation of peroxides, aldehydes and ketones. Although sensory evaluation of foods is the most important quality assessment, taste evaluations are not practical for routine quality control. It is always preferable to have a quantitative method for which rejection points may be established by sensory means (Brühl and Matthäus, 2008).

In this study, the three types of the blending ratio under go for sensory evaluation, where the selection was based on the oxidative stability involved in the blending. Besides, not to lose panelists motivation and evaluation ability, only three samples were given for evaluation in one session. Affective testing method was applied for this analysis, so that 9-point hedonic scale with expressions stretching from dislike extremely to like extremely were used. Thus, 1= dislike extremely and 9= like extremely (M.K. Sharif *et al.*, 2017). Sensory analysis of blended $N_8F_{20}S_{72}$, $N_{34}S_{66}$ and F_{100} from each treatment was evaluated by 70 untrained sensory panelists and provided with samples in plates coded with three-digit numbers and they were asked to rinse their mouth with water after tasting each sample in order not affecting the previous taste for the next.

The percent respondents for color, odor, flavor, taste, mouth feel and overall acceptability for the prepared salad with $N_8F_{20}S_{72}$ oil was found 1.4% respondents were who dislike slightly the salad-50% respondents were who like very much the salad, 10% respondents were who like salad slightly-54% respondents were who like salad very much, 2.9% respondents were who neither nor dislike the salad-54.3% respondents were who like salad very much, 4.3% respondents were who neither nor dislike the salad-42.9% respondents were who like very much the salad, 4.3% respondents were who like slightly the salad-60% respondents were who like very much the salad and 4.3% respondents were who neither nor dislike the salad-50% respondents were who like very much the salad, respectively.

Similarly, the percent respondents for color, odor, flavor, taste, mouth feel and overall acceptability for the prepared salad with $N_{34}S_{66}$ oil was found in the range 1.4% respondents who like moderately the salad-62% respondents who extreme like the salad, 8.6 % respondents who like moderately the salad-47.1% respondents who like very much the salad), 7.1% respondents who like moderately the salad-47.1% respondents who extreme like the salad, 2.9% respondents who neither nor dislike the salad-50%

respondents who like very much the salad, 1.4% respondents who neither nor dislike the salad-47.1% (respondents who like very much the salad and 5.7% respondents who like slightly the salad-45.7% respondents who like very much the salad, respectively.

Furthermore, the percent respondents for color, odor, flavor, taste, mouth feel and overall acceptability for the prepared salad with F_{100} oil was found in the range 2.9% respondents were who dislike moderately the salad-25.7% respondents were who extreme like moderately the salad, 5.7 % respondents were who dislike moderately the salad-25.7% respondents were who like very much the salad, 2.9% respondents were who dislike very much the salad-24.3% respondents were who like very much the salad, 7.1% respondents were who dislike very much the salad-30% respondents were who like very much the salad), 10.0% respondents were who dislike very much the salad-34.3% respondents were who like very much the salad and 5.7% respondents were who dislike very much the salad-21.5% respondents were who like very much the salad, respectively.

Generally, the salad was prepared for the sensory test that salad prepared with $N_8F_{20}S_{72}$ oil is the highest overall acceptance 50.0 % of the respondents like the salad very much and the lowest overall acceptance was observed using flax seed oil F_{100} 21.4% for all test parameters based on 9-hedonic scale, although the respondent response vary with test parameters. This showed that blending can influence the color, odor, flavor, taste and mouth feel of the single flax seed oil. The details of the percent respondents to each parameter based on the 9-hedoinc scale are given in Table 5.

Table 5. The percent of respondents to each testing parameters according to hedonic scale test.

Type of oil	hedonic scale	1	2	3	4	5	6	7	8	9
	color				1.40	2.90	5.70	21.4	50.0	18.6
	odor						10.0	11.4	54.3	24.3
	flavour					2.90	1.40	17.1	54.3	24.3
	taste					4.30	10.0	14.3	42.9	28.6
	mouth feel						4.30	14.3	60.0	21.4
$N_8F_{20}S_{72}$	overall acceptance					4.30	10.0	14.3	50.0	21.4
	color							1.40	35.9	62.9
	odor							8.60	47.1	44.1
	flavour							7.10	45.7	47.1
	taste					2.90	5.70	12.9	50.0	28.6
	Mouth feel					1.40	8.60	10.0	47.1	32.9
$N_{34}S_{66}$	overall acceptance						5.70	12.9	45.7	35.7
	color			2.90	8.60	14.30	20.0	25.7	24.3	4.30
	odor			5.70	18.60	14.30	15.70	12.9	25.7	7.10
	flavour		2.90	5.70	8.60	17.10	14.30	14.3	24.3	12.9
	taste		7.10	7.10	10.00	12.90	10.00	15.7	30.0	7.10
Flax seed	Mouth feel		10.0	2.90	10.00	14.30	12.90	8.60	34.3	7.10
(F_{100})	overall acceptance		5.70	4.30	5.70	18.60	12.90	20.0	21.4	11.4

Note: 1 = Extreme dislike; 2 = Dislike very much; 3 = Dislike Moderately; 4 = dislike slightly; 5 = neither nor dislike; 6 = Like slightly; 7 = Like moderately; 8 = Like very much and 9 = Extreme like.

As shown in Figure 2, the color, odor, flavor, taste, mouth feel and over all acceptability were shown in radar chart. The average score for color, odor, flavor, taste and mouth feel were vary from oil to oil. The variation in the color, odor, flavor, taste and mouth feel for different oil types might be due to the different chemical composition of oils and their mixing ratio, although, further study was required to identify the main responsible factor for. Besides, the variation in the color, odor, flavor, taste and mouth feel also affect the overall acceptability the oil. ANOVA test was applied that reviled that all parameters have showed a significant difference across the three-oils types. The detail of the ANOVA test is given in appendix II and III. Generally, from this study, one can concluded that the acceptance of all tested parameter is better in using tertiary blend oils followed by the binary blend and single flax seed oil.

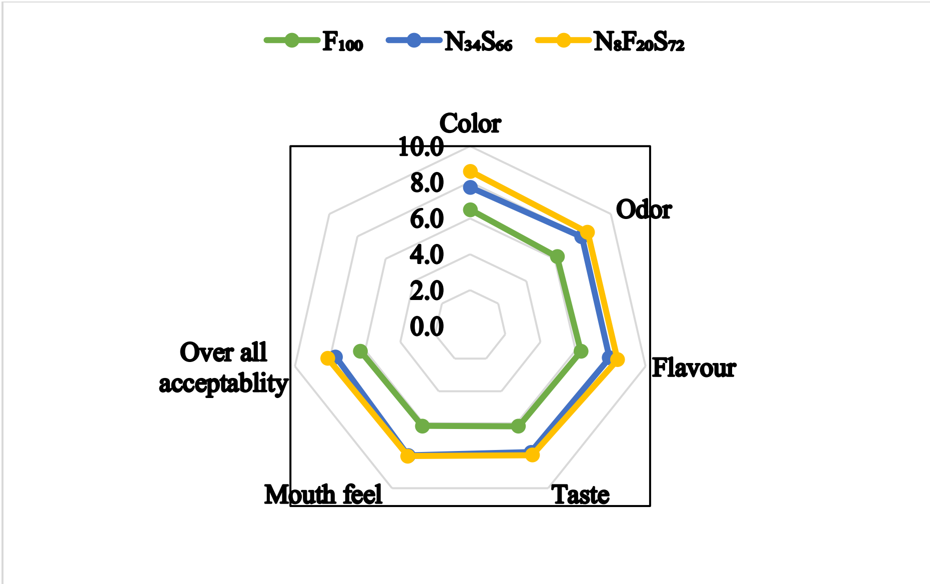


Figure 2. The radar chart for all test parameters based on 9-hedonic scale.

4.1.6.1 Color analysis

Color of oil was one of the most important physical properties that attract consumer appellant (Ahmad *et al*., 2010). The average scores for color of the three-oil types were found in the ranged 6.5-8.61, the highest score was resulted for the $N_8F_{20}S_{72}$ blended oil (8.61), while the lowest score was resulted for the color of was F_{100} (6.5). The variation in the color might be due to the difference in chemical composition in the tested oils.

4.1.6.2 Odor analysis

The odor of an oil is the most important indicator of quality oil. It can caused by one or more volatilized chemical compounds that are generally found in low concentrations that humans and animals can perceive by their sense of smell. An odor is also called a "smell" or a "scent", which can refer to either a pleasant or an unpleasant odor. An oil with off-odor will be quickly rejected by consumers. This is the reason why the majority of published studies are on the shelf-life of processed oils rather than on freshly processed oils. Hence, in this study the odor for selected blended oil were analyzed and the results revealed that the average scores for odor of the oils studied were found in the ranged 6.5-

8.36, the highest score was resulted for the $N_8F_{20}S_{72}$ blended oil (8.36), while the lowest score was resulted for the color of was F_{100} (6.5). The change in odor of the oils varies from oil to oil due to the change on the level of processing, the length and conditions of storage of the oils https://en.wikipedia.org/wiki/odor).

4.1.6.3 Flavor analysis

Flavor is one of the most important qualities of foods. The generation of off-odors and off flavors in foods especially in oils/fats and oils/fats based foods are usually associated with oxidative and/or hydrolytic degradations of the lipid components. These flavor qualities are directly correlated with the value of the oil for the consumer and determine the success or failure of a product on the market. Therefore, a precise method was needed to assess the sensory qualities of virgin oils for the producer, the food surveillance and the consumer (Brühl and Matthäus, 2008). Hence, flavor taste was done in this study and the results showed that the highest score was resulted for the $N_8F_{20}S_{72}$ blended oil (8.40), while the lowest score was resulted for the color of was F_{100} (6.31). Such variation might be due to the variation in the degree of unsaturation of lipids; exposure to light, oxygen, or certain enzymes (lipoxygen-ases); presence of transition metal ions, pro-oxidants, or antioxidants, and so on, while the lipid hydrolysis pathway usually involves hydrothermal or enzymatic (lipase) reactions oils that affects lipid oxidation in oil (Yang and Boyle, 2016).

4.1.6.4 Taste analysis

Taste is the sensation produced or stimulated when a substance in the mouth reacts chemically with taste receptor cells located on taste buds in the oral cavity, mostly on the tongue. Taste, along with smell (olfaction) and trigeminal nerve stimulation (registering texture, pain, and temperature), determines flavors of food and/or other substances. Humans have taste receptors on taste buds (gustatory calyculi) and other areas including the upper surface of the tongue and the epiglottis (https://en.wikipedia.org/wiki/Taste). Similar to other parameters such as odor, color and flavor taste also one of the major factors affecting the acceptance of the oil by the consumers. The average scores taste for the selected oil type were found in the ranged 6.18-7.96, the highest score was resulted for the $N_8F_{20}S_{72}$ blended oil (7.96), while the lowest score was resulted for the color of was F_{100} (6.18). The variation might be due to variation in chemical composition of oils and subjective perceptions of the panelists.

4.1.6.5 Mouthfeel

Mouth feel (sometimes called, texture) refers to the physical sensations in the mouth caused by food or drink, as distinct from taste. It is a fundamental sensory attribute which, along with taste and smell, determines the overall flavor of a food item (https://en.wikipedia.org/wiki/Mouthfeel). The average scores taste for the selected oil type were found in the ranged 6.15-8.01, the highest score was resulted for the $N_8F_{20}S_{72}$ blended oil (8.01), while the lowest score was resulted for the color of was F_{100} (6.15).

4.1.6.6 Analysis of overall acceptability

Overall acceptability was calculated based on the average score give to the color, odor, flavor, taste and mouth feel that the overall acceptability the studied oils were found in the range of 6.15-8.01. The highest and the lowest average score values were observed at F_{100} and $N_8F_{20}S_{72}$, respectively. Although, further study was required to identify the main responsible factor for the variation in the color, odor, flavor, taste and mouth feel for different oil types might be due to the different chemical composition of oils and their mixing ratio. Besides, the variation in the color, odor, flavor, taste and mouth feel also affect the overall acceptability the oil. Generally, from this study, one can concluded that the acceptance of all tested parameters is better in using tertiary blend oils followed by the binary blend and single flax seed oil.

CHAPTER 5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

It is well known that blending of different types of oils can improve physicochemical properties of oils. Thus, in this study the quality and properties of the oils and blends were evaluated through using different parameters. For instance, the general trend showed the level of AOS (accelerated oxidative stability) found in the order of 0.87< 0.93< 1.02< 1.32< 2.31< 2.45< 2.52< 3.06< 3.38< 4.52 <4.69. The highest AOS was recorded sesame oil, whereas the lowest values were measured flax seed oil that sesame oil was found to be the most stable against oxidation. Sesame oil has the natural potential of hindering the oxidation of niger and flax seed oils. Though for the other oils these phenomena are almost the same, for both the binary and tertiary blend were found to have more resistance against oxidation. Thus, this study will help the oil producing industry to find the most economically viable oil blends for cooking purposes, with maximum nutrition as well as desirable physicochemical properties. Therefore, blending is a good choice by which any stake holder can manufacture edible oils of good characteristics and ensure their quality. The food value of the oils and blends can also be predetermined to provide the safest food for consumers. Generally, it can be concluded that the flax oil stability can enhance through blending without using synthetic mechanism such as hydrogenation that can cause for the formation of toxic fatty acid which is trans fatty acid.

Moreover, the ested oils were found in the range of 0.9207-0.9467 mg/L, 46.2-63.6 centipoise, 1.467-1.482, 171-177 mg of KOH/g of oil, respectively. The highest value for density, viscosity, refracti $_8F_{20}S_{72}$, F_{100}, S_{100} and both ($N_{47}F_{53}$ and $N_{74}F_{23}S_3$), respectively, whereas their lowest value was observed at F_{100}, S_{100}, F_{100} and both (S_{100} and $N_{34}S_{66}$), respectively. The PV for pure oils after the 15 days of storage (denoted at PV at day one), 45 day of storage (PV at day 45) and 75 day of storage (PV at day 75) were found in the range 1.56-3.19, 2.47-5.01 and 3.49-7.78 meq O_2/kg, respectively. The highest PV value for pure, binary and tertiary oils at all storage time was observed in S_{100}, $N_{34}S_{66}$ and $N_{12}F_{81}S_7$, respectively. Where their lowest value was seen at F_{100}, $F_{49}S_{51}$ blends, except at day 75 which is $N_{47}F_{53}$ and $N_8F_{20}S_{72}$ respectively.

The sensory evaluation based on the 9-hedonic scales were done and the results showed that the overall acceptance of the tested parameters are better in using tertiary ($N_8F_{20}S_{72}$) blended oils followed by the binary blended ($N_{34}S_{66}$) and single flax seed oil (F_{100}).

5.2 Recommendation

Measuring the fatty acid composition and different antioxidant properties for unblended and blended oils was not performed in this study, although it has used for estimating of the benefits of such oils. So, we are highly recommended to interested people to do the research in this area. Moreover, the results of this study could be used as a benchmark for future research on the examination of health issues related to the oils. Since Ethiopia is the world known producer of niger seed, the government will use the result of the study to promote the niger seed oil.

Furthermore, as far as our knowledge is concerned there are no small and large-scale production of blended oils that people who interested in the area will produce blended oils after completing the remaining research works (such as fatty acid composition and frying stability).

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APPENDIX I:

ANOVA test for density, RI, SV, AOs and oxidative stability of both pure and blended oils.

		Sum of Squares	Df	Mean Square	F	Sig.
SV	Between Groups	94.431	10	9.443	1.048	.439
	Within Groups	198.280	22	9.013		
Density	Between Groups	.004	10	.000	1.039	.445
	Within Groups	.008	22	.000		
Viscosity	Between Groups	965.138	10	96.514	26.035	.000
	Within Groups	81.556	22	3.707		
Refractive index	Between Groups	.000	10	.000	45058.344	.000
	Within Groups	.000	22	.000		
AOS	Between Groups	56.115	10	5.612	933.927	.000
	Within Groups	.132	22	.006		
PV at day 15	Between Groups	10.281	10	1.028	25.776	.000
	Within Groups	.877	22	.040		
PV at day 45	Between Groups	23.260	10	2.326	22.128	.000
	Within Groups	2.313	22	.105		
PV at day 75	Between Groups	67.636	10	6.764	26.607	.000
	Within Groups	5.593	22	.254		

APPENDIX II:

ANOVA test for color, odor, flavor, taste, mouth feel and over all acceptability of tested oils.

		Sum of Squares	Df	Mean Square	F	Sig.
Color	Between Groups	162.086	2	81.043	69.808	.000
	Within Groups	240.314	207	1.161		
Odor	Between Groups	187.800	2	93.900	64.222	.000
	Within Groups	302.657	207	1.462		
Flavor	Between Groups	156.867	2	78.433	49.161	.000
	Within Groups	330.257	207	1.595		
Taste	Between Groups	142.467	2	71.233	33.059	.000
	Within Groups	446.029	207	2.155		
Mouth feel	Between Groups	158.514	2	79.257	39.701	.000
	Within Groups	413.243	207	1.996		
Over all acceptability	Between Groups	126.438	2	63.219	34.120	.000
	Within Groups	383.543	207	1.853		

APPENDIX III:

Post hoc ANOVA test (based on Tukey HSD) for color, odor, flavor, taste, mouth feel and over all acceptability of tested oils.

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Color	$N_8F_{20}S_{72}$	F_{100}	1.243*	.182	.000	.81	1.67
		$N_{34}S_{66}$	-.900*	.182	.000	-1.33	-.47
	F_{100}	$N_8F_{20}S_{72}$	-1.243*	.182	.000	-1.67	-.81
		$N_{34}S_{66}$	-2.143*	.182	.000	-2.57	-1.71
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.900*	.182	.000	.47	1.33
		F_{100}	2.143*	.182	.000	1.71	2.57
Odor	$N_8F_{20}S_{72}$	F_{100}	1.757143*	.204388	.000	1.27464	2.23964
		$N_{34}S_{66}$	-.428571	.204388	.093	-.91107	.05393
	F_{100}	N8F20S72	-1.757143*	.204388	.000	-2.23964	-1.27464
		$N_{34}S_{66}$	-2.185714*	.204388	.000	-2.66821	-1.70322
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.428571	.204388	.093	-.05393	.91107
		F_{100}	2.185714*	.204388	.000	1.70322	2.66821
Flavour	$N_8F_{20}S_{72}$	F_{100}	1.5714*	.2135	.000	1.067	2.075
		$N_{34}S_{66}$	-.4429	.2135	.098	-.947	.061
	F_{100}	$N_8F_{20}S_{72}$	-1.5714*	.2135	.000	-2.075	-1.067
		$N_{34}S_{66}$	-2.0143*	.2135	.000	-2.518	-1.510
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.4429	.2135	.098	-.061	.947
		F_{100}	2.0143*	.2135	.000	1.510	2.518
Taste	$N_8F_{20}S_{72}$	F_{100}	1.6714*	.2481	.000	1.086	2.257
		$N_{34}S_{66}$	-.1429	.2481	.833	-.729	.443
	F_{100}	$N_8F_{20}S_{72}$	-1.6714*	.2481	.000	-2.257	-1.086
		$N_{34}S_{66}$	-1.8143*	.2481	.000	-2.400	-1.229
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.1429	.2481	.833	-.443	.729
		F_{100}	.8143*	.2481	.000	1.229	2.400
Mouth feel	$N_8F_{20}S_{72}$	F_{100}	1.82857*	.23883	.000	1.2648	2.3924
		$N_{34}S_{66}$	-.02857	.23883	.992	-.5924	.5352
	F_{100}	$N_8F_{20}S_{72}$	-1.82857*	.23883	.000	-2.3924	-1.2648
		$N_{34}S_{66}$	-1.85714*	.23883	.000	-2.4209	-1.2933
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.02857	.23883	.992	-.5352	.5924
		F_{100}	1.85714*	.23883	.000	1.2933	2.4209
Over all acceptability	$N_8F_{20}S_{72}$	F_{100}	1.42857*	.23008	.000	.8854	1.9717
		$N_{34}S_{66}$	-.37143	.23008	.242	-.9146	.1717
	F_{100}	$N_8F_{20}S_{72}$	-1.42857*	.23008	.000	-1.9717	-.8854
		$N_{34}S_{66}$	-1.80000*	.23008	.000	-2.3432	-1.2568
	$N_{34}S_{66}$	$N_8F_{20}S_{72}$	.37143	.23008	.242	-.1717	.9146
		F_{100}	1.80000*	.23008	.000	1.2568	2.3432

*. The mean difference is significant at the 0.05 level.