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KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

OXIDATIVE STABILITY OF THE RED PEPPER SEED OIL

ADNAN ALI MUSTAFA

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ, TURKEY, 2018

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ADNAN ALİ MUSTAFA
ÖZET

Daha yüksek değere sahip faydalı ürünlere dönüştürme, hammadde veya biyolojik işlemlerden sonra gıda ya da yem olarak kullanma potansiyele sahip olan işlenmiş gıda atıkları, çeşitli endüstriler için yeni, alternatif ve ucuz biyoaktif veya gıda bileşenleri kaynağı olarak birçok çalışmaya konu olmuştur. Bu çalışma, kırmızı biber çekirdek yağının depolama sırasında oksidatif stabilitesinin araştırılmasını amaçlamıştır. Kırmızı biber çekirdeğindeki yağ çözücü ekstraksiyon tekniği kullanılarak ekstrakte edilmiştir. Yağlar karanlık ve ışık ortamında 90 gün boyunca oda sıcaklığında depolanmıştır. Kırmızı biber çekirdek yağının yağ asit kompozisyonu belirlenmiş ve depolama süresince peroksit, serbest yağ asitleri, konjuge dien (K_{232}) ve konjuge triene (K_{270}) analizi ile yağın oksidatif stabilite izlenmiştir. Kırmızı biber yağının yüksek miktarlarda linoleik asit içerdiği belirlenmiştir (yaklaşık %72). Depolama süresi arttıkça yağın oksidatif stabilitesi önemli ölçüde, özellikle ışık ortamında, azalmıştır. Başlangıçtaki yağın peroksit değeri 1,4 meq O_2/kg yağ'dır. 90 günlük depolamanın sonunda, ışık ortamında peroksit değeri 4,65 meq O_2/kg yağ ve karanlık ortamda 3,8 meq O_2/kg yağ'dır. Yağın, başlangıçta serbest yağ asitleri içeriği (oleik asit yüzdesi) %2,35 olarak bulunmuştur. Son depolama periyodunda, serbest yağ asitleri içeriği ışıkta %5,32 karanlıkta ise %4,08 yükselmiş ve konjuge dien (K_{232}) içeriği karanlık ortamda 1,54 ışık ortamında ise 2,06 iken konjuge triene (K_{270}) içeriği karanlık için 0,7 ve ışık için 1,66 olduğu belirlenmiştir.

Anahtar Kelimeler: kırmızı biber çekirdek yağı, oksidatif stabilite, peroksit, serbest yağ asidi, yağ asit komposizyonu

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OXIDATIVE STABILITY OF THE RED PEPPER SEED OIL

(M.Sc. THESIS)

ADNAN ALI MUSTAFA

ABSTRACT

Food processing wastes; which have a potential for conversion into useful products of higher value as by product, or even as raw material for other industries, or for use as food or feed after biological treatment, have been the subject of many studies as an attempt to find new, alternative and cheap resources of bioactive or food components with application in several industries. Red pepper seed being waste in red pepper industry contains %15-20 oil. The present study aimed at investigation of the oxidative stability of red pepper seed oil during storage. The oil in red pepper seed was extracted by using solvent extraction technique. The oil samples were stored at room temperature for 90 days in the dark and light medium. The fatty acid composition of the oil was determined, and the oxidative stability of the red pepper seed oil during the storage were monitored by analysis of peroxide, free fatty acids, conjugated diene (K_{232}) and conjugated triene (K_{270}). It was determined that the red pepper oil contained the high amounts of linoleic acid (approximately 72%). The oxidative stability decreased significantly, especially in the light as the storage period increased. The initial peroxide value was 1.4 meq O_2/kg oil. At the end of 90-day storage the value was 4.65 meq O_2/kg oil for the light storage and 3.8 meq O_2/kg oil for the dark storage. The initial free fatty acids content (percent oleic acid) in the oil was found 2.35%. It raised to 5.32% in the light and 4.08% in the dark at the final storage period, and also it was determined that conjugated diene (K_{232}) content was 1.54 in the dark medium and 2.06 in the light medium as conjugated triene (K_{270}) contents were 0.7 for the dark and 1.66 for the light.

Keywords: fatty acid composition, free fatty acid, oxidative stability, peroxide, red pepper seed oil

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Adnan Ali Mustafa

TABLE OF CONTENTS

Page No

LIST OF FIGURES

Page No

LIST OF TABLES

Page No

1. INTRODUCTION

Red pepper (*Capsicum annuum* L.) is a very common and widely utilized pepper spices in Turkey. It can be utilized for different purposes including seasoning, flavoring, imparting aroma, colorant, or as a pungent product (Arslan and Özcan 2011; Omolo *et al.* 2014; Korkutata and Kavaz 2015). The number one producer of this pepper is China which produces 612,800 ha (36% of the area) for growth or 12,531,000 ton/year (50% of the production) followed by Turkey which produces 1,745,000 ton/year (Omolo *et al.* 2014). It is the most cultivated and consumed spice worldwide, particularly in temperate and semi temperate countries (Ananthan *et al.* 2018; Bekir *et al.* 2016; Aydin *et al.*, 2007). Chilli peppers (*Capsicum annuum* L.) belong to the Solanaceae family (Doymaz and Pala, 2002; Korkutata and Kavaz, 2015; Sung *et al.* 2015a). Pepper is a valuable tropical and subtropical crop considering the fact that its high consumed, nutritious and cash value to farmers and consumers both in developed and developing countries (Rasul, 2015).

Chilli pepper is grown commonly in various areas of the world particularly in cultivated tropical and temperate parts (Zou *et al.* 2015; Arslan and Özcan, 2011). The chili is a self-pollinating, diploid, facultative crop which is tightly connected to family of potatoes, tomatoes, eggplants, tobacco, and petunia. It is believed that chili pepper is one of the ancient domesticated products in the Western hemisphere and the most widely grown spices in the world as it is a main constituent in many cuisines (Surh *et al.* 1998; Omolo *et al.* 2014). Red pepper (*Capsicum annuum* L.) is an excellent source of antioxidant compounds such as carotenoids (Di and Crapiste, 2008; Vega-Gálvez *et al.* 2009).

Chili peppers utilization history belongs to prehistoric times. According to preserved peppers evidence it has been assumed that Americans grew and utilized aji (chili in English), since 2500 B.C. The peppers become more common and combined to the diet of particular cultures. Whereas, Hot peppers and analogous spices stayed confined to these cultures until the 13th century, when they became prevalent to nations. The importance of chili peppers is attributed to the generation of capsaicinoids (also called capsinoids, a group of naturally generated constituents that are uncommon to the Capsicum) (Omolo *et al.* 2014).

Health benefits, nutritional profile and sensory characteristics make pepper a

commonly utilized vegetable, which is a rich source of polyphenols, particularly flavonoids. These compounds proved antioxidant property, anticancer potency against certain cancers, improving the immune system, reducing the risks of cardiovascular diseases, and slowing the aging process. Human health, well-being and diet can be improved via consumption of various phytochemicals and different bioactive compounds in foods. Hot peppers possess various of applications , including nutraceuticals, natural coloring material, and cosmetic usages, as an ornamental plant, and as the bioactive compound in most defense repellants (i.e. pepper sprays) (Chen *et al*., 2012; Cagno *et al.* 2009; Omolo et al., 2014). Capsicum species are utilized very commonly globally not only as a food stuff, but also in traditional medicine against some diseases (health problems) such as gastric ulcers, rheumatism, alopecia, toothache and diabetes mellitus (Tundis *et al.* 2013).

Despite its valuable potential and uses as non-animal oil, there is a little information present on the production of pepper for seed oil purposes. Also there are no studies out there on the growth of pepper in particular for oil production. Rather, pepper seed oil is normally prepared from the waste products of pepper produced for other applications. It has been stated that seed of a domestically grown pimento variety produced ~132.5 L of oil per 0.9 (wet weight) of seed when compressed. In addition, 32,000 MT (metric tons of wet weight) of pimento pepper was harvested in the southeastern US in 1964, and those fruits contained approximately 1320 MT (metric tons of non-dried weight) of seed that would be anticipated to produced 20% oil. Fruit of some hot pepper species are dried prior to processing, and in that sense, seeds constitute ~ 60% of the total dry weight (Jarret *et al*., 2013).

Hot pepper seeds are attained as waste processed products in the preparation of red pepper color and powder. They account for 450–500 g/kg of the total pepper weight (Gu *et al.* 2016; Jinyan *et al.*, 2014). Oil is present in hot seed ranges from 12 to 26% in different Indian species and averaged about 24.4% in a number of Greek and Italian species and described the fatty acid composition of *(C. annuum*) seed oil as 67.0% C18:2, 14.8% C18:1, 11.3% C16:0 and 4.4% C18:0 (Jarret *et al*., 2013; Wang *et al.*, 2017).

Oil of vegetable are a valuable source of fatty acids and have been well utilized in cosmetic products. This can be attributed to their oiling; softening, smoothing and protective properties contain a large number of essential compounds for human being. In addition to that there are a number of ingredients of high-quality cosmetic materials for different purposes such as skin and hair care. In cosmetics, they are utilized for both base and active constituents. Majority of natural compounds were high biological activity such as vitamins A, D, E, provitamin A. All aforementioned benefits of vegetable oils and fatty acids may illustrate the importance of these ingredients for skin and hair care (Zielinska and NowAK, 2014).

Smashed and powdered red pepper is amongst the most utilized spices globally (Aydin *et al.* 2007). In Turkey, generally peppers planted can be classified into five groups, according to their general morphology and/or initial application. These types include bell-type Dolmalık peppers (used for stuffing), long pointed Sivri peppers, long blunt-ended Charleston type Çarliston peppers (consumed raw), small-fruited "fancy" Süs peppers (eaten fresh or pickled) and paste Salçalık peppers (processed into a paste). All of them is multi-usage which means not used exclusively for only one purpose (Keceli, 2008; Frary *et al.* 2008).

In particular, red pepper for spice is of the most important income source for farmers of the south and southeast cities of Turkey (Tursun *et al.* 2012).

Pepper is one of the majorly cultivated in Anatolia, and in the Marmara areas of Turkey. The majority of chili pepper planted in South Anatolia is used to make spices, but it is also used as a fresh eating and red pepper paste. Red pepper is planted in some cities of turkey such as in the Kahramanmaras, Gaziantep and Sanliurfa districts of Turkey are well known for their unique flavors and tastes. Kahramanmaraş is one of the most important cities in connection to chilli plant in Turkey (Figure 1.1). Fresh chilli peppers production is about 80.000 tons per year in Kahramanmaras and neighboring countries. These products are dried and processed to about 16-20 tons milled or powdered chillies. According to values of exported organic products, dried crops have a high percentage (Bekir *et al.* 2016; Özkan *et al.* 2015).

Figure 1.1. Kahramanmaras red pepper.

Even though the final quality of processed red pepper is assessed through a number of various indices, colour and pungency levels are the most widely used quality characters. Pepper quality is commercially assessed on red colour intensity. Pepper of a dark red colour is classified as Grade A; pepper of a normal red colour is labeled as Grade B; and pepper of red colour mixed with yellow and brown colours is labeled as Grade C. The colour of red pepper is under control of a number of carotenoids (capsanthin, capsorubin, and xanthophylls for the red colour, and carotene and zeaxanthin for the yellow-orange colour) (Duman, 2010; Keceli, 2008).

Linoleic acid and oleic acid contents can make over 90% among unsaturated fatty acids, which are crucial for human being and can diminish low-density lipoprotein cholesterol which is known as bad cholesterol. Some medical research considers that red pepper seeds can reduce the risks of cardiovascular diseases and regulate the nervous system. The seeds also contain dissimilar minerals and vitamins like A, D, E, and K, (Jinyan *et al.* 2014).

A number of researches have stated that hot pepper seeds are excellent sources of proteins and minerals. These dietary compounds; especially essential fatty acids and amino acids, are crucial nutrients for the maintenance of the healthy body. Therefore, red hot pepper seeds may be an inexpensive source of dietary proteins and minerals (Zou *et al.* 2015; Özyıldız *et al.*, 2012).

Nowadays there have been shed lights on the applications of processing waste-products food processing, as well as on under-utilized agricultural products. The problems of industrial by-products are becoming difficult to solve, and more effort is required in developing the nutritional and industrial potential of these non-used or under-used compounds. Only a small part of plant material is used directly for human consumption, but most of it has to be processed. During processing, a lot of waste is generator, which may contain important compounds suitable for further utilization either in human nutrition or for technical applications (Matthäus and Özcan 2009).

The purpose of the present study was to determine fatty acid composition and to investigate of the oxidative stability of red pepper seed oil in the two different storage conditions.

2. LITERATURE REVIEW

2.1. Origin and History of Pepper

Red hot pepper (*Capsicum annuum* L.) is an ancient known edible vegetables. The origin of hot pepper species extends from North Mexico to South Bolivia. They combined into the human diet since about 7500 BC. Majority of the bell peppers grwon in tropical and temperate parts (Duman, 2010; Arslan and Özcan, 2011). Chili pepper (Capsicum species) is affiliated to the Family Solanaceae, Genus Capsicum, and species frutescence L., vegetables. Cultivated peppers are all belongs to capsicum species. There is nearly 1,600 dissimilar species of red pepper globally with five main local species including C. *pubescens* R., C. *Chinensis*., C. *baccatum* L., C. *annum* L., and C. *frutescens* L.. (Delelegn, 2011).

2.2. Species of the Genus Capsicum

Red chilli (*Capsicum* sp.) is one of the most commonly planted spices worldwide valued for their sensory characteristics of appearance, flavor and pungency (Ananthan *et al.*, 2014).

Capsicum species are small perennial herbs originated to tropical South America. The majority of academic professionals assume that this genus is composed of more than twenty species. The first five most common geniuses believed to be a consequence of domestication include C. *annuum*, C. *baccatum*, C. *frutescens*, C. *chinense* and C. *pubescens*. Other species are considered exotic and not as commonly distributed like other five aforementioned geniuses (Omolo *et al.*, 2014).

Capsicum peppers on commercial scale are classified according to the concentration of capsaicin ($C_{18}H_{27}NO_3$) which determines a variety's chilliness "hotness". Capsicum species are diploid, most possessing twenty four chromosomes (2n=24). On the other hand, recent studies stated that chromosome number for non-pungent species is n=13. They are different in size, shape, color, flavor, and degree of chilliness, from moderate to very hot (Delelegn, 2011).

The hot pepper is a kind of vegetable which is introduced to its rich content antioxidant. Fresh hot red peppers contain high ascorbic acid which is around 116 mg per 100 g. Their impressive color is attributed to the a number of carotenoid pigments, which includes b-carotene with pro-vitamin A activity and oxygenated carotenoids, such as capsanthin, the commercial value of paprika rely on directly on its relative richness color resulting because of carotenoids (Sim and Sil, 2008; Ananthan *et al.* 2018).

The intense red colour of ripe peppers is because of presence of carotenoid pigments. *β*- Carotene is also known as provitamin A as it is the main source of vitamin A for people worldwide. Vitamin A is needed for normal development, growth, and eyesight. Thus, dietary *β*-carotene is essential for humans (Romo-Hualde *et al.* 2012).

Fresh peppers have been appreciated for its rich source of vitamins C and E, provitamin A, carotenoids and phenolic compounds, metabolites with well-known antioxidant activity. These ingredients possess useful impact on human well-being, such as anti-carcinogenic activity against number cancers, protecting activity against gastric ulcer, improve the immune system, reducing the risks of cardiovascular diseases and slowing down age-connected macular degeneration and cataracts. Peppers have shown to protect the oxidation of docosahexaenoic acid and cholesterol (Silva *et al.,* 2013).

2.3. Characteristics and Composition Seed Oil

Because of the increasing demand for edible oils, many studies have been conducted on the traits of plant seed and fruit oils (Youzbachi *et al.,* 2015). Hot pepper seeds comprise 45%-50% of the total pepper weight (Jinyan *et al.* 2014).

Seed oil comprises main source of dietary constituents because of their fatty acid components and tocopherol content. Seed oil is high in unsaturated fatty acids, which are assumed to possess positives influence, and with a rich content of constituents like tocopherols are now supplemented into infant diet and dissimilar food products. They are also available as pharmaceutical supplements in many countries (Bozan and Temelli., 2008).

Hot pepper seeds can be taken into account as by-products of chilli pepper product processing (Özyıldız *et al.*, 2012). The ability of Capsicum seed oil for salad dressing and/or for cooking purposes was recognized early. There a current interest for the development of alternative sources of oil for use as fuel and other commercial applications. Furthermore it is also intended to utilize of plant by product processing products that otherwise would be thrown away, the potential for the increased usage of oil extracted from pepper (Jarret *et al.*, 2013).

The global oilseed production will face an increasing demand in the upcoming three decades as a result of the combination of some industrials sectors, including a higher usage for palatable oil, the development of the biodiesel industry, and the demand for green chemistry. Nowadays, the annual global oil production is reached to 135 MT (Metric Ton) with palm, soybean and rapeseed oils representing 31%, 24% and 15% of the total production respectively. Vegetable oils have gained a particular interest to be utilized as raw materials for a number of industries like food (for their nutritional value), energetic (through their conversion in renewable biofuels), or chemical (detergents or materials industry, film-forming substances like varnishes, paints, and so on) (Avram *et al.*, 2014).

Vegetable oils compose one of the main components of human diets, comprising as much as 25% of average caloric intake. The ratio of saturated fatty acid to unsaturated is very crucial for human diet. A high level of saturated fatty acids is appreciably recognized to improve oil stability. Seed oil is high in unsaturated fatty acids, which are assumed to be beneficial constituents, and with a high content of tocopherols are now added into children diet and different food stuffs and available as pharmaceuticals supplements in many countries. In addition, not only oil components but also remaining parts after oil extraction are the significant source of protein, carbohydrate and non-nutritive but bioactive compounds such as polyphenolics (Zambiazi et al., 2007).

Chemical components and nutritional profile of chilli pepper seeds (*Capsicum annuum*) planted in Northeast of China were studied. The proximate analysis showed that ash, moisture, crude protein, crude oil and total dietary fiber contents were as following 4.48, 4.94, 23.65, 21.29 and 38.76 g/100g, respectively. The main amino acids showed to be glutamic acid and aspartic acid (more than 2 g/100g) in the second place came histidine, phenylalanine, lysine, arginine, cysteine, leucine, tryptophan, serine, glycine, methionine, threonine and tyrosine (0.8-2 g/100g). Proline, alanine, valine, and isoleucine comprised

below 0.8 g/100 g. The fatty acid profile revealed that linoleic acid, palmitic acid, oleic acid, stearic acid and linolenic acid were above (0.55 g/100g) as the most prevailed fatty acids followed lauric acid, arachidic acid, gondoic acid and behenic acid (0.03-0.15 g/100 g). Analysis of mineral content referred that the most present mineral was potassium, followed by magnesium, calcium, iron, zinc, sodium, and manganese. The chemical composition of chilli pepper seeds recommends that they could be regarded as good sources of food constituents and as new alternative sources of palatable oils (Zou *et al.*, 2015).

Fatty acids are of great interest in cosmetology, improves the lipid barrier of the epidermis, prevent transepidermal water loss. Further, they normalizes the skin metabolism and becoming more and more predominantly used substances of many cosmetic coompstion used for daily care of the body skin. On the other words, lack in these ingridients might consequences in excessive drying of the skin. Vegetable oils, works as a cosmetic base, controls water loss through the skin, primerely by means of adding a protective layer on the epidermis. Moreover, they soften the stratum corneum and diminish the inflammation of the skin, thereby decreasng the pain sensation. Furthermore, they play a very crucial role in the proper working of the human organs. Also medicine appreciates the positive impacts of vegetable oils, partiualrly in the biological synthesis of components of cell membranes or eicosanoids (prostaglandins, prostacyclins, thromboxanes, leukotrienes). Oils are also particiapte in the transportation and oxidation of cholesterol. The absence of these oils contained in the fatty acids negativley infuences vascular fragility, decreases the immune system workflow, intervenes with the clotting process and also increases the likelihood of the development of atherosclerosis (Zielinska and Nowak, 2014).

The charactestics of the oils mainly depend on the fatty acids composition which has gained much attention owing to its beneficial implications for human health. In addition, the existence of polyunsaturated fatty acids improves the potential beneficial traits of the oils. Moreover, oleic acid and palmitoleic acid, as monounsaturated fatty acids, posses an important physiological benefits (Youzbachi *et al.* 2015).

Even though vegetable oils, the optimum cooking material of the daily routine, include beneficial and popular as a result of their cholesterol lowering properties, some significant issues in relation to their judicious utilization are ignored to a large extend by people and the medical community. In contrast non-plant based oil, which are predominantly saturated and hence do not react readily with other chemicals, especially oxygen (Naz *et al.* 2004).

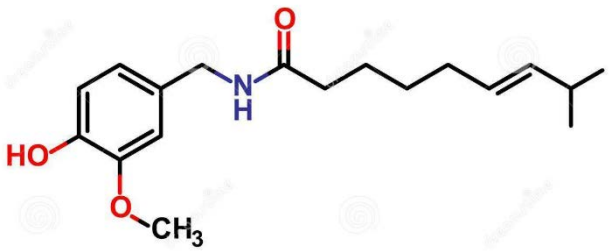
Medical studies have showed that hot seeds of chilli pepper could possess precautions and protect cardiovascular disease and improve and regulate the central nervous system. Moreover, various minerals and vitamins like A, D, and K present in red hot pepper seeds. They are also rich vitamin E content (Zou *et al.* 2015; Lin *et al.*, 2013). It is worth mentioning that their rich level of vitamin E is particularly useful to healthcare and anti-aging (Gu *et al.*, 2016). As well as vitamin C (up to 6 times the concentration of an orange) (Omolo *et al.*, 2014).

Red hot pepper seed has been showed to be rich in protein (25.91%) content. It has been found that the red chilli pepper seeds are high in anti-oxidants (Özyıldız *et al.* 2012). Seeds are rich in amino acids and essential fatty acids, which are very crucial nutrients for the sustain of body well-being (Zou *et al.*, 2015; Özyıldız *et al.*, 2012).

Red chilli pepper seeds contain fatty acids, which majorly composed of linoleic acid, oleic acid, hexadecanoic acid, stearic acid and linolenic acid (Jinyan *et al.*, 2014; Yilmaz *et al.*, 2015). Whereas, the seeds and central core of *Capsicum annuum* could contain some capsaicin (Sung *et al.*, 2015).

The basic pungent constituent available in chilli red pepper (*Capsicum annum* L.) and hot pepper *Capsicum frutescence* L. is the polyphenolic compounds known as capsaicin (IUPAC Name: (E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methylnon-6-enamide) structure shown in Figure 2.1. The capsaicin quantity of hot peppers is ranged from 0.1% to 1% (Surh *et al.* 1998).

2D Structure



3D Conformer

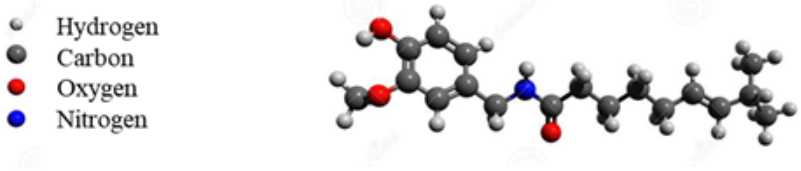


Figure 2.1. Chemical structures of capsaicin

A component like Capsaicin, is well-documented for chemical substances of the Capsicum and one of the pungent capsaicinoids discovered in hot peppers, has already demonstrated a high degree of bioactive influencing the nervous, cardiovascular, and digestive systems (Omolo *et al.,* 2014). It can also be utilized as a food supplement and in food fortification since people prefer natural functional ingredients. It is also used in food preservatives because of its antimicrobial activity and the exploitation the nutraceutical industry and biological properties (Matthäus and Özcan., 2009). Capsaicin is recognized as food preservatives and in pharmaceutical applications. Additionally, it has been revealed that capsaicin can act anti-carcinogenic agent and proved wide applications against various ranges of cancers (Chapa-Oliver and Mejía-Teniente, 2016; Sarkar *et al.* 2015; Ogawa *et al.*, 2015; Sricharoen *et al.*, 2017).

The chemical traits of hot pepper seed oil were stated as similar to those of safflower or certain members of the Onagraceae family. The oil has been well-documented to have a pleasant flavor that compares favorably with peanut oil. The similarity of hot pepper seed oil to sunflower oil has also been studied (Jarret *et al.*, 2013).

In any case, the capability of Capsicum seed oil usage as a salad oil dressing or for use in daily routine cooking was recognized. Additionally, The rich content of linoleic acid makes the oil to be nutritious and useful in the production of margarine and mayonnaise (Jarret *et al.*, 2013).

2.4. Oil Extraction

Solvent extraction is a standard and common method to extract oil from seeds (figure 2.2). Solvent extraction methods require a high volume of solvents (typically several hundred milliliters) and long extraction period of times (8–16 h) to extract the oil from the seeds. Solvent extraction method is one of the abundant and most efficient techniques, applied to produce oil from seeds. Once the oil is removed from the product, a quantity of oil in the seeds can be determined, and the chemical components of the oils can be investigated. Accelerated solvent extraction is an automated extraction method that uses increased temperature and pressure to quicken the removal of analyses from different matrices (Kettle, 2013).

Figure 2.2. The Seed and the oil extracted from red pepper seeds.

The main seed-oil extraction technologies are mechanical pressing, chemical extraction and supercritical CO_2 extraction. Mechanical technique requires a large amount

of energy, and the extraction capacity is insufficient. Chemical extraction technique commonly used solvents including hexane, which is utilized on the large quantity production scale of seed oils. It is when solvent extraction follows mechanical pressing to accelerate yield extraction. However, the oil must be heated to distill it; at the same time, the oil is oxidatively unstable and prone to rancidity during the separation process. Supercritical CO_2 extraction possesses useful characteristics such as non-toxicity, no left residuals organic solvent, and better selective extraction. Whereas, the technique has disadvantages such as a high compress and a long extraction period of time are required. Another alternative method is subcritical butane extraction (Gu *et al.*, 2016).

Using particular extraction methods depends on the seed type, some European processors use mechanical extraction, especially for soft seeds (e.g. sunflower and rapeseed) because the consumers prefer naturally processed oil (Bayz, 2016).

The extraction process shares many objectives including obtaining intact oil. Oils that would have as high as possible yield production, economically efficient and high-quality oil residue. This is in order to gain a high economic price of the extraction process. Extraction rate relies on the thickness and area of the solid phase, temperature, solvent and moisture content (Avram *et al* . 2014).

2.5 Oxidative Stability

Oxidative stability (OS) is one of the most common and dependent parameters of palatable oils in order to evaluate the quality of fats and oils (Ratusz *et al.* 2016). OS is often defined as any organic substance that resists oxidation, and more detrimental changes which lead to change in chemical properties and loss of quality. Moreover, various techniques have been proposed to notice the OS of lipids, and many tests have been developed to quicken the normal oxidation process. In addition, the protocol has made it pragmatic to understand how the lipid may become deteriorated when exposed to oxidation. Understanding the lipid deterioration oxidative kinetics can aid for correct prevention procedure to minimize the deterioration as much as possible. For study purposes, the OS of an oil is usually tested by exposing it to an energized process via increasing temperatures as well as inserting oxygen bubbling (Chander, 2010). The oxidation of oils results in different products via two main stages, primary and secondary.

The primary products of oxidation are hydroperoxides which then transfers to the second stage and consequently breaking down in a number of complicate reactions to generate secondary products such as alcohols and carbonyl substances. These can be further oxidized substances like carboxylic acids (Chandrasekara and Shahidi, 2011).

The oxidation is sophisticated process since many factors intervene such as light, temperature, enzymes, and metals. Furthermore, it happens through very similar mechanism, chain reactions in the participation of free radicals, which are named autoxidation. The oxidation process occurs with presence of light which is called photooxidation which by energy is transferred from light to the sensitizer and into oxygen (Rasul, 2014).

Oils storage at high temperatures is normally applied for observation of OS of pepper oils. The level of oil oxidation is frequently assessed by quantifying the peroxide value (PV). This index is connected to the hydroperoxides and the primary oxidation products, which are changeable and quickly decomposed to form majorly mixtures of volatile aldehyde substances. The oxidative break down of substances that are taken from break down of hydroperoxides is commonly known as secondary oxidative products which are quantified in fats and oils through methods like panisidine (AV) (Abdelazim *et al.* 2013; Yun and Surh, 2012).

The oxidation extent of oil is a crucial quality parameter for food professionals. As explained before, oil oxidation is not merely results in a rancid flavor but it also detriments its nutrition profile, quality and safety via the accumulation of oxidized products. From the health perspective, lipid free radicals and oxidized substances involve occurrence of some undesirable health problems such as aging, coronary heart diseases, DNA damage, carcinogenesis, and tumor (Rasul, 2014).

Fats and oil-containing foods encounter oxidation at different levels which leads to alteration. Oil oxidation changes sensory characteristics and influences the expiration of the product. It also not only generates unpleasant odors, undesirable flavors, and color chnges but also deteriorates the nutritional quality (Abdelazim *et al.* 2013). One of the most significant criterions that affect lipid oxidation is the level degree of unsaturation of fatty acids. Therefore, palatable oils with larger extent of unsaturation, especially polyunsaturated fatty acids (PUFA) are more subjected to oxidation. The existence of

natural substances with dissimilar chemical composition that exhibit antioxidant activity may also affect oxidation rate. Another lipid parameter that changes lipid is hydrolysis, with subsequent accumulation of free fatty acids (FFA), through enzymic chemical and/or action. This phenomenon is of great interest in water-containing lipid systems particularly butter and virgin olive oil during olive processing. Despite the fact the initial causes and the result of oxidative and hydrolytic break down processes are quite dissimilar , they seem to interact with each another and decrease the expiration of palatable oils (Chandrasekara and Shahidi, 2011).

OS of palatable vegetable oils can greatly influence sensory and nutritional value. Moreover, the breakdown of hydroperoxides, the initial generated oxidation, to secondary oxidation products with aldehydic and ketonic functions negatively influences their sensory attribute. Fortunately, the occurrence rancidity is sensible enough to avoid consumers from consuming these oils in foods. The exposure of certain oils to oxidation, e.g. hot red pepper, diminishes their usage as edible oils, which results to large economic losses. The instability of vegetable oils also reduces their usage in products like cosmetics and skin care products. Stable vegetable oils could have important future implementations like diesel oils that can also use non-traditional oils. Moreover, vegetable oils possess various sources and compositions. They are extracted from seeds, nuts, grain germs, fruit mesocarps, etc. Chemically, vegetable oils composed majorly from components like triacylglycerols (ca. 95%) which roles as a solvent for sterols, fat-soluble vitamins (mainly tocopherols/tocotrienols), pigments-including chlorophylls and carotenoids, phenolic compounds including lignans, phospholipids, free fatty acids, and mono- and diacylglycerols. Additionally, the oils are different in the level of unsaturation, to what level fatty acids contribute to their triacylglycerols, as well as in the quantity and quality of the substances in their non-saponifiable ingredients. Dissimilarly in the structure are manifested into differences in stability, sensory and technical qualities of the oils (Kamal-eldin, 2006).

Hot pepper seed is also a source of oil for industrial implementation and ingredients in the formulation of UV protectants. Pepper seeds contain a higher amount of antioxidant substances, particularly polyphenols, vitamin C, flavonoids and carotenoids (e.g., capsaicin), with free radical scavenging capacity. Capsaicin and capsanthin are significant ingredients of red hot pepper that are commonly utilized in the fields of food, pharmacy, cosmetics among others. Pepper seed possess high quantity of oxidative stability (Jarret *et*

al. 2013).

Carotenoids, especially β-carotene, can control oil oxidation by light filtering, singlet oxygen quenching, sensitizer inactivation, and free radical scavenging. The physical quenching mechanism of carotenoids is based on their low singlet energy state, which facilitates the acceptance of energy from singlet oxygen. In the inexistence of light, carotenoids and their oxidation products may act as prooxidants in vegetable oils. The presence of oxygen is significant parameters in the breakdown of carotenoids, even a low concentration of oxygen results in the loss of this color. Carotenoids are more sensitive to temperature than chlorophylls (Rasul, 2014).

OS is not a sole dependent parameter on initial raw material chemical composition. Following criterion are important as well including level of maturity of seeds, physical damages, moisture, microbial contaminations; seeds' processing conditions, like preliminary humidifying, seeds' heating and cold-pressing conditions-press pressure, temperature of pressed oil, time and temperature of sedimentation and filtration (Ratusz et al., 2016; Abuzaytoun and Shahidi, 2006).

2.6. Chemical Properties of Pepper Seed Oil

2.6.1. Peroxide Value

Oils and fats undergo oxidation under the action of some factors expressed in peroxide value (PV). Consequently, hydroperoxides are produced because of primary oxidation. They can be degraded to volatile and nonvolatile secondary oxidation products. PV is commonly used to assess shelf life of oils through determining the number of hydroperoxides. However, at the end of the oxidation process, they can attach to each other and produce other products that cannot be longer determined. The PV is defined as milli-equivalents of oxygen per one kilogram of fat (meq/kg). Oils with peroxide value more than 10 meq/kg are considered to have a short shelf life (Bayz, 2016).

2.6.2. Free Fatty Acids (FFAs).

Quality of nutritional oil is not merely useful but can also improve flavor to meals.

The quality of palatable oil is decided by number of parameters including free fatty acids (FFA) commonly evaluated quality criterion in the time production, storage, and marketing (price dictated by FFA content). FFA is normally one unwanted parameters in palatable oils, it leads in poor flavor quality and stability of the oil, and too high content of FFA will lead to in rancid oil. Thus, quantitative assessment of FFA is significant to the control the quality, trading and storage of the edible oils (Li *et al.* 2011).

Some unrefined oils may possess as much as several percent free fatty acids. The content of free fatty acids is reduced in the refining process. Fully refined fats and oils usually have a free fatty acid content of less than 0.1%. FFAs are spontaneously generated from oils while in storage because of hydrolysis. This phenomenon triggers what is known as oil rancidity. The hydrolyzing agents are either enzymes or certain chemical species that inadvertently were exposed to the oils during storage/ processing. The amount of FFAs is quantified in terms of the parameter known as "Acid number" which expressed as the number of milligrams of potassium hydroxide required to neutralize the alkali reactive groups in one gram of oil under the conditions of the test (Mohammed, 2016).

2.6.3. Conjugated Diene, Trine

Determination of the specific absorption coefficients (specific extinction) in the ultraviolet region is needed for estimating the oxidation stage of olive oil. The absorption at specified wavelengths at 232 and 270 nm in the ultraviolet region is connected to the formation of conjugated diene and triene in the oil matrix, because of oxidation or refining processes. Compounds of oxidation of the conjugated dienes involve to K_{232} while substances of secondary oxidation (aldehydes, ketones etc.) involve to K_{270}. They are generated in the oxidized oil as a consequence of a shift in the conjugated unsaturated fatty acids. This is because of already taking place hydroperoxides in the system. Therefore , it can be indicative of oxidation in oil (Bayz, 2016; Yildirim, 2009).

2.6.4. Fatty Acid Composition of Oil

Fatty acids in vegetable oils are comprised of a complex of saturated (SFAs) and unsaturated (UNFAs) fatty acids classified based on the number of unsaturated bonds as monounsaturated (MUFAs) or polyunsaturated fatty acids (PUFAs). Therefore, every

vegetable oil has specific fatty acid according to their plant sources. So, their influence on human health could be evaluated based on individual fatty acids due to their various influences on human health and risks of serious diseases (Orsavova *et al.* 2015).

Higher unsaturated level oils are oxidized more quickly than fewer ones. Since the degree of unsaturation increases, both the rate of formation, fatty acid and the amount of initial oxidation substances accumulated at the end of the induction period increase (Yildirim, 2009).

Fatty acids are very important in cosmetology because of their beneficial influence, especially on the skin, becoming more and more commonly used components of many cosmetic formulations intended for daily care of the face and body. Deficiency in these compounds can cause excessive drying of the skin. Vegetable oils, helping as a cosmetic material, control of water loss through the skin, mainly meaning making a preventive layer on the epidermis. Additionally, they make the stratum corneum soft and decrease inflammation of the skin. Thereby, it controls the sensation of pain. Furthermore, they act a very significant role in the proper working of the human body. In addition, there is medical usefulness influencing vegetable oils, majorly in the biological synthesis of substances of cell membranes or eicosanoids. Oils are also contributed in the transport and oxidation of cholesterol. The inexistences of these oils contained in the fatty acids largely influences vascular fragility, weakening the immune system workflow, interferes with the clotting process and also increases the likelihood of the development of atherosclerosis (Zielinska and Nowak, 2014). Linoleic acid is considered beneficial for health, especially because it contributes to the reduction of fat body and total cholesterol (Teixeira, Gisele Sora *et al.* 2015).

Fatty acids are grouped into two groups; saturated and unsaturated fatty acids according to nature double bond in their alkyl chains. Fatty acids are derived from plant sources contain higher quantity of unsaturated fatty acids; whereas saturated fatty acids are more abundant in animal world. Essential fatty acids (EFAs) are fatty acids that should be up take to sustain proper health. They are named essential because the human body is unable to make them on its own. Alpha-linolenic acid (ALA) is an essential fatty acid and the main omega-3 fatty acid 20 found in food. Linoleic acid (LA) is also an essential fatty acid and is the main omega-6 fatty acid revealed in food. Pre-agricultural humans ate diets that were high in like fish, lean meat, (Mandal, 2013; Zambiazi *et al.* 2007).

3. MATERIALS AND METHODS

3.1 Materials

Spice red pepper seed was used in this study. It was obtained from Müsan Food Inc., Kahramanmaraş, Turkey. All the solvents (chloroform, diethyl ether, ethanol, methanol, and n-hexane) and reagents (acetic acid, potassium iodide, potassium hydroxide, sodium thiosulfate, starch soluble and phenolphthalein) used were of analytical grade and are purchased from Merck, Istanbul, Turkey.

3.2. Oil Extraction and Storage

The oil in red pepper seed was extracted by using solvent extraction. The seeds from the pepper were separated and ground (figure 3.1) with an electrical grinder. For the extraction of oil from the seeds 1 gram of seed powder was mixed with 4 milliliters of hexane as a solvent. The mixture was mixed at 50ºC for 30 minutes (figure 3.2). The solution was then filtrated. The solvent was removed by evaporation using rotary evaporator. The extracted oil seemed to be 12.7 g/100g seeds. It was packed in 25 mL containers made of transparent glass bottles and amber colored glass bottles (no head space). The samples were stored at room temperature (approx. 25°C) under two different conditions (dark and light) for 90 days. A separate container was used for each 30 days. The samples in colored glass bottles for storage in the dark were put in cartoon box without any light. The samples in transparent glass bottles for storage in the light being diffused daylight were stored in a white painted room.

Figure 3.1. Electrical grinder machine and pepper powder

Figure 3.2. The mixture (powder seed and n-hexane) on the heater

Figure 3.3. The oil during precipitation process

3.3. Determination of Peroxide Value

The peroxide value was determined according to the AOAC method 965.33 (AOAC, 2005). As 5 grams of fat or oil sample was weighed in a 250 mL Erlenmeyer flask, and 20 mL of glacial acetic acid and chloroform mixture (3:2v/v) was added and shaken. Then, 0.5 mL of saturated KI solution was added, and the solution was stirred for 1 minute, then 30 ml of distilled water was added. The solution was titrated with 0.01 N sodium thiosulfate until the yellow color almost disappeared. 0.5 mL of one percent starch indicator was added until the blue color just disappeared. Blank experiment has the same procedures, but no oil or fat was used.

The PV (meq/kg oil) was calculated as:

$$Peroxide\ value = \frac{(V_2 - V_1)x\ 0.01N\ x\ 1000}{Weight\ of\ sample(g)}$$

V_1 = is the volume of sodium thiosulfate 0.01 titrated with the blank.

V_2 = is the volume of sodium thiosulfate 0.01 titrated with the sample.

3.4. Determination of Free Fatty Acids

Free fatty acids in crude and refined oils were determined by the AOAC method 940.28 (AOAC, 2005) This method measures the amounts of sodium hydroxide that is required to neutralize the acids that are formed during oil extraction and refining processes. For analysis of free fatty acids, 7.5 g of the unrefined oil sample was weighed into a 250 mL conical flask. To this, 50 mL alcohol and 2 mL of phenolphthalein solutions were added and 0.1-0.2 mL of 0.1 N Sodium hydroxide solution was added to produce a temporary faint pink color. This solution was titrated against a 0.25 N sodium hydroxide solution with vigorous shaking until pale permanent pink appears and persists more than 1 minute. The titer value of sodium hydroxide (mL of 0.25 N NaOH) corresponds to the % free fatty acids (expressed as oleic acid). The deodorized samples were analyzed with the same procedure followed for determining free fatty acids in unrefined oils. The calculations are as follows:

Free fatty acids, in terms of oleic acid, percent by mass

$$Free\ Fatty\ Acid\ = \frac{28.2 * V * N}{M}$$

Where*:*

V = Volume in ml of standard potassium hydroxide solution used,

N = Normality of standard potassium hydroxide solution, and

M = Mass in g of the material taken for the test

3.5. Determination of Conjugated Diene and Triene Contents

Content of conjugated dienes as absorbance at 232 nm (K_{232}) and content of conjugated trienes as absorbance at 270 nm (K_{270}), were determined by dissolving weighed-out samples in isooctane (0.1%) and reading the sample absorbance at 232 nm (K_{232}) and 270 nm (K_{270}), using a UV/VIS double-beam scanning spectrophotometer.

The specific K_{232} and K_{270} extinction coefficient were used to determine the level of the associated ions found in the oil. It is known that the oxidation products of oils and fats,

which may result from decomposition, display distinct spectra in the ultraviolet region and at about 232 nm and 270 nm (Paraskevopoulou *et al*., 2006).

3.6. Determination of Fatty Acid Composition

Fatty acids were determined by the analytical methods described in the European Parliament and the European Council in EEC regulation 2568/91(EEC 2568/1991). The fatty acids were converted to fatty acid methyl esters (FAMEs) before being analyzed. This is done through shaking off a solution of 0.2 g of oil and 3 ML of hexane with 0.4 ML of 2 N methanol potassium hydroxide. The FAMEs were then analyzed in a Hewlett-Packard model 4890D Gas Chromatograph furnished with HP-INNOWax fused silica capillary columns (Cross-Linked PEG), 30m-0.25mm-0.25m and a flame ionization detector (FID). Inlet and detector temperatures were held at 230 ºC and 250 ºC, respectively. The initial oven temperature was held at 120 ºC for 1 min and then it was raised to 240 ºC at a rate of 4.0 ºC/min for 4 min. The FAMEs injected volume was 1 L and nitrogen (N2) was used as the carrier gas at 1 mL/min with a split inlet flow system at a 1:100 split ratio. Then, heptadecanoic acid C17:0 was added as an internal standard before methylation, so as to measure the amount of fatty acids. Eventually, the fatty acid contents were measured using a 4890A Hewlett-Packard integrator. The FAMEs peaks were identified by comparison with the retention times of a standard mixture. The peak areas were computed, and the percentages of the FAME were obtained as area percentages by direct normalization.

3.7. Statistical Analysis

Results of chemical analysis (peroxide value, free-fatty acids) were subjected to Linear Regression and sensory data were subjected to two-way ANOVA at α=0.05 and Duncan's Multiple Range Test (DMRT) with 5% level of significance were performed as a post-hoc test to compare means of significantly different treatments. Statistical analyses were done using SPSS for windows version 20. Data were presented as means ± standard deviation (von Hippel., 2004).

4. RESULTS AND DISCUSSION

4.1. Changes in Peroxide Value

The quickly deteriorating components of food ingredients are of great concerns by food technologists and professionals. This is because food ingredients affect chemical, nutritional and sensorial attributes and eventually the desirability of the end product. Stored oils are exposed to oxidation and the peroxide value and free fatty acids changes.

For the PV test, the results showed that the value was 1.4 on day 0 and as the starting point. Furthermore, it was noticed that the value has increased to 1.8 on day 30 and in the light but decreased to 1.3 in dark but significantly according to the statistical analysis. Nevertheless, this value was noticeably increased to 3.1 in the light and to 2.5 in the dark in day 60. Finally, the data showed that the PV went up on day 90 to 4.65 in the light and to 3.8 in the dark (Table 4.1). Figure 4.1 shows graphically changes of peroxide values

Table 4.1. Changes in peroxide value (meq/kg) of chili pepper seed oil during storage

Storage time (day)						
0	30		60		90	
$1.4^{a,A*}$	Light	Dark	Light	Dark	Light	Dark
	$1.8^{a,B}$	$1.3^{a,A}$	$3.1^{b,B}$	$2.5^{b,A}$	$4.65^{c,B}$	$3.8^{c,A}$

*values followed by the same letter are not significantly different and level of 5% (p>0.05)
series "a-d" for days in one stage medium (light or dark)
series "A-B" for comparison of the samples in one storage period

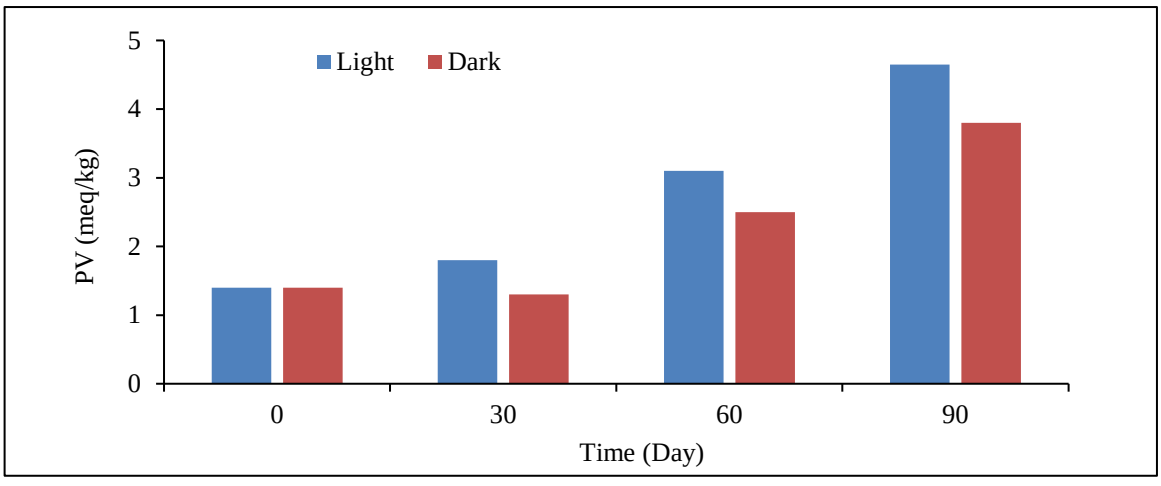


Figure 4.1. Changes in peroxide value (meq/kg) of chili pepper seed oil during storage.

It can be seen from the data that over time, the PV increased. This is not incongruent with the previous studies which referred that the PV is increasing overtime (Ratusz *et al.*, 2016; Jung, Yoon and Jung, 2012; Jung *et al.*, 1999; Zhang *et al.*, 2017). Despite this time of storage, it is obvious that the PV of seed oil of red pepper might be ranging between 1-6 (meq/kg) (İnanç *et al*., 2010; Prabhat *et al*., 2010). -

The samples which are stored in the dark reveaeled a progressive increase in the peroxide value. This means greater primary oxidation, while the samples exposed to light showed a greater peroxide value, which could be attibuted to assess from secondary to primary oxidation.

In a study about camelina oil, it was reported that the camelina oils PV was around 0.79-2.04 and considered a very high-quality edible oil (Ratusz *et al.* 2016). It can be seen that the PV of red pepper seed oil is even less than the aforementioned ranges. PV below the standard range could mean that the quality of the edible oil is acceptable (Besbes, 2004; Yildirim, 2009).

4.2. Changes in Free Fatty Acid

The quality of edible oil is assessed through different methods, and free fatty acids (FFA) are one of the most abundantly assessed quality indices during production, storage, and marketing (price dictated by FFA content). FFA is usually one of unwanted content in oils, it leads to lower flavor quality and stability of the oil, and too high quantity of FFA will lead to rancid oil. Thus, quantitative determination of FFA is equally significant to the quality control, trading, and storage of the edible oils. Therefore, it is quite crucial to assess them. As explained earlier during storage oil exposed to oxidation and consequently FFAs are changed. For the FFAs test, the results showed (Table 4.2) that the value of FFAs was 2.35 at the beginning of storage. Furthermore, the data revealed that FFAs went up to 3.24 in the light and to 2.8 in the dark after 30 days of storage. Moreover, FFAs has increased to 4.2 in the light and to 3.64 in the dark in the 60 days storage. Finally, in the third month (90 days), the FFAs value increased to 5.32 in the light and to 4.08 in the dark.

Table 4.2. Changes in free fatty acid (%) of red pepper seed oil during storage

Storage time (day)						
0	30		60		90	
	Light	Dark	Light	Dark	Light	Dark
$2.35^{a,A*}$	$3.24^{b,B}$	$2.8^{b,A}$	$4.2^{c,B}$	$3.64^{c,A}$	$5.32^{d,B}$	$4.08^{d,A}$

*values followed by the same letter are not significantly different and level of 5% (p>0.05)
series "a-d" for days in one stage medium (light or dark)
series "A-B" for comparison of the samples in one storage period

It is can be expected that over time because of the change in chemical composition of the oil, the release of free fatty acid could increase (figure 4.2).

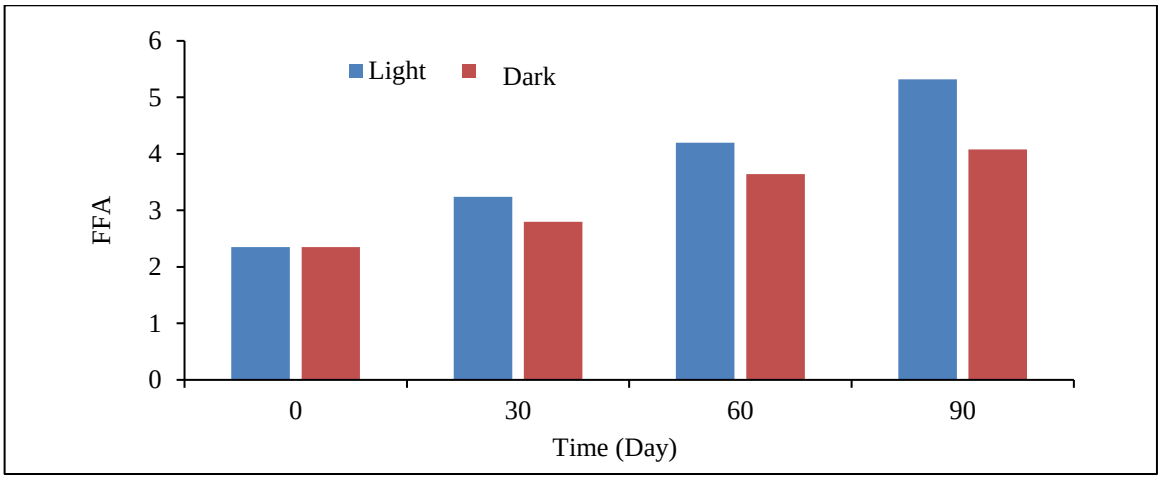


Figure 4.2. Changes in free fatty acid (%) of red pepper seed oil during storage.

This is compatible with the previous studies that indicated FFAs are accumulating and increasing overtime (Yildirim, 2009), Despite the fact that the range is different due to the different chemical composition. Furthermore, it can be seen from the literature that different storage conditions have different impact on FFAs. Oil storage at ambient temperature could enhance and stimulate more FFAs but storing in the refrigerator could slow the process (Li *et al.*, 2011).

4.3. Changes in Conjugated Diene (K_{232}) and Triene (K_{270}) During Storage

The specific K_{232} and K_{270} extinction coefficient was utilized to determine the level of the connected diene determined in the oil. K_{232} is to quantify of the initial oxidation products, conjugated dienes. K_{270} is the indicative of conjugated triens (the primary

oxidation products) and secondary oxidation products such as aldehydes and ketones. It is well-documented that the oxidation products of oils and fats, which may lead to structure decomposition, display distinct spectra in the ultraviolet region and at about 232 nm and 270 nm. Thus, the determination of absorbance at 232 nm and 270 nm is a reference to the oxidation status of fat and oil.

The conjugated diene content (K_{232} value of the oil sample that were storage in light and dark, were determined for 0, 30, 60, and 90 days at room temperature. Table 4.3 shows statistically the changes in K_{232} and k270 specific coefficient over time. It was found that increasing the period of oil storage significantly affected the K_{232} and K_{270} specific extinction coefficient ($P<0.05$). It can be seen from figure 4.3 and figure 4.4 that the K_{232} and K_{270} values are increasing with increasing storage time. This result is in agreement with the previous studies which found that conjugated diene and triene increases with the time of storage (Baştürk *et al.*, 2018; Abdelazim *et al.* 2013).

Table 4.3. Changes in main K_{232} and K_{270} of red pepper seed oil during storage

	Storage time (day)						
	0	30		60		90	
		Light	Dark	Light	Dark	Light	Dark
K_{232}	$0.302^{a,A*}$	$0.706^{b,B}$	$0.216^{a,A}$	$1.6^{c,B}$	$0.68^{b,A}$	$2.06^{d,B}$	$1.54^{c,A}$
K_{270}	$0.190^{a,A}$	$0.382^{b,B}$	$0.140^{a,A}$	$0.7^{c,B}$	$0.31^{b,A}$	$1.66^{d,B}$	$0.70^{c,A}$

*values followed by the same letter are not significantly different and level of 5% ($p>0.05$)
series "a-d" for days in one stage medium (light or dark)
series "A-B" for comparison of the samples in one storage period

The initial K_{232} specific extinction coefficient of oil was 0.302. When storage time increased, it caused to increasing the K_{232} significantly ($P<0.05$) and reached to 1.54 at 90 days in the ambient temperature.

Every 30 days increase from 30 days to 90 days caused in 4 to 8 times increase in the K_{232} specific extinction coefficient, referring to that storing time was more effective on the generation of conjugated dienes in oil (Besbes, 2004; Abuzaytoun and Shahidi 2006).

It was observed that when the period time was increased, the K_{232} and K_{270} specific extinction coefficient in the oil sample increased significantly ($P<0.05$).

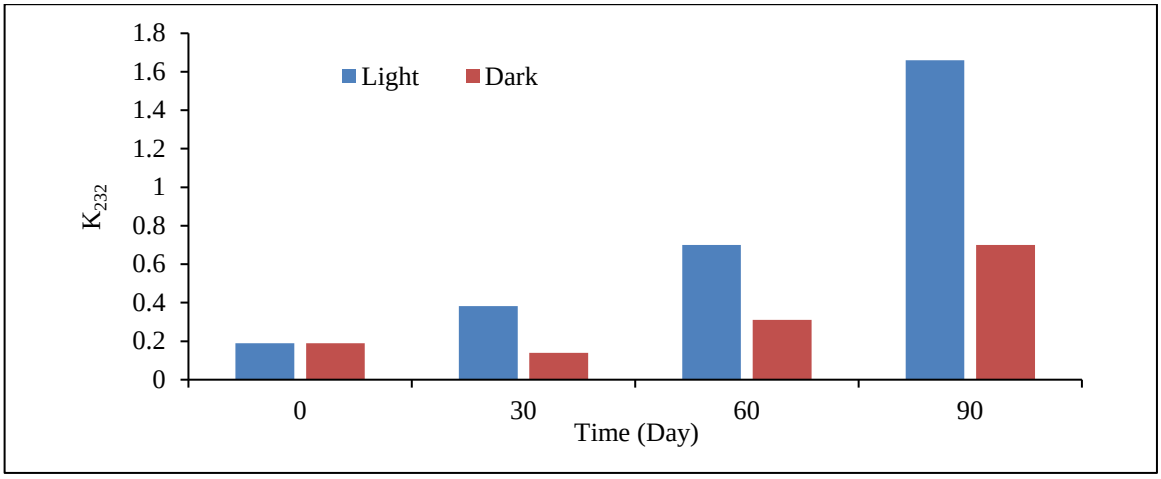


Figure 4.3. Changes in Kj_{232} of red pepper seed oil during storage.

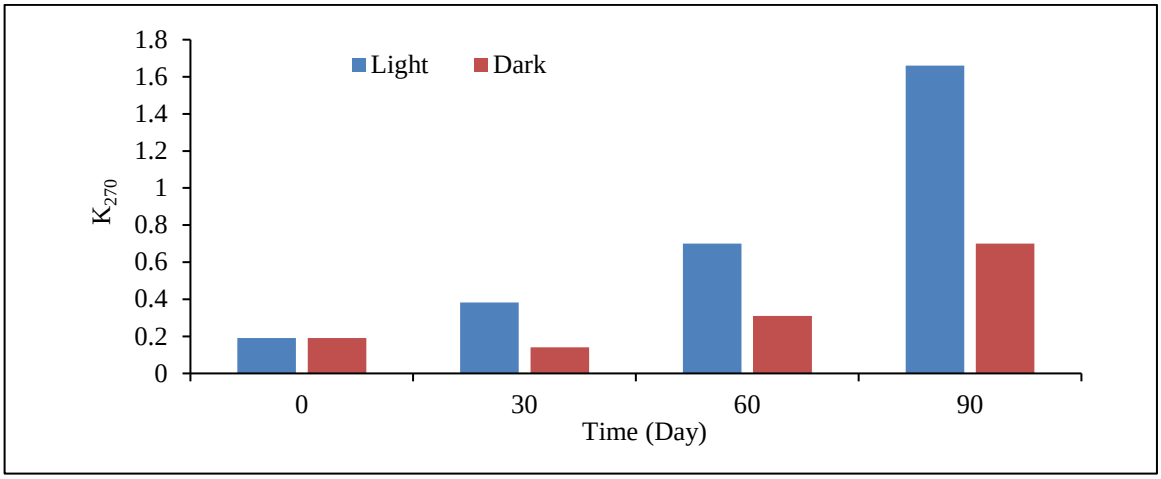


Figure 4.4. Changes in K_{270} of red pepper seed oil during storage.

It was observed that when the period time was over, the K_{270} specific extinction coefficient in the oil sample increased significantly ($P<0.05$). When the increase the period time increased from 0 to 30 days the K_{270} value specific extinction coefficient increased around 0.14 to 0.28 times. However, the increase rate of K_{270} value specific extinction coefficient did not change with the 30 days increment in the heat treatment ($P<0.05$), indicating that the formation of conjugated triene in VOO occurred almost at the same rate.

These experimental results are in agreement with the expectations since the formation of conjugated -C=C-C=C-C=C- the double bond system is improbable even in the case of linolenic acid oxidation. The –C=C-C=C-C=O systems are more probable and could interfere with the measurement at 270 nm (Abdelazim *et al.* 2013; Yildirim, 2009).

The oil showed higher initial K_{232} (2.05 for light and 1.54 for dark) compared to K_{270}

(1.66, light and 0.7, Dark) due to its high C18:2 content. Also, these outcomes were in compliance with the outcomes showed by previous study (Baştürk *et al.* 2018).

(K_{232}, K_{270}) values showed were generally fluctuated during the storage but increased at higher levels compared to the initial values.

It has been reported that PV, FFA, K_{232}, and K_{270} has increased over time. It was also stated that the oil exposed to oxidation more under the light condition and showed increase comparing to dark condition (Vacca *et al.* 2005).

4.4. Correlation of Period Storage with K_{232} and K_{270} Specific Extinction Coefficients

Regression analysis assesses the relationship between concentration of conjugated diene (K_{232}) and triene (K_{270}) with storage condition (light and dark). Moreover, it can be seen from the Figure 4.4. a, that the concentration of K_{232} specific extinction coefficient is reversibly correlated with the storage at light. Similarly, the correlation between K_{270} with the storage at light showed something in Figure 4.4.a. Furthermore, it can be seen that the K_{270} specific extinction coefficient is reversibly correlated with the storage period of time at dark (Figure 4.4.a). Similarly, this happened to the K_{232} at dark (Figure 4.4.b).

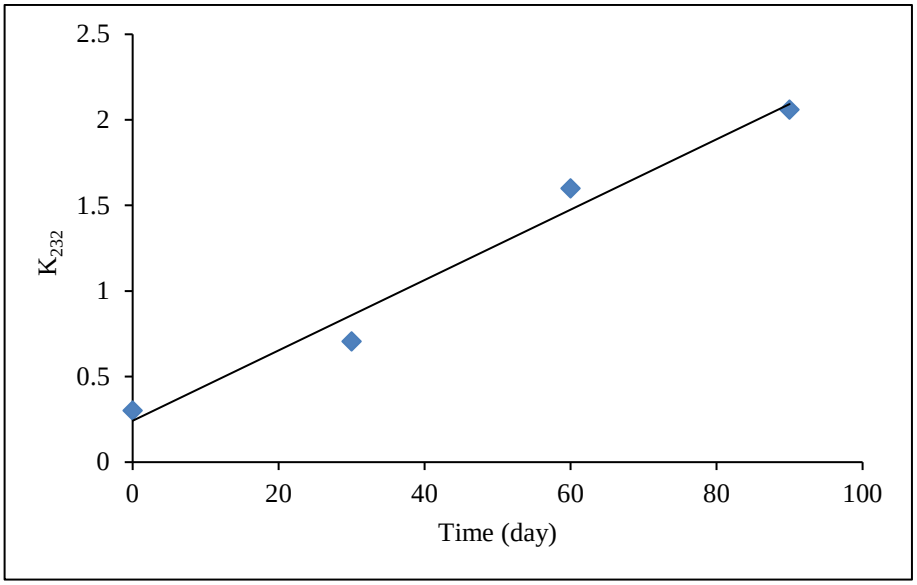


Figure 4.5. Linear correlation between storage time (light) and K_{232} specific extinction coefficient

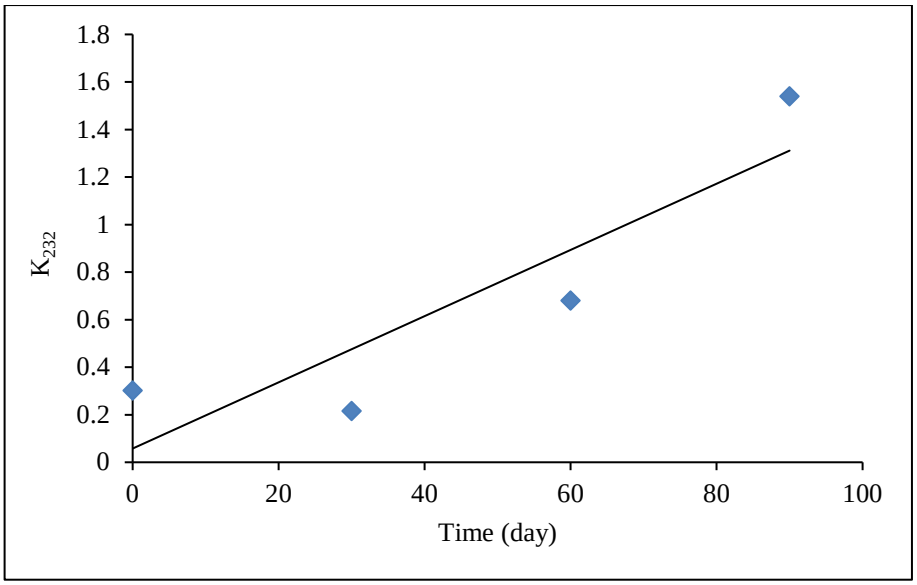


Figure 4.6. Linear correlation between storage time (Dark) and K_{232} specific extinction coefficient

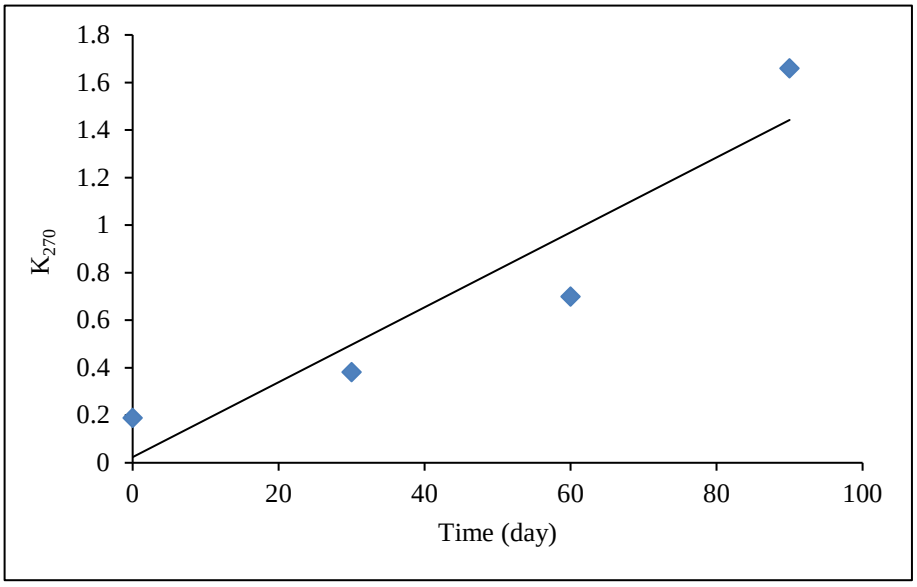


Figure 4.7. linear correlation between storage time (light) and K_{270} specific extinction coefficient

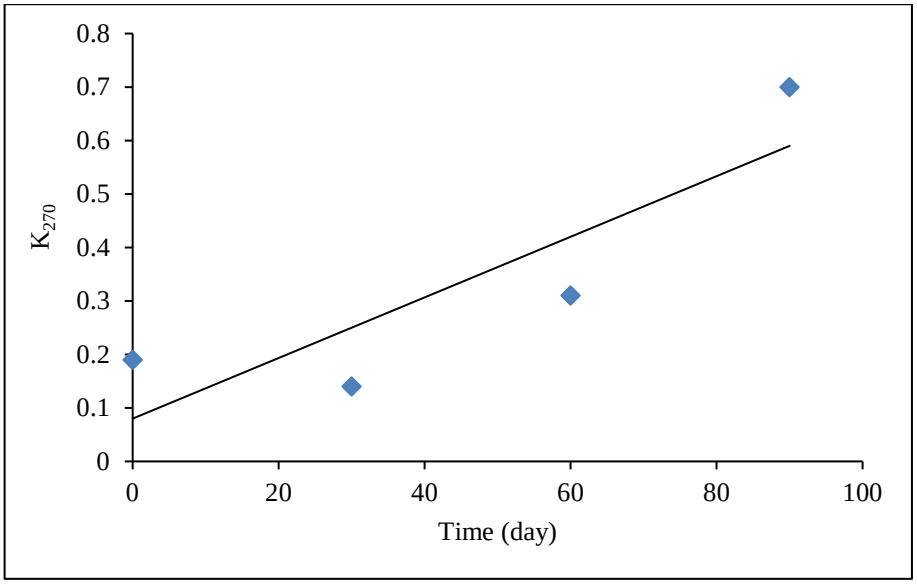


Figure 4.8. linear correlation between storage time (Dark) and K_{232} specific extinction coefficient

The coefficient of determination (R^2) was higher than 0.95 for K_{232} specific extinction coefficient and 0.88 for K_{270} specific extinction coefficient, indicating a good correlation between chlorophyll content and K_{232} and K_{270} specific extinction coefficients (Table 4.4). Our findings are in agreement with those of Malheiro *et al.* (2009).

Table 4.4. Linear regression analysis between period storage and K_{232} and $_{K270}$ specific extinction coefficients

	K_{232}		K_{270}	
Storage	Equation	R^2	Equation	R^2
Light	y = 0.0206x + 0.2418	0.9777	y = 0.0158x + 0.0238	0.8742
Dark	y = 0.0139x + 0.0578	0.7951	y = 0.0057x + 0.08	0.7491

R^2: Coefficient of determination

4.5. Fatty Acid Composition

Table 4.5 shows the fatty acid composition (%) in red pepper seed oil. Gas chromatogram from the GC-MS analysis of the red pepper seed oil is shown in appendix. The composition of red pepper seed oil revealed that the main fatty acids were linoleic acid ($C_{18:2}$), palmitic ($C_{16:0}$) oleic ($C_{18:1}$) and stearic ($C_{18:0}$). On the other hand, the other fatty acids found in very little amounts. Moreover, the chemical composition of the fatty acid was as following (linoleic 71.77%, palmitic 11.65%, oleic 10.62% and stearic 3.34%). Similar results were found in the previous studies (Jarret *et al.*, 2013; Özyıldız *et al.*, 2012). Furthermore, In a study about different types of pepper seeds *Capsicum*, the data showed the incomparably higher amount of the above-mentioned fatty acid comparing to our red pepper seed apart of oleic acid which was higher in red pepper (Teixeira, Gisele Sora et al., 2015; Yilmaz et al., 2015; Wang *et al.*, 2017). The difference between fatty acid compositions of different seed oil could be due to environmental and genetically factors (Zou *et al*., 2015; Mandal, 2013).

Table 4.5. Percent fatty acid composition (%) in red pepper seed oil.

Saturated fatty acid (SFA)	%	Unsaturated fatty acid (SFA)	%
Butyric acid ($C_{4:0}$)	0.03	Myristoleic acid ($C_{14:1}$)	0.01
Caproic acid ($C_{6:0}$)	0.03	Cis-10-pentadecenoid acid methyl ester ($C_{15:1}$)	0.05
Caprylic acid ($C_{8:0}$)	0.02	Cis-10-heptadecenoic acid ($C_{17:1}$)	0.06
Capric acid ($C_{10:0}$)	0.05	Palmitoleic acid ($C_{16:1}$)	0.29
Lauric acid ($C_{12:0}$)	0.06	Hexadecadienoic acid ($C_{16:2}$)	0.11
Myristic acid ($C_{14:0}$)	0.35	Oleic acid ($C_{18:1}$)	10.63
Palmitic acid ($C_{16:0}$)	11.66	Linoleic acid ($C_{18:2}$)	71.78
Stearic acid ($C_{18:0}$)	3.35	Alfa-Linolenic acid ($C_{18:3}$)	0.29
Arachidic acid ($C_{20:0}$)	0.01	Cis -11- eicosenoic acid ($C_{20:1}$)	0.26
Behenic acid ($C_{22:0}$)	0.12	Erucic acid ($C_{22:1}$)	0.06
Tricosylic acid ($C_{23:0}$)	0.03	Cis-11,14,17-Eicosatrienoic acid ($C_{22:3}$)	0.21
Lignoceric acid ($C_{24:0}$)	0.26	Cis-5,8,11,14,17-Eicosapentaenoic acid ($C_{20:5}$)	0.20
		Nervonic acid ($C_{24:1}$)	0.10
Total SFA	16.42	Total UFA	83.58

Linoleic acid was the only polyunsaturated fatty acid (PUFA) determined in this study and comprised 71.78% of total fatty acids in seeds oil. This could indicate that the nutritional value is high. This substance is crucial in the diet, is a significant ingredients of cell membranes and is contributed in the generation of various compounds in human body, such as those contributed in the sustenance of blood pressure and inflammatory response. Recently it has implementation and interest on its impact in diabetes mellitus prevention and a number of cancers(Ruiz-Rodriguez *et al.,* 2010). Linoleic acid is largely applied industrially; for example, in the nutraceuticals industry it is utilized as diminisher of cholesterol levels(Ruiz-Rodriguez *et al.* 2010) and in food industry as food preservatives because of its antimicrobial activity (Silva *et al.* 2013; Nicholls *et al.* 2005). In a study about olive oil fatty acid profiles, it was found that $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ were 16.5, 2.3, 66.4 and 16.4 respectively. This means that olive oil unlike pepper contains more palmitic, oleic whereas it contains lower stearic and linoleic comparing to red pepper seed oil.(Mišurcová *et al.* 2011; Stefanoudaki *et al.* 1999)

Red pepper seed oil presents similarities to those of sunflower and safflower oils, which have linoleic acid as the most prevalent fatty acid, followed by oleic acid. This showed a high degree of unsaturation in red pepper seed oil.(Özyıldız *et al.* 2012).

5. CONCLUSIONS

Overall, the study it was conducted to determine the fatty acid composition and to monitor the oxidative stability of the red pepper seed oil extracted by the solvent (without applying any oil refining process) in the storage conditions of light and dark at room temperature. the critical chemical parameters of the oil increased significantly after 90 days. The storage conditions had an influence on the chemical composition of the oil at room temperature storage. The oil quality parameters were affected slightly in the dark medium as compared to the light medium. The fatty acid profiles had effect on the quality of the oil. Because the red pepper seed oil contains high amount of unsaturated fatty acid, especially linoleic acid. But it can be considered that red pepper seed oil has positive potential on human health because of a rich source of unsaturated fatty acid. In future it can be performed the works on red pepper seed oil such as using physical extraction or investigation of antioxidant properties.

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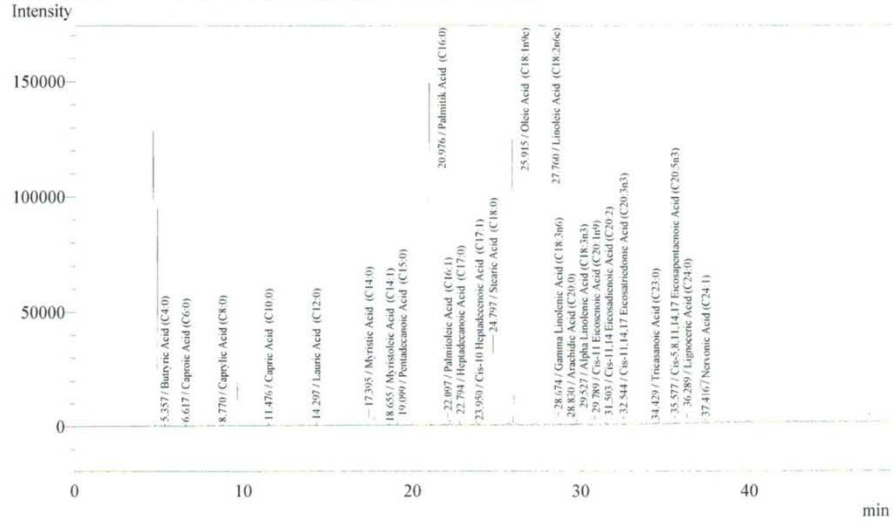
APPENDIX

Text

Analysis Date & Time : 21.10.2016 13:54:24
User Name : Admin
Vial# : 2
Sample Name : biber
Sample ID :
Sample Type : Unknown
Injection Volume : 1.00
ISTD Amount :

Data Name : C:\GCsolution\Data\Project1\21.12.gcd
Method Name : C:\GCsolution\Data\Project1\Metotlar\FAME.gcm



Peak#	Cmpd Name	Ret.Time	Area	Area%
1	Butyric Acid (C4:0)	5.357	1649	0.0299
2	Caproic Acid (C6:0)	6.617	1261	0.0229
3	Caprylic Acid (C8:0)	8.770	939	0.0170
4	Capric Acid (C10:0)	11.476	2851	0.0518
5	Lauric Acid (C12:0)	14.297	3545	0.0644
6	Myristic Acid (C14:0)	17.395	19019	0.3453
7	Myristoleic Acid (C14:	18.655	812	0.0147
8	Pentadecanoic Acid (C	19.099	2755	0.0500
9	Palmitik Acid (C16:0)	20.976	642135	11.6586
10	Palmitoleic Acid (C16:	22.097	15753	0.2860
11	Heptadecanoic Acid (C	22.794	5800	0.1053
12	Cis-10 Heptadecenoic A	23.950	3314	0.0602
13	Stearic Acid (C18:0)	24.797	184502	3.3498
14	Oleic Acid (C18:1n9c)	25.915	585428	10.6291
15	Linoleic Acid (C18:2n(	27.760	3953294	71.7762
16	Gamma Linolenic Acid	28.674	16113	0.2925
17	Arachidic Acid (C20:0)	28.830	500	0.0091
18	Alpha Linolenic Acid ((	29.527	14241	0.2586
19	Cis-11 Eicosenoic Acid	29.789	6397	0.1161
20	Cis-11,14 Eicosadienoi	31.503	3372	0.0612
21	Cis-11,14,17 Eicosatrie	32.544	11633	0.2112
22	Tricasanoic Acid (C23:(	34.429	1702	0.0309
23	Cis-5,8,11,14,17 Eicosa	35.577	10791	0.1959
24	Lignoceric Acid (C24:0	36.289	14439	0.2622
25	Nervonic Acid (C24:1)	37.416	5559	0.1009
Total			5507804	100.0000

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