EVALUATION AND ANALYSIS OF ALMOND OIL IN MEDITERRANEAN

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF HEALTH SCIENCES

OF

NEAR EAST UNIVERSITY

BY

ABEIDA KHALED BAHRI MOHAMED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN ANALYTICAL CHEMISTRY

NICOSIA 2016

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I hereby declar that all information in this document has been obtained and presented in accordance with academic rule and ethical conduct. I also declare that, as required by thes rules and conduct , I have fully cited and referenced all material and results that are not original to this work

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Date : 2/6/2016

ABEIDA KHALED BAHRI MOHAMED : EVALUATION AND ANALYSIS OF ALMOND OIL IN MEDITERRANEAN REGION

We certify that this thesis in satisfactory for the award of the degree of master of science in analytical chemistry.

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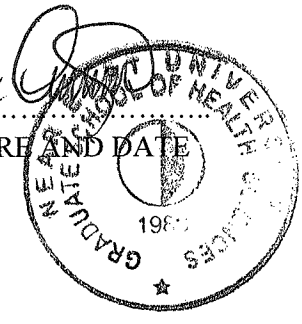

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TO my parents and my lovely wife . To my grandfather and my brothers and sisters . To the martyrs of Bani walid city .

إهداء

أهدي هذا العمل المتواضع إلى أبي الذي لم يبخل علي يوماً بشيء

وإلى أمي التي ذودتني بالحنان والمحبة

وإلى زوجتي سندي في الحياة

إلى منارة العلم جدي

وإلى إخوتي وأسرتي جميعاً

و إلى أرواح شهداء مدينة بني وليد

أقول لهم: أنتم وهبتموني الحياة والأمل والنشأة على شغف الاطلاع والمعرفة

ثم إلى كل من علمني حرفاً أصبح سنا برقه يضيء الطريق أمامي

أ. عيدة خالد خري غيث

Abstract

Almond (*Prunus amygdalus*) is an important medicinal plant known since ancient times. Almond trees are very common in Mediterranean countries and is also cultivated in similar climates such as California-USA. The nutritional importance of almond fruit is related to its kernel oil. Other parts of fruit such as shells and hulls were used as livestock feed and burned as fuel.

Almond oil contain oleic, palmitic, palmitoleic, stearic, linoleic, α-linoleic, arachidic, eicosenoic, behenic, erucic acids and vitamin E, thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), vitamin B6, Folate (B9), vitamin C, calcium, iron, magnesium, phosphorus, potassium, zinc. Percent of these substances can change more or less, according to the region where they grow and the methods of obtaining almond oil.

In this study, publications on almond trees and almond oil have been compiled. In this contribution, biological activates of almond oil are also reviewed. Besides information on the traditional obtaining methods and using, especially current obtaining methods (supercritical CO2 exraction) and analysis methods results are reviewed.

Keywords: *Almond , Fatty acids , Mediterranean region , Prunus amygdalus*

ÖZET

Badem (*Prunus amygdalus*) eski çağlardan beri bilinen önemli bir tıbbi bitkidir.. Badem ağaçları Akdeniz Bölgesi ülkelerinde yaygındır ve benzeri iklimlerde örneğin Kaliforniya'da da kültürü yapılmaktadır. Bademin önemi, meyve tohumlarının içerdiği yağından dolayıdır. Meyvenin diğer kısımları, kabukları hayvan yemi,çekirdek kabukları ise yakacak olarak kullanılır.

Badem yağı, oleik, α-linoleik, palmitik, palmitoleik, stearik, eikosenoik, behenik, erucic asitler ile vitamin E, thiamin (B1), riboflavin (B2), niacin (B3), pantotenik asit (B5), vitamin B6, Folate (B9), vitamin C, kalsiyum, demir, magnesyum, fosfor, potasyum, çinko içermektedir. Bu bileşiklerin oranı badem ağacının yetiştiği bölgelere ve elde edilme yöntemlerine gore az ya da çok değişmektedir.

Bu çalışmada badem ağaçları ve badem yağları ile ilgili makaleler derlenmiştir. Badem yağlarının biyolojik aktiviteleri üzerindeki çalışmalar da derlenmiştir. geleneksel elde edilme yöntemleri yanında özellikle güncel elde edilme yöntemleri (super kritik CO_2 ekstraksiyonu) ve analiz metotları da derlenmiştir.

Anahtar Kelimeler: *Prunus amygdalus, Badem , Yağ asitleri , Akdeniz Bölgesi*

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1.Introduction

Almond (*Prunus amygdalus* Batsch, syn. *Prunus dulcis* Miller, syn. *Amygdalus communis* L.) is a species native of South-West Asia , that belongs to the Rosaceae family, Amygdaloideae subfamily . According to phylogenetic studies based on chloroplast DNA analysis, almond and peach (*Prunus persica*) appear to be the most closely related species among cultivated Prunus and are classified into the subgenus *Amygdalus*. Actually almond and peach are thought to have evolved from the same primitive stock: peach seems to have evolved eastward into China at lower elevations in regions of higher humidity, whereas almond spread along the deserts and lower mountain slopes to the West, developing many subspecies along the way . In 3,000 BC, domesticated almond (sweet-seeded) was in use in Mediterranean civilizations. With regard to Italy, almond was first brought to Sicily by the Greeks, in the 5th century B.C. [1]

Almond is the most important nut crop worldwide. According to the last available FAO data (FAOSTAT), the USA were the first almond producing country in 2010, with 1,413,800 tons of shelled almonds (mainly from California), followed by Spain with 221,000, Iran with 158,000 and Italy with 108,160 tons .[1].

Most of traditional varieties are self-incompatible and early-blooming, both facts causing them to have a little production, due to low fertility and frost damage to the flowers. That caused, in the last decades, a regression of traditional almond cultivation in Italy both in terms of lands and production. Such a decline is also due to the application of agronomic techniques not suitable to the needs of a modern almond cultivation.[1] .

Nuts are known as a source of nutritious food with high lipid content. Replacing half of the daily fat intake with nuts has been known to lower total and LDL cholesterol levels significantly in humans. The observed blood cholesterol lowering effects of nuts were far better than what was predicted according to their

dietary fatty acid profiles. Research also shows a connection between regular nut consumption and decreased incidence of coronary heart disease These beneficial physiological effects suggest that bioactive compounds of nuts may possess lipid altering activities due to additive/synergistic effects and/or interactions with each other. Dietary antioxidants provide protection against oxidative attack by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals, binding of metal ion catalysts, decomposing primary products of oxidation to non-radical compounds, and chain breaking to prevent continuous hydrogen removal from substrates .[2]

Almonds when incorporated in the diet, have been reported to reduce colon cancer risk in rats and increase HDL cholesterol and reduce LDL cholesterol levels in humans . Extracts of whole almond seed, brown skin, shell, and green shell cover (hull) possess potent free radical-scavenging capacities .These activities may be related to the presence of flavonoids and other phenolic compounds in nuts. Almond hulls have been shown to serve as a rich source of three triterpenoids (about 1% of the hulls), betulinic, urosolic, and oleanolic acids . as well as flavonol glycosides and phenolic acids . In addition, and , isolated catechin, protocatechuic acid, vanillic acid, p-hydroxybenzoic acid, and naringenin glucoside, as well as galactoside, glucoside, and rhamnoglucoside of 3β-O-methylquercetin and rhamnoglucoside of kaempferol. The production of almond hulls, which are mainly used in livestock feed, is estimated to exceed 6 million tons annually, thus being a potentially good source from which to extract antioxidants that are present, if any, in high quantities , [2] .

Almond oil an excellent moisturizer and lubricant, which prevents the skin from drying and keeps you free from chapped and peeling skin. For centuries, almond oil had been used, as a soothing remedy for skin allergies, and to treat minor cuts and wounds. Another common use of almond oil is in massages because it is an

excellent skin lubricant. Its properties make it popular with massage therapists' worldwide. It does not have any greasy effect and will take a little bit of time before it is absorbed by the skin. Using it for a massage makes your body feel relaxed and your skin looking healthy. It will definitely relieve the stress you have from a hard day's work. The newly pressed sweet almond oil is a mitigator of pain and all manner of aches; therefore it is good in calming of head, brain, pleurisy and colic.[3] .

According to the 3rd edition of the European pharmacopeia , this oil is { the fatty oil obtained by cold expression from the rip seeds of Prunus dulcis (Miller) D.A. Webb var . dulcis or Prunus dulcis (Miller) D.A. Webb var . amara (D.C) Buchheim or a mixture of both varieties } The pharmacopeia also describes refined almond oil . [4]

1.1.Habitat and History of Almond

The Almond tree is a native of the warmer parts of western Asia and of North Africa, but it has been extensively distributed over the warm temperate region of the Old World, and is cultivated in all the countries bordering on the Mediterranean. It was very early introduced into England, probably by the Romans, and occurs in the Anglo-Saxon lists of plants, but was not cultivated in England before 1562, and then chiefly for its blossom [5] .

The early English name seems to have been Almande: it thus appears in the Romaunt of the Rose. Both this old name and its more modern form came through the French amande, derived from the late Latin amandela, in turn a form of the Greek amygdalus, the meaning of which is obscure [5] .

The tree grows freely in Syria and Palestine: it is mentioned in Scripture as one of the best fruit trees of the land of Canaan, and there are many other biblical

references to it. The Hebrew name, shakad, is very expressive: it signifies 'hasty awakening,' or 'to watch for,' hence 'to make haste,' a fitting name for a tree, whose beautiful flowers appearing in Palestine in January, herald the wakening up of Creation. The rod of Aaron was an Almond twig, and the fruit of the Almond was one of the subjects selected for the decoration of the golden candlestick employed in the tabernacle. The Jews still carry rods of Almond blossom to the synagogues on great festivals [6].

As Almonds were reckoned among 'the best fruits of the land' in the time of Jacob we may infer they were not then cultivated in Egypt. Pliny, however, mentions the Almond among Egyptian fruit-trees; and it is not improbable that it was introduced between the days of Jacob and the period of the Exodus[5].

Almonds, as well as the oil pressed from them, were well known in Greece and Italy long before the Christian era. A beautiful fable in Greek mythology is associated with the tree. Servius relates that Phyllis was changed by the gods into an Almond tree as an eternal compensation for her desertion by her lover Demophoon, which caused her death by grief. When too late, Demophoon returned, and when the leafless, flowerless and forlorn tree was shown him, as the memorial of Phyllis, he clasped it in his arms, whereupon it burst forth into bloom - an emblem of true love inextinguishable by death [6].

During the Middle Ages, Almonds became an important article of commerce in Central Europe. Their consumption in medieval cookery was enormous. An inventory, made in 1372, of the effects of Jeanne d'Evreux, Queen of France, enumerates only 20 lb. of sugar, but 500 lb. of Almonds[6].

The ancients attributed many wonderful virtues to the Almond, but it was chiefly valued for its supposed virtue in preventing intoxication. Plutarch mentions a great drinker of wine, who by the use of Bitter Almonds escaped being intoxicated, and Gerard says: 'Five or six, being taken fasting, do keepe a man

from being drunke.' This theory was probably the origin of the custom of eating salted Almonds through a dinner . This oil has been traditionally used by massage therapists to lubricate the skin during a massage session, being considered by many to be an effective emollient. Sweet Almond Oil exhibits excellent penetrating qualities and incredible spread-ability on the skin, making it ideal as a massage oil or as a carrier oil for treatment products. Sweet Almond Oil adds moisturizing attributes to creams and lotions and bar soaps. This particular grade of oil offers superb clarity and light color, desirable attributes for today's high end formulations. Because of its moderate cost, it may be used as a substitute for petroleum based oils. Exhibits low comedogenicity on the skin. May be used in cosmetics, toiletries, bar soaps, massage oils, hair care and sun care applications[6] .

1.2 Botanical information

The peach-like edible almonds fruit (P. *amygdalus* L) have three distinct parts: the inner kernel or meat, the middle shell portion, and an outer green shell cover or hull. Almond varieties vary in shell texture; therefore they are termed hard or soft shelled. The harvesting procedure starts when the almonds are partly dried on the trees. In addition the sweet almond is a stone fruit which have several unique features. It is commercially cultivated where there are long, hot, and Mediterranean like summers, such as those in Spain, Morocco, Armenia, Iran, Italy, California (USA), and Australia. It is unique, in that unlike others in its botanical family, such as peach, apricot and plum, where the flesh (mesocarp) of the fruit is eaten and the seed within its shell, or stone (endocarp) is discarded, the reverse is true for the almond early in its maturation cycle, for a period of a few weeks, the entire fruit (seed, endocarp and mesocarp) can be, and is, eaten, in several parts of the world. As the maturation cycle continues, the hull splits open.

When dry, it may be readily separated from the shell. The almond pit, containing a kernel or edible seed, is the nut of commerce, the endocarp (shell), and mesocarp are separated for low value uses, such as cat litter and animal feed .Shelled almonds may be sold as whole natural almonds or processed into various almond forms. The whole natural almonds have their shells removed but still retain their brown skins; blanched whole almonds have both their shells and skins removed. Usually, the removed skins are discarded [7] .

1.3 Obtaining Almond Oil

1.3.1 Cold Press Method

Cold pressed method is one of the best methods to extract essential oils. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. Cold pressed method is also known as scarification method. No external heat is required to let the process go, rather the high temperature to carry out the process is obtained internally. Though it is not considered a practical method of extraction for all vegetable oils but it is highly regarded as the extraction method of choice.[8].

Cold pressed almond oil is an excellent emollient. It also helps to balance the loss and absorption of moisture, making it particularly effective for dull and irritated skin, soothing it while nourishing and protecting. Almond oil high lubricating quality makes many people to use it as their daily skin care product. It well combines with other natural ingredients , such as bee products, essential oils, that makes almond oil a perfect carrier oil for massage.

Cold pressed almond oil has also been proven to have a calming effect on irritated or allergic skin. It has both the ability to soothe as well as treat skin inflammation, therefore it is frequently used in babies and children cosmetic products [9].

1.3.2 Supercritical CO2 Extraction Method

Supercritical CO2 was found to be selective in the separation of desired compounds without leaving toxic residues in extracts and without the risk of thermal degradation of processed products. Through the exploitation of the solvating power acquired by fluids near their critical points and the sensitivity of this power to small perturbations in temperature, pressure and modification of the solvent with the addition of entrainers, solvent-free extracts were readily obtained due principally to the high volatility of these solvents at ambient conditions. The favorable transport properties of fluids near their critical points also allows deeper penetration into solid plant matrix and more efficient and faster extraction than with conventional organic solvents. For the past three decades, the commercial application of supercritical fluid technology remained restricted to few products due to high investment costs and for being new and unfamiliar operation. With advances in process, equipment and product design and realization of the potentially profitable opportunities in the production of high added value products, industries are becoming more and more interested in supercritical fluid technology . The extraction is carried out in high-pressure equipment in batch (Figure 1) . In both cases, the supercritical solvent is put in contact with the material from which a desirable product is to be separated. The supercritical solvent, now saturated with the extracted product, is expanded to atmospheric conditions and the solubilized product is recovered in the separation vessel permiting the recycle of the supercritical solvent for further use. Supercritical fluid extraction (SFE) of seed oil has been studied by several authors from the processing point of view and a wide range of seed species has been explored: wheat germ [10].

Despite the large number of species processed, only some models of the SFE of seeds have been published. They all agree with the fact that at least the first part

of the SFE process is governed by the solubility equilibrium between the oil and the fluid phase. The equilibrium relationship has been generally supposed to be linear since more precise information is not avail- able in such complex systems. From the mathematical point of view, all models proposed are based on differential mass balance integration. used a shrinking core model to describe a variable external resistance where the solute balance on the solid phase determines the thickness of the mass transfer layer in the external part of the particles. considered the solid phase as divided between broken and intact cells. Two separate mass balances were written and different mass transfer resistances in the solvent phase for the broken cells and in the solid phase for the intact ones were considered. This model was based on sound physical hypotheses and represented the first attempt to introduce a description of the structure of the vegetable matter by a mathematical model. However, it required several parameters related to physical properties of the seed which were difficult to be measured or calculated and, therefore, were adjusted by the authors to fit the experimental results.[11]

In the first part of the extraction experiments the solvent exits the extractor saturated with the almond oil. This period is longer for smaller particles. This extraction feature can be explained by considering two different phases in which the oil can be found within the seeds .a freely available oil phase contained within broken cavities (cells) on the surface of the crushed particles and a tied oil phase, either contained within closed cells inside the particles or somewhat tied to their internal structure. An extraction model considering these two different phases was proved to describe fairly well the whole extraction process. It is significant that only the mass transfer coefficient, among the many parameters involved,. In particular, scanning electron microscopy was used to evaluate the volume of the free oil phase. This reduction of the arbitrary parameters involved in the model makes the validation of the model itself more significant. Reduction of

arbitrary parameters is important, particularly, if modeling is aimed at determining the most relevant physical phenomenon in a complex process, like the extraction of natural matrices with supercritical fluids. The concentration profiles within the extractor calculated with our model confirm the existence of a marked concentration shock wave during the first extraction stages [11].

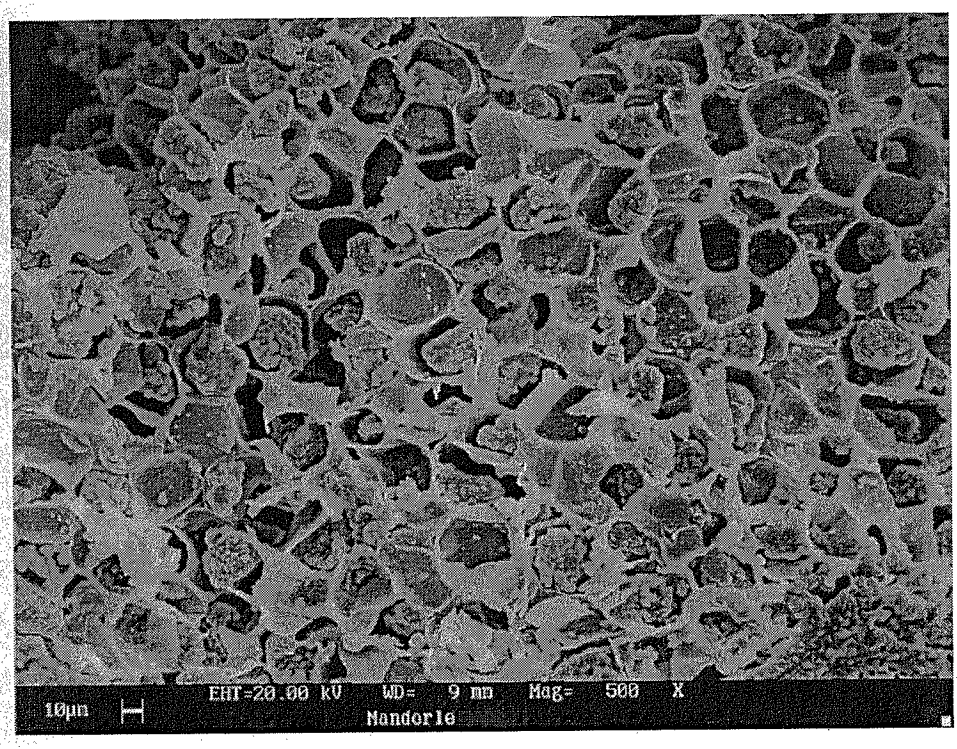

Fig. 1. Scanning electron microscope image of the surface of an almond particle extracted with supercritical CO_*.Broken vegetable structures (cells) that contained the free oil phase are clearly evident. Inside the cells it is possible to see the presence of non-extractable starch grains. The mean cell diameter can be estimated around 20 µm .(Chemical Engineering Science, Vol. 53, No. 21, pp. 3711Ð3718, 1998)

2.Quality and Analysis of Almond and Almond Oil

Almond quality comprises commercial, industrial, organoleptic and nutritional aspects related to the nut and kernel. Almond (*Amygdalus communis* L., syn *Prunus amygdalus* Batsch., syn *Prunus dulcis* Miller), the kernel is the edible part of the fruit.

2.1.Quality Characterization of Almond Production

Commercial quality includes characteristics regarding the external presentation, like size, texture, absence of double kernels etc., as well as marketable yield. Industrial quality refers to the cultivar's attitude to handling, transportation, processing and storage. Organoleptic quality is highly variable and subjective as it consists of those parameters related to consumer preferences. Nutritional quality refers to the specific nutrients provided and the contribution to consumer health on the whole.

Until recently, as far as fruit is concerned, almond breeding has been focused on selecting fruits of a high physical quality (mainly related with commercial quality). For this reasons, information about the chemical composition of the almond oil kernel and their variability are scarce. Including such analyses in the evaluation of almond varieties would be of great interest to explore the possible utilizations of the product. Almond have been used in different ways: they are consumed raw, roasted, peeled or unpeeled; processed into food items, such as marzipan, nougat ("torrone") and other traditional sweets, typical in the Mediterranean basin, and into almond milk. Additionally almond is used in the pharmaceutical and cosmetics industries. Thus the same kernel trait may be considered positive or negative depending on its final utilization [12].

2.2. Composition of Almond Oil

Almond oil includes five major fatty acids: three UFAs (oleic acid, monounsaturated, palmitoleic and linoleic acid, polyunsaturated), that account for the 90% of the total lipids; and two SFA (palmitic and stearic acid).

Almond oil also contains eight minor fatty acids . Among them it is worth mentioning the polyunsaturated α-linolenic acid (= ALA) that, like linoleic acid, is an essential fatty acids: they are not synthesized by the human organism, so they must be taken in through the diet. They are the starting point, respectively, of omega-3 and omega-6 fatty acid families. Among the several functions of these compounds, the omega-3s have anti-inflammatory and anti-thrombotic effects and the omega-6s reduce the blood cholesterol concentration . As nowadays' diet is poor in fish, the primary source of omega-3 fatty acid family, their intake is scarce if compared to omega-6 fatty acid family. Thus some alternative, vegetable, sources of omega-3s are precious [12].

Finally, the unsaponifiable fraction of almond oil contains sterols, aliphatic alcohols hydrocarbons and liposoluble vitamins . Besides the nutritional value, lipid content and composition is also important for determination of oil stability, since component fatty acids differ in their vulnerability to oxidation , thus influencing the resistance to rancidity. As polyunsaturated fatty acids (PUFAs) are more susceptible to oxidation than MUFAs, suggested that oleic acid/linoleic acid ratio is a good index of resistance to oil rancidity, with higher ratios indicating better resistance [12] .

Resistance to oil rancidity also depends on the presence of natural antioxidant, such as tocopherols, that protect PUFAs against peroxidation. In almond the tocopherols lengthen the storage time by playing an important role against fat oxidation. Several studies have shown that almond nuts can be stored for nine months with little quality compromission .Storage is possible up to one year for

cultivars with high concentration of natural antioxidants such as α-tocopherol (more than 400 mg/ kg oil, . Tocopherols are also important for human health: α-tocopherol is the form of vitamin E that is most efficiently used by the human body, yet it is often deficient in modern diets [28] Vitamin E along with the antioxidant polyphenols and fibers may help to prevent heart diseases and cancer . Almond is the nut with the highest α-tocopherol content. For this reason almonds was included in the recommendations of The Dietary Guidelines for Americans (USDA, 2005) in the context of enhancing the intake of this vitamin [12].

2.2.1. Gas Chromatographic Analysis of Fatty Acids

GC analysis of fatty acids can be realized after methylation process. To transform the oil fatty acids to the corresponding methyl esters (FAMEs): 0.5 g aliquots of oil, were dissolved in 6 ml hexane, and 250 μ L of KOH 2N in methanol was added. After moderate shaking, tubes were centrifuged at 2000 x g for 10 min. were recovered and transferred in vials for gas chromatography (GC) analysis [13] .

The fatty acids methyl esters were analyzed by gas chromatography. Gas chromatography was performed with a capillary column (capillary column which was 25 m in length, 0.25 μm inner diameter and at a 25 micron film thickness) using nitrogen as a carrier gas (flow rate 0.8 mL/min.). During analysis, the column temperatures, detector, and injection valve were 120-220, 240, and 280°C, respectively. Before the fatty acid methyl esters analysis of the samples, the standard mixture of fatty acid methyl esters by injection and each fatty acid retention time were determined. Identification of the individual methyl esters were performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions .The fatty acid content of the almond seed samples was determined according to the standard fatty acid chromatogram (Figure 2). All of the almond genotypes gas chromatographic

analyses were carried out as shown in Figure 2. The results obtained as averages through triplicate with standard deviation and percentages of genotypes values [13] .

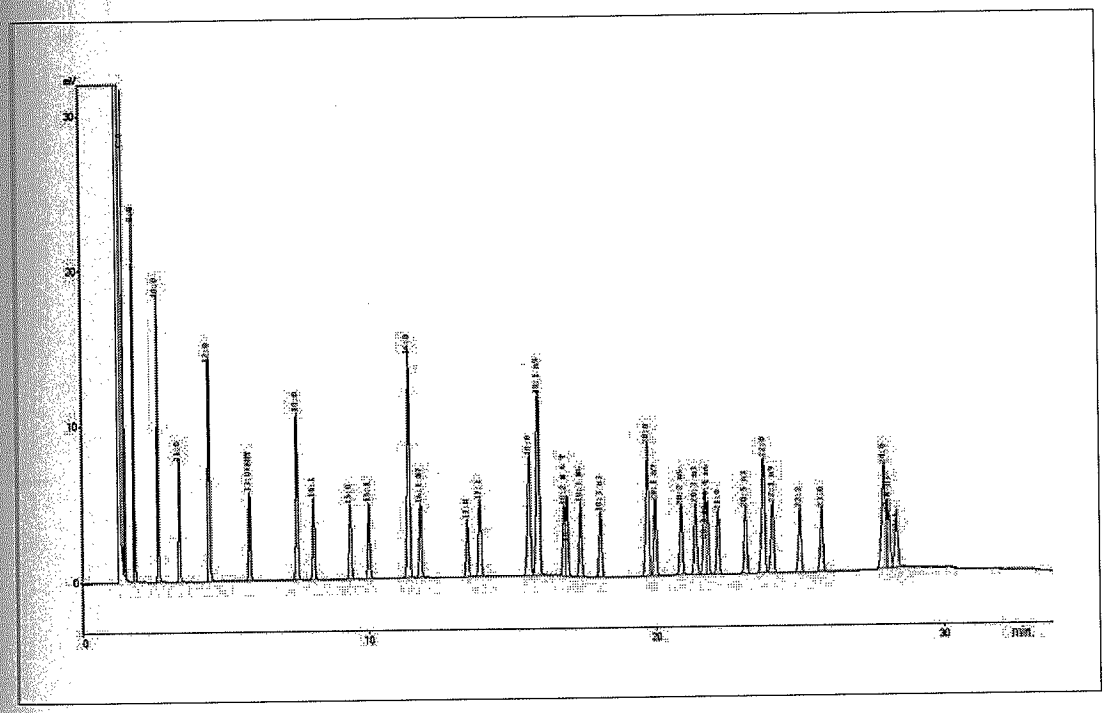

Figure 2. Standard fatty acids chromatogram (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39(3):307–316 doi: 10.5505/tjb.2014.55477)

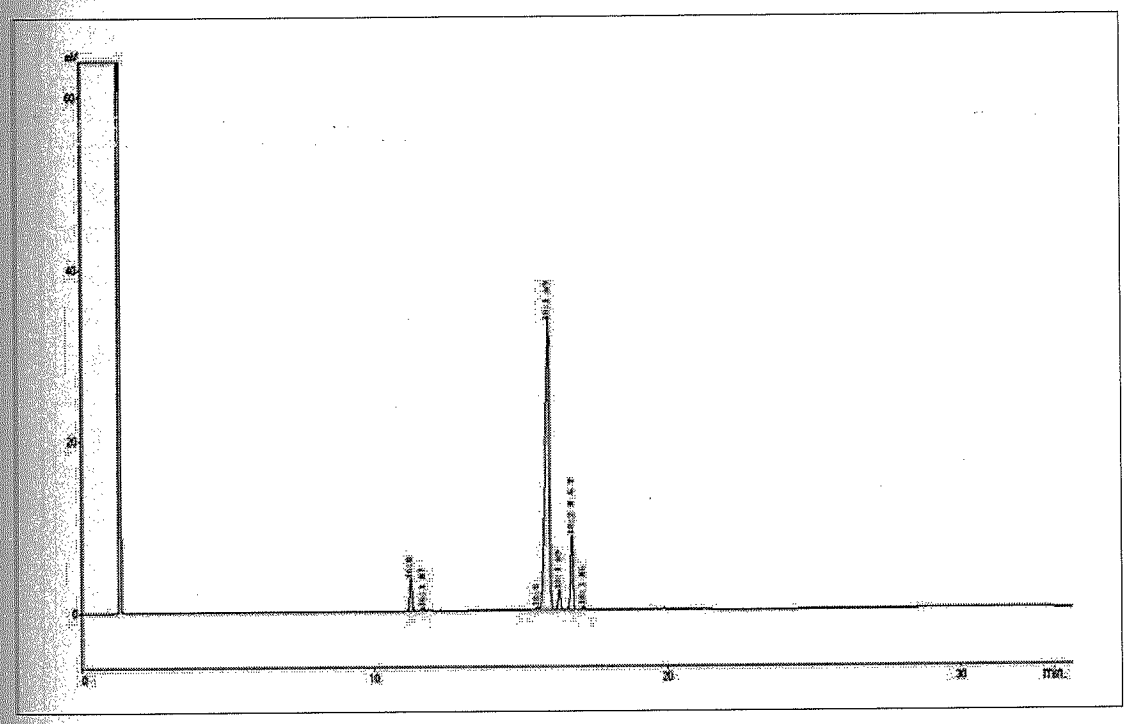

Figure 3. One of the almond fatty acids chromatogram example (1. reputation of 18 numbered genotype (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39(3):307–316 doi: 10.5505/tjb.2014.55477)

Fatty acids		Range	Typical
Palmitic	C16:0	3.0-9.0 %	5.5 %
Palmitoleic	C16:1	2.0 % max	0.2 %
Stearic	C18:0	0.5 - 3.0%	2.8 %
Oleic	C18:1	60.0 -75.0 %	70.0 %
Linoleic	C18:2	20.0 - 30.0 %	21.0 %
Alpha Linoleic	C:18:3	0.4 % max	0.1 %
Arachidic	C20:0	0.2 % max	0.1 %
Eicosenoic	C20:1	0.2 % max	0.1 %
Behenic	C22:0	0.2 % max	0.1 %
Erucic	C22:1	0.1 % max	n.d.

Tabe 4 : The fatty acids extraction from almond oil (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39(3):307–316 doi: 10.5505/tjb.2014.55477)

2.2.2 Statistical Analysis of Fatty Acids

For statistical analysis the SPSS 17.0 software program was used. The analysis was performed in triplicate. As a result of the analysis, to compare differences between samples $p<0.05$, $p<0.01$ and $p<0.001$ probability level analysis of variance (ANOVA) and Duncan tests were per- formed. The mean values of the genotypes were detected by standard deviation and standard errors of averages. In analysis, fatty acid compositions, % rates and correlations were performed by comparing the averages. Furthermore, each fatty acid composition (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid) in the seed except for existing trace amounts of almond fatty acids was combined and identified usingcluster analysis.

In this study, in material and methods, while column temperature was set between 4°C /min 200°C to 220°C with 35 minutes (in this study 34 minutes were used) analysis with starting 130°C of column temperature and termal expansion applied 215-230°C at 4°C/min gradually. and were use

Almond Samples	Palmitic (16:0)	Palmitolei (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Omega-3	Omega-6
2	5.97±0.27	0.76±0.21	0.67±0.05	71.42±1.97	20.10±2.06	1.08±0.03	0.00	21.18±2.05
4	5.18±0.09	0.79±0.02	0.86±0.09	75.74±0.16	16.73±0.19	0.70±0.01	0.00	17.43±0.18
6	4.92±0.26	0.71±0.14	0.74±0.04	77.26±1.65	15.67±1.88	0.70±0.02	0.00	16.38±1.88
8	5.94±0.38	0.70±0.19	0.90±0.01	76.91±1.54	14.99±1.71	0.55±0.01	0.00	15.54±1.70
11	**4.42±0.16**	0.48±0.15	1.34±0.04	77.46±2.18	15.50±2.20	0.81±0.03	0.00	16.31±2.19
12	5.77±0.96	0.78±0.19	0.44±0.76	70.47±2.99	21.64±2.45	0.90±0.16	0.00	22.54±2.61
13	5.41±0.34	0.66±0.22	1.03±0.07	79.92±1.52	12.67±1.46	**0.32±0.2**	0.00	12.99±1.74
14	6.70±0.16	**0.95±0.02**	1.14±0.04	66.50±1.57	23.98±1.01	0.73±0.63	0.00	24.71±1.62
16	4.87±0.11	0.55±0.01	0.35±0.61	74.16±2.09	19.24±1.73	0.83±0.01	0.00	20.07±1.73
17	4.65±0.09	**0.42±0.14**	1.78±0.01	79.88±1.63	12.78±1.75	0.49±0.00	0.00	13.27±1.75
18	4.63±0.05	0.53±0.12	0.71±0.01	79.92±1.75	13.70±1.70	0.51±0.00	0.00	14.21±1.70
19	5.73±0.05	0.72±0.02	1.32±0.02	77.86±1.99	13.74±2.02	0.62±0.02	0.00	14.37±2.01
21	5.20±0.05	0.71±0.01	0.44±0.76	73.34±0.93	19.46±0.21	0.86±0.01	0.00	20.32±0.22
22	6.02±0.28	0.63±0.19	0.94±0.01	71.38±1.13	20.22±1.12	0.80±0.05	0.00	21.02±1.11
24	5.03±0.21	0.60±0.17	1.21±0.04	76.29±1.53	16.21±1.72	0.66±0.00	0.00	16.86±1.72
27	5.19±0.03	0.70±0.01	0.34±0.59	75.15±0.66	17.83±0.25	0.78±0.01	0.00	18.61±0.25
28	5.08±0.28	0.69±0.07	0.30±0.52	77.28±0.24	15.95±0.25	0.63±0.01	0.00	16.65±0.28
30	5.73±0.27	0.93±0.02	0.30±0.52	78.59±0.18	13.71±0.21	0.49±0.00	0.00	14.46±0.36
33	5.20±0.08	0.63±0.01	0.83±0.04	75.92±0.03	16.70±0.11	0.72±0.00	0.00	17.42±0.11
35	5.53±0.10	0.75±0.01	1.00±0.04	**64.05±2.1**	**27.00±2.0**	**1.67±0.0**	0.00	**28.67±2.1**
37	5.26±0.50	0.79±0.09	0.57±0.99	71.70±0.55	20.38±0.64	1.30±0.32	**0.30**	21.38±0.70
39	5.14±0.06	0.67±0.00	0.83±0.10	68.09±1.68	24.08±1.76	1.18±0.02	0.00	25.26±1.75
40	5.46±0.15	0.83±0.03	0.27±0.46	71.55±0.31	20.85±0.12	1.05±0.10	0.12	21.78±0.12
42	5.42±0.12	0.86±0.18	0.67±0.01	71.67±1.56	20.77±1.21	0.61±0.53	0.00	21.38±1.73
43	5.06±0.02	0.53±0.15	0.92±0.03	75.44±1.73	17.56±1.36	0.50±0.43	0.00	18.06±1.79
44	5.87±0.08	0.81±0.01	1.01±0.01	66.56±1.79	24.48±1.80	1.28±0.01	0.00	25.75±1.79
46	4.68±0.05	0.82±0.12	**0.26±0.4**	79.46±2.59	14.32±2.03	0.46±0.40	0.00	14.78±2.43
47	4.97±0.09	0.83±0.42	0.75±0.03	78.92±1.73	14.19±1.29	0.34±0.29	0.00	14.53±1.58
49	4.65±0.09	0.82±0.02	0.84±0.03	**80.68±2.0**	**12.65±1.8**	0.36±0.31	0.00	**13.01±2.1**
52	5.30±0.69	0.65±0.17	1.09±0.02	76.19±6.32	16.20±5.22	0.57±0.24	0.00	16.77±5.46
53	5.71±0.66	0.52±0.04	**1.67±0.5**	71.94±6.93	19.51±5.64	0.65±0.19	0.00	20.16±5.83
54	**6.25±0.39**	0.61±0.18	1.60±0.38	70.89±2.51	19.76±2.72	0.82±0.05	0.00	20.66±2.56
General Average	**5.34±0.58**	**0.70±0.17**	**0.85±0.5**	**74.46±4.7**	**17.89±4.1**	**0.75±0.3**	**0.013**±0.06	**18.64**±4.38

Table 2. The average, minimum (bold written values)-maximum (bold and taken into boxes values), percentage and standard deviation values of the main fatty acids of almond seeds samples (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014)

Almond Samples	SFA	USFA	USFA/SFA	MUFA	PUFA	MUFA/ PUFA	Oleic/ Linoleic
2	6.64±0.24	93.36±0.2	14.07±0.54	72.18±1.9	21.18±2.0	3.43±0.40	3.58±0.44
4	6.04±0.01	93.96±0.0	15.54±0.02	76.53±0.1	17.43±0.1	4.39±0.0	4.53±0.0
6	5.66±0.30	94.34±0.3	16.70±0.93	77.96±1.6	16.38±1.8	4.81±0.6	4.98±0.6
8	6.84±0.38	93.16±0.3	13.64±0.83	77.61±1.4	15.54±1.7	5.04±0.6	5.19±0.7
11	5.76±0.16	94.24±0.1	16.38±0.48	77.94±2.1	16.31±2.1	4.84±0.7	5.07±0.8
12	6.21±0.20	93.79±0.2	15.12±0.53	71.25±2.8	22.54±2.6	3.20±0.5	3.30±0.5
13	6.43±0.41	93.57±0.4	14.58±1.02	80.58±1.5	**12.99±1.7**	6.30±1.0	6.38±0.9
14	7.84±0.11	92.16±0.1	**11.76±0.19**	67.45±1.5	24.71±1.6	2.74±0.2	2.78±0.1
16	5.22±0.51	94.78±0.5	18.29±1.77	74.71±2.0	20.07±1.7	3.75±0.4	3.88±0.4
17	6.43±0.10	93.57±0.1	14.56±0.25	80.30±1.7	13.27±1.7	6.14±1.0	6.35±1.0
18	5.34±0.05	94.66±0.0	17.73±0.18	80.45±1.6	14.21±1.7	5.73±0.8	5.91±0.9
19	7.05±0.04	92.95±0.0	13.18±0.07	78.58±2.0	14.37±2.0	5.56±1.0	5.77±1.0
21	5.64±0.72	94.36±0.7	16.92±2.12	74.05±0.9	20.32±0.2	3.65±0.0	3.77±0.0
22	6.97±0.27	93.03±0.2	13.36±0.58	72.01±1.1	21.02±1.1	3.43±0.2	3.54±0.2
24	6.25±0.25	93.75±0.2	15.03±0.64	76.89±1.6	16.86±1.7	4.60±0.6	4.75±0.6
27	5.53±0.58	94.47±0.5	17.20±1.79	75.86±0.6	18.61±0.2	4.08±0.0	4.22±0.0
28	5.38±0.25	94.62±0.2	17.61±0.83	77.97±0.2	16.65±0.2	4.68±0.0	4.85±0.0
30	6.03±0.26	93.97±0.2	15.61±0.69	79.52±0.1	14.46±0.3	5.50±0.1	5.73±0.1
33	6.03±0.11	93.97±0.1	15.60±0.29	76.55±0.0	17.42±0.1	4.39±0.0	4.55±0.0
35	6.53±0.06	93.47±0.0	14.32±0.14	**64.80±2.1**	**28.67±2.1**	**2.27±0.2**	**2.39±0.2**
37	5.83±0.50	94.17±0.5	16.23±1.40	72.49±0.4	21.68±0.9	3.35±0.1	3.52±0.1
39	5.97±0.16	94.03±0.1	15.76±0.44	68.77±1.6	25.26±1.7	2.73±0.2	2.84±0.2
40	5.73±0.32	94.27±0.3	16.50±0.93	72.37±0.3	21.90±0.0	3.30±0.0	3.43±0.0
42	6.10±0.13	93.90±0.1	15.41±0.35	72.52±1.6	21.38±1.7	3.41±0.3	3.46±0.2
43	5.98±0.03	94.02±0.0	15.73±0.08	75.96±1.8	18.06±1.7	4.24±0.5	4.32±0.4
44	6.88±0.07	93.12±0.0	13.54±0.16	67.37±1.7	25.75±1.7	2.63±0.2	2.73±0.2
46	**4.93±0.40**	**95.07±0.4**	**19.35±1.58**	80.28±2.6	14.78±2.4	5.56±1.2	5.65±1.0
47	5.71±0.06	94.29±0.0	16.50±0.19	79.75±1.5	14.53±1.5	5.54±0.7	5.60±0.6
49	5.48±0.06	94.52±0.0	17.23±0.18	**81.51±2.1**	13.01±2.1	**6.41±1.3**	**6.49±1.1**
52	6.39±0.69	93.61±0.6	14.75±1.61	76.84±6.1	16.77±5.4	4.94±1.6	5.07±1.7
53	7.37±1.16	92.63±1.1	12.81±2.38	72.46±6.9	20.16±5.8	3.94±1.7	4.04±1.8
54	**7.85±0.64**	**92.15±0.6**	11.80±1.10	71.49±2.5	20.66±2.5	3.51±0.5	3.65±0.6
General Averag	**6.19±0.78**	**93.81±0.7**	**15.40±1.98**	**75.16±4.7**	**18.65±4.3**	**4.32±1.2**	**4.45±1.2**

Table 3. Samples of fatty acid composition of almond seeds and for the mean of % rates with standard deviation and minimum maximum values. (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014)

injection temperature at 250°C close to our work. Mainly fatty acids amounts were determined in almond genotips. The average amounts of fatty acids of almond genotypes with minimum-maximum values and standard deviation are shown as percentages in Table 2 and Table 3. The averages of palmitic acid (16:0) 5.34%, palmitoleic acid (16:1) 0.70% stearic acid (18:0) 0.85%, oleic acid (18:1) 74.46%,

linoleic acid (18:2) 17.89% and linolenic acid (18:3) 0.75%, omega 3 and omega 6 were found. In addition, eicosenoic acid (20:3), docosahexaenoic acid (22:6) and tridecanoic acid (13:0) were encountered in very small amounts (<0.5%). Apart from these, SFA 6.19%, USFA 93.81% and a rate of USFA/SFA of 15.40, MUFA 75.16 %, PUFA 18.65 % and a MUFA/PUFA ratio of 4.32 were found. The ratio of oleic/linoleic acid was found to be 4.45 and the sum of oleic + linoleic acid was found to be between 90.48% - 93.78%. Omega-3 fatty acids were found in very low amounts in the majority of genotypes. According to Table 2, overall average of omega-3 was detected as 0.013% with 0.068 standard deviations, 0.005 variance and 0.007 standard error of average. The overall mean of omega-6 of genotypes was found as 18.64% with 4.38 standard deviations, 19.16 varience and 0.447 standard error of mean was detected. Despite the fact that low levels of omega-3 in total in almond genotypes, omega 6 fatty acids were detected in high values. It cannot be produced in human body, omega-3, and omega-6 hold important place in every period of human life and deficiency of its can cause disease in humans body. Therefore, with respect to omega-3 fatty acids, 35 and 40 numbered genotypes and with respect to omega-6 fatty acids, primarily 35 numbered genotype, 44, 39, 14 and 12 numbered genotypes are important. From these, the 35 numbered genotype have the highest degrees terms of valuable fatty acids that the oleic, linoleic and linolenik acids. Substantial differences were found between almond genotypes at α= 0.05 significance level and the $p<0.001$ probability level according to the proportion of fatty ac- ids. Different sub-groups were found between the genotypes to which the Duncan test was applied. Palmitic acid between 4.42% (genotype number 11) - 6.70% (genotype number 14), palmitoleic acid between 0.42% (genotype number 17) - 0.95% (genotype number 14), stearic acid between 0.26% (genotype number 46) - 1.78% (genotype number 17) and oleic acid between 64.05% (genotype number 35) – 80.68% (genotype number 49) were

found. Linoleic acid was found to be between 12.65% (genotype number 49) - 27.00% (genotyp number 35) and linolenic acid was found to be between 0.32% (genotype number 13) - 1.67% (genotype number 35) which are essential PUFA. The genotype numbered 35 was one of the important genotypes among all almond genotypes in terms of linoleic and linolenic acid [13] .

A higher proportion of USFA is prefered to SFA because of its benefical effect on human health for almond seed. While the lowest amount of SFA was detected to be 4.93% in the genotype numbered 46, the highest SFA rate was found to be 7.85% in the almond genotype numbered 54. USFA was found to be between 92.15% (genotype number 54)- 95.07 (genotype number 46) in terms of average values of each of the genotypes. The ratio of USFA to SFA (USFA/SFA) is an important feature of oil quality in almond seeds. According to this feature, the almond genotype had rates between 11.76 (genotype number 14) and 19.35 (genotype number 46). Unsaturated fatty ac- ids to saturated fatty acid ratio (USFA/SFA) were higher of overall average of the 32 almond genotypes. It will provide important quality improvement as weel as the effort intensified on these almond genotypes especially 46 numbered genotype that has USFA ratio 95.07% and USFA/SFA ratio 19.35, 16 (USFA/SFA ratio 18.29) and 18 numbered genotype (USFA/SFA ratio 17.73). In terms of MUFA, relative to USFA, the genotypes had ratios be- tween 64.80% (genotype number 35) - 81.51% (genotype number 49). Also, PUFA received between 12.99% (genotype number 13) - 28.67% (genotype number 35). The MUFA/PUFA ratio was evaluated because MUFA is more high quality than PUFA. Therefore, the MUFA/PUFA ratio was found to be between 2.27 (genotype number 35) and 6.41 (genotype number 49) in the genotypes. Like-wise, a high oleic/linoleic acid ratio is also prefered. In this respect, the highest rate (6.49) was seen in the genotype numbered 49 numbered. In addition to these,

USFA which is based on oleic and linoleic acids is also known as the dominant fatty acid. In terms of sum USFA percent- age, the genotypes numbered 46, 18, 16, 28, 49, 27, 47 and 43 have more than 94% (94.02-95.07%) which have high rates while the genotypes numbered 54, 14, 53 and 19 have low values (92.15% and 92.95%) [13]. According to the evaluation of the most important fatty acid compositions such as PUFA, oleic acid, MUFA, USFA/PUFA and oleic/linoleic acids of the almond genotypes, the highest levels were identified in the genotypes numbered 46, 49, 18, 16, 28 and 27. We can say that these genotypes are suitable for agriculture in terms of the quality of the fatty acid. SFA, which has less desirable content such as palmitic and stearic acids, was identified in the genotypes numbered 54, 14, 53 and 19 at high rates. In other words, the genotypes that have low rates of total USFA can be eliminated for breeding programs. Some important results have been obtained in terms of the fatty acid composition, and the rates and correlations of almond genotypes (Table 4).

Fattv	Palmi (16:0)	Palmitol (16:1)	Steari (18:0	Olei (18:	Linol (18:2)	Linole (18:3)	SFA	USF	USF SFA	MUF	PUF	MUF PUFA	Oleik/ Linole
Palmitic	1.000												
Palmitolei	0.362	1.000											
Stearic	0.022	-0.400	1.000										
Oleic	-0.579	-0.212	-	1.00									
Linoleic	0.481	0.191	-	-	1.000								
Linolenic	0.274	0.077	-	-	0.757	1.000							
SFA	0.759	0.009	0.668	-	0.347	0.143	1.00						
USFA	-0.759	-0.009	-	0.47	-	-0.143	-	1.00					
USFA/SFA	-0.725	-0.005	-	0.46	-	-0.158	-	0.98	1.00				
MUFA	-0.570	-0.177	-	0.99	-	-0.760	-	0.48	0.46	1.00			
PUFA	0.477	0.188	-	-	0.999	0.790	0.33	-	-	-	1.00		
MUFA/PU	-0.468	-0.152	0.046	0.95	-	-0.760	-	0.318	0.30	0.95	-	1.000	
Oleic/Lino	-0.472	-0.167	0.046	0.95	-	-0.737	-	0.321	0.31	0.95	-	0.998	1.000

Table 4. Significant positive and negative correlations between fatty acids and their proportions .(Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014)

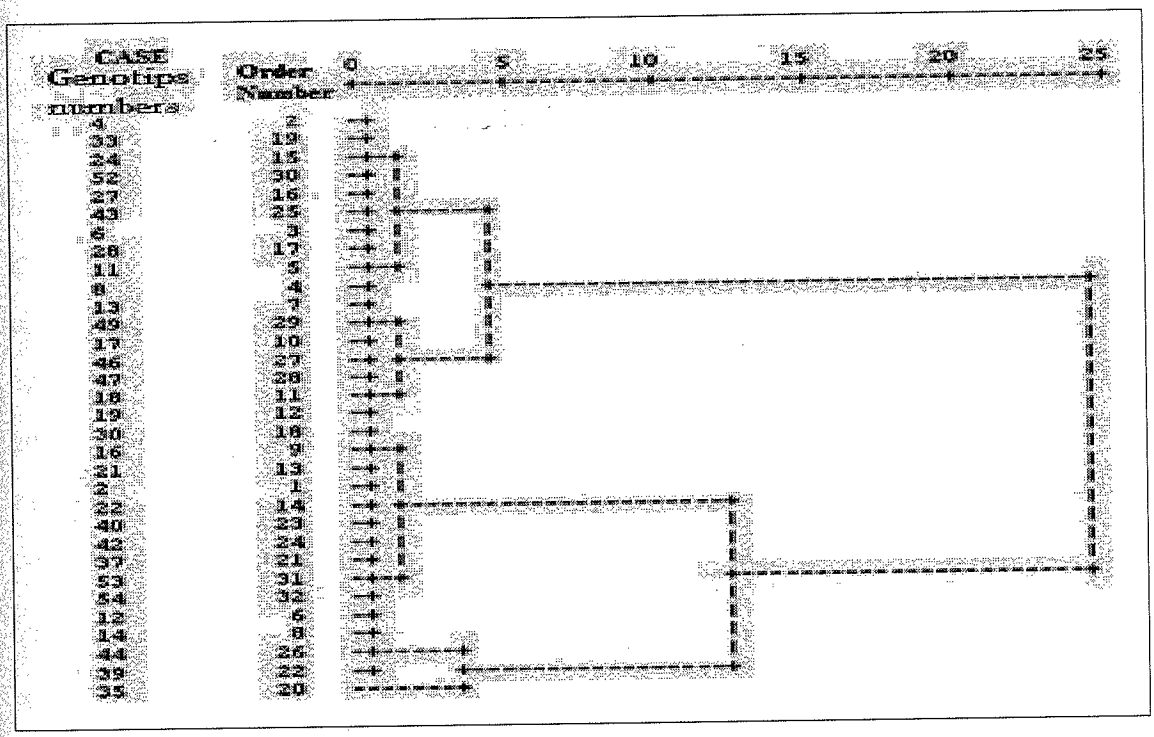

Figure 3. Dendrogram created according to the combination and rate of fatty acids and their smilarities of genotips (Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014)

The highest negative correlation (r=-0.988) was identified between oleic acid and linoleic acid according to the correlation analysis of fatty acid compositions of almond genotypes. stated that there is a negative correlation between oleic and linoleic acids. This result confirmed our findings. Also, a positive correlation (r=0.757) was found between linoleic and linolenic acid. Both fatty acids tend to increase or decrease. Likewise, a negative correlation (r=-0.758) was determined between oleic and linolenic acid as well. In the other words, when oleic acid increased, linoleic and linolenic acid decreased, and vice-versa. A negative

correlation coefficient (r=- 0.579) was found at a lower level between palmitic acid and oleic acid. In the same way, there were lower positive correlations between palmitic acid and linoleic acid (r=0.481) and between palmitic acid and linolenic acid (r=0.274) contrary to between palmitic acid and oleic acid. The corelations between stearic acid and the other fatty acids generally had very low values. However, we can say that there is a negative correlation between stearic acid and palmitoleic acid at a low-level (r=-0.400). Generally, except for this correlation coefficient, positive correlations (r=0.95 and above) were found with the oleic acid/linoleic acid ratio and oleic acid, MUFA, MUFA/ PUFA ratio. The existance of a high ratio of specific fatty acids has a significant effect on correlation coefficients [13] .

The presence of variations and relationships between different origins of almond were exhibited by the dendrogram created using the composition of fatty acids of the genotypes (Figure 3). According to this, two major groups of origins occurred. Also, one of groups was divided into two sub-groups. From these, while the genotypes numbered 44, 39, and 35 numbered created a distinct sub- group, the genotypes numbered 4, 33, 24, 52, 27 and the other genotypes that existed in other groups had high re- lationship affinites. In the dendrogram, the bitter almonds which were the genotypes numbered 37, 42 and 53 were in the same group and were seen to be close relatives. Some of the genotypes that were collected from similar geographic regions were involved in the same sub-groups as double or triple genotypes. Relationships of almond samples were partially identified in the cluster analysis. Furthermore, some almond genotypes having essential fatty acids and its ratios such as 46, 16 and 18 numbered were determined for using breeding programs. The dendrogram that consisted of fatty acids composition shows a lower

level relationship than the dendgrogram consisting of the sum of the band profiles of SDS-PAGE protein sub- fractions [13] .

The wider variations were identified in the almond genotypes distributed in Eastern and Southeastern Anatolia regions than the other research results surveyed in the different regions according to some USFA such as oleic and linoleic acids. Of them, the higher rate USFA that are useful for human health were identified than the other studies conducted outside our country. Because of these important quality features, the identified almond varieties can be evaluated in breeding programs. Furthermore, there are low levels SFA which reduce the oil quality such as palmitic acid and stearic acid in this study especially.

Almond genotypes having different fatty acid composition brings about alternatives to use them for different purposes. Fatty acids are particularly important in the cosmetic and pharmaceutical making industry. There have been many studies of the fatty acid composition of almonds.. In this study, in general, a large number of genotypes and more comprehensive fatty acid compositions of almond geno- types were studied. Our findings were found to be similar to and especially results. showed that genotypes' oleic/linoleic acid ratio is an important factor in determining the stability of almond oil. In addition, they indicated that this rate can be used as distinction of genotypes because this rate does not change over the years and linoleic acid is less stable and less saturated than oleic acid. In our study, the oleic acid ratio was found to be 64.05-80.68% and the linoleic acid ratio was found to be 12.65-27.00%, similar to results. Previous studies indicated that synthesis of fatty acids may vary according to genetic, ecological, morphological, physiological and cultural factors. But, recently studies .revealed that oil composition the mostly depend on the genetic factor. Oil content in almond kernel shows a high heritability value of 57% , confirming that the genetic factor is

the most determinant for oil content in almond kernels. This trait appears to be under polygenic control with a clear environmental effect indicated that the magnitude of the effect of the external factors such as the climatic condition of the year probably depends on the genetic background of each cultivar, explaining the significant effect of the interaction genotype X year. According to the information received from the studies in Europa , maximum USFA/SFA was seen as 14.17 and minimum as 10.24 in studies in certain cultivars selected from Italy, France and Spain. In this study, this ratio is quite high determined with an average of 15.40 and maximum and minimum value of 19.35 and 11.76 respectively. Therefore, it can be say that almond genotypes seeds collected Eastern and South eastern Anatolia have high USFA ratio have more quality features. High oleic and low linoleic acid leads to increase of the kernel oil stability and nutritional value. That is rich in terms of USFA; almond oil reduces the risk of heart diseases . In our study, high USFA values were obtained from the almond genotypes numbered 46, 16, 18, 28 and 49. In addition to these, MUFA consumption can be arranged according to low-density lipoprotein, cholesterol, and total cholesterol levels [14,26,31,32]. Among the works of many fatty acids nuts, the MUFA and PUFA content of almond seeds were found to be higher than in other popular foods such as walnuts, peanuts, pine nuts, Brazil nuts and olives . This is one of the important factors of almond seed. With regard to our study, the genotype numbered 49 can be recommended for its MUFA percentage (81.51%). High MUFA and PUFA ratio (MUFA/PUFA) is an important parameter for the stability of USFA . The average MUFA/PUFA ratio is found at the highest rate as 4.32 in our study found the oleic acid level to be between 69-78% and 72-80% in wild and cultivated almonds, respectively. Also, similarly, we found the oleic acid values to be between 64.05-80.68% and the average to be 74.46%. In our study, as mentioned above, the almond genotypes that were found to be at higher rates in terms of USFA may be

subject to breeding of almond seeds for human nutrition. Locally, in many regions of Turkey, almonds pomologic characters, especially the hull percentage (hull wt/fruit wt x 100), kernel percentage (kernel wt/nut wt x 100), kernel length, widh, thickness, dual rate, protein, ash and total fat content were studied. Of these, one of the breeding by through the selection study carried out on the naturally grown almond tree in the district of Kemaliye –Er- zincan. In this study, only mentioned of the total amount of fat content were given in almond fruits [36]. However, amount of fatty acids and its compositions in the oil have been emphasized in the world generally. In this composition, desired substanstially USFA such as oleic, linoleik, linolenic, omega-3 and omega-6 have become important. Because of its importance, proportions of fatty acids were focused on in almond genotypes oil instead of the total amount of fat content. In this way, the almond genotypes that have rich for most important fatty acids can be selected and used for breeding programs in Turkey. Thus, it will be opened pat way of high quality almond production extended to the world countries [13] .

The USFA/SFA of almond genotypes collected from Eastern and Southeastern Anatolia were detected higher than the almond varieties values detected by Sabudak in Çorlu region . Likewise, in this study, general average of oleic acid and linolenik acid value that are desirable for high quality fat were found as 74.46% and 0.75%. These proportions are higher than Çorlu region . almond varieties values that are 68.63% and 0.06% respectively. High percentage of SFA in the oil is undesirable for quality oil. In addition, low amounts of SFA (palmitic and stearic acid) were found in East and Southeastern regions than Çorlu. This situation shows that the almond genotypes collected our working areas have more quality fatty acids contain [13] .

2.3. α-Tocopherol Extraction

Samples for T extraction were prepared by dissolving 0.1 g of oil in 1.9 mL acetone (about 2 ml of total sample), shaking and filtering through a 0.22 μm syringe cellulose filter. T determination was performed using a Waters HPLC, equipped with a Waters 600 Controller pump unit and a 717 plus auto sampler. The chromatography column, kept at 25°C, was a Spherisorb ODS2 250 x 4.6 x 5 μm, with a Phenomenex pre-column (cartridge C18 AJO-4287). The mobile phase was a 1:1 mixture of acetonitrile and methanol, at a flow rate of 1 mL/min. Detection of T was carried out using a Waters 996 photodiode array detector (PDA) at 295 nm wave length, within a run time of 18 min. Results were recorded and processed by Enpower 2 Work Station.

T concentration was quantified in mg/l basing on a calibration curve with α-tocopherol (from Sigma-Aldrich) as external standard. From the oil weight in the 2 ml sample (see above), T value was then expressed as mg/kg oil [14] .

2.4. Other Compounds in Almond Oil

Besides fatty oil ,almonds also contain a variety of phenolic compounds which are localized principally in their skin, including flavonols (isorhamnetin, kaempferol, quercetin, catechin and epicatechin), flavanones (naringenin), anthocyanins (cyanidins and delohinidin), procyanidins, and phenolic acids (caffeic acid, ferulic acid, P-coumaric acid and Vanillic acid) [15] .

The active constituents of almonds are globulins such as amandine and albumin and amino acids such as arginine, histidine, lysine, phenylalanine, leucine, valine, tryptophan, methionine and cystine. Almonds contain proteins and certain minerals such as calcium and magnesium. They are also rich in dietary fiber, B vitamins, essential minerals and mono unsaturated fat. Almonds also contain phytosterols

which are associated with cholesterol-lowering properties. The phytosterol content of almonds is 187 mg/100mg [15] .

An example of nutrition analysis of an almond example is given below [14]

Almond Nut, Raw [based on 100g]

Energy	2420kJ	Thiamin (B1)	0.24mg (0.18%)
Carbohydrates	20g	Riboflavin (B2)	0.8mg (53%)
-Sugars	5g	Niacin (B3)	4mg (27%)
-Dietary Fibre	12g	Pantothenic	0.3mg (6%)
Fat	51g	Vitamin B6	0.13mg (10%)
-Saturated	4g	Folate (B9)	29µg (7%)
-Monounsaturated	32g	Vitamin C	trace
-Polyunsaturated	12g	Calcium	248mg (25%)
Protein	22g	Iron	4mg (32%)
b		Magnesium	275mg (74%)
		Phosphorus	474mg (68%)
		Potassium	728mg(15%)
			3mg (30%)

Table 5. showing the nutritional constituents together with the recommended nutritional intake of vitamins and micronutrients (Cah iers Option s Méditerran éen n es; n . 56)

3.Pharmacological Activities of Almonds

The edible portion of the *Prunus amygdalus* is it nuts, which are commonly known as "almond" or "badam", and it is a popular, nutritious food .The almond, which is known as the king of nuts, is a highly nutritious food. Almonds are rich in healthy fats, proteins, minerals and vitamins. In addition to its nutritional values, it has some medicinal values that may be helpful for treating certain diseases and health problems. The almond is an effective health building food, both for the body and the mind; it is also a valuable food remedy for several common ailments. The nuts of *Prunus amygdalus* are found to possess various pharmacological properties, such as anti-stress , anti-oxidant , immunostimulant , lipid lowering , and laxative . The almond is highly beneficial in preserving the vitality of the brain, strengthening the muscles and prolonging life. Almonds are a useful food remedy for anaemia, as they contain copper, iron and vitamins.

3.1. The Cholesterol Lowering Activity

CE Berryman et al have found that almonds have a consistent LDL- cholesterol lowering effect in healthy individuals and in individuals with high cholesterol and diabetes, in the controlled and free – living settings. Almonds are low in saturated fatty acids and rich in unsaturated fatty acids and contain fiber, phytosterols, plant protein, α-tocopherol, arginine, magnesium, copper, manganese, calcium and potassium. The mechanism which is responsible for the LDL-cholesterol reduction which is observed with almond consumption is likely to be associated with the nutrients which are provided by the almonds, i.e., decreased absorption of cholesterol and bile acid, increased bile acid and cholesterol excretion and an

increased LDL-cholesterol receptor activity. The nutrients which are present in almonds regulate the enzymes which are involved in cholesterol synthesis and bile acid production [16] .

David J.A. et al shown that almonds reduced the biomarkers of lipid per oxidation in hyper lipidaemic patients [17] .

The dose response effects of whole almonds which are considered as snacks, were compared with low saturated fat (<5% energy), whole –wheat muffins (control) in the therapeutic diets of hyperlipidaemic sub- jects. In a randomized cross over study, 27 hyperlipidaemic men and women consumed 3 isoenergetic (mean 423 kcal/d or 1770 kj/d) supplements, each for 1 month. The supplements consisted of full-dose almonds (73 ± 3g/d), half-dose almonds plus half- dose muffins (half dose almonds), and full dose muffins (control). The subjects were assessed at weeks 0, 2 and 4. Their mean body weights differed (≤ 300g) between the treatments, although the weight loss on the half-dose almond treatment was greater than the weight loss on the control ($P<0.01$). At 4 weeks, the full-dose almonds reduced the serum concentrations of malondialdehyde (MDA) ($P= 0.040$) and the creatinine-adjusted urinary isoprostane out put ($P=0.026$), as compared to the control. The serum concentrations of ∞- or α- tocopherol, which were adjusted or unadjusted for total cholesterol, were not affected by the treatments. The anti-oxidant activity of almonds was demonstrated by their effect on 2 biomarkers of lipid peroxidation, serum MDA and urinary isoprostances, and this finding supported the previous finding that almonds reduced the oxidation of LDL-C. Their anti-oxidant activity provides an additional possible mechanism, in addition to lowering cholesterol, that may account for the reduction in CHD risk with nut consumption [18] .

Olivia J. et al, in their study, found that almond consumption was associated with improvements in the serum lipid profiles . They reported that the influence of almonds on the lipid parameters could help in defining the role of almonds as lipid modulators. Manual controlled trails (totaling 142 participants) met all the inclusion criteria. Upon meta-analysis, almond consumption, which ranged from 25 to 168g/day was found to significantly lower cholesterol (weighted mean difference-6.95 mg/dL (95% confidence interval [CI]-13.12 to -0.772) (0.18 m mol/L [95% Cl-0.34 to -0.02)] and this showed a strong trend towards reducing LDL cholesterol [weighted mean difference -5.79 mg/dL (95% CI-11.2 to0.00])] (0.15 m mol/L [95% CI-0.29 to 0.00])]. No significant effect on HDL cholesterol, triglycerides or the LDL: HDL ratio was found. No statistical heterogenicity was observed for any analysis [18] .

3.2. Hypoglycaemic Activity

David J.A. Jenkins et al showed that almonds lowered post-prandial glycaemia, insulinaemia and oxidative stress. The nut consumption in the Seventh Day Adventists study, the nurses health study, the physicians health study, the health professionals study and the Iowa women's health study were all associated with the same actions which are mentioned above. Almonds decrease post-prandial glycaemia and oxidative damage in healthy individuals [19] .

Fifteen healthy individuals, 7 men and 8 women, with an age of 26.3 ± 8.6 years were studied. All the subjects completed 5 study sessions, each lasting 4hours, with a minimum 1 week washout between the tests. The subjects consumed the control meal on 2 occasions, and the almond, parboiled rice, and mashed potato meals only once. The blood glucose concentration over the 4 hour testing for each meal

revealed that the almond (55±7) and rice meals (38± 6) showed lower values than that of the instant mashed potato meal (94± 11) ($p \leq 0.003$). The almond and rice meal glycaemic index values did not differ ($P = 0.25$). Similarly, the post-prandial glucose peak heights for the almond (5.9 ± 0.2 m mol /L) and rice (5.8 ± 0.1 m mol/L) meals were lower than the peak heights for the potato meal (6.6 ± 0.2 m mol/L) and the control white bread (6.9 ± 0.2 mmol/L) ($P < 0.001$). Shah KH, et al have shown in their study, that the ethanolic extract (250 and 500mg/kg) of the leaves, flowers and seeds of almonds was taken up to evaluate its anti diabetic activity against normal and streptozotocin induced diabetic mice. The oral administration of the extract for 21 days resulted in a significant reduction in the blood glucose levels. At the end of the experiment (15th day), the blood glucose levels were 80.6 ± 1.8 and 77.6 ± 1.4 mg/dl in the diabetic mice which were treated with 250 and 500 mg/kg b. w. of the leaf extract respectively. The flower and seed extracts, at a dose of 500mg/kg b. w., also showed significant reduction ($P < 0.001$) in the blood glucose levels of the diabetic mice on the 15th day of the study [20] .

3.3. Immunostimulant Activity

Adriana Arena, et al, evaluated in their study, that with almonds, high levels of cytokine production were observed i.e., interferon-α (INF-α), interleukins (IL-12), INF-gamma and tumour necrosis factor (TNF-α). Their data suggested that almonds improved the immune surveillance of the peripheral blood mono nuclear cells towards viral infections. Almonds also were found to induce a significant decrease in the *Herpes simplex* virus (HSV-2) replication [21] .

3.4, Effect on Amnesia

Kulkarni, et al, in their study, suggests that almonds possess a memory enhancing activity in view of its facilitatory effect on the retention of special memory in scopolamine induced amnesia. They concluded that almonds lowered the serum cholesterol in rats. They were also found to elevate the Ach level in the brain and ultimately improve the memory (special and avoidance) of rats. In the light of the above findings, it may be worthwhile to explore the potential of this plant in the management of cognitive dysfunction . The paste of the PA nuts was administered orally at three doses (150, 300, and 600 mg/kg) for 7 and 14 consecutive days to the respective groups of rats. Piracetam (200mg/kg) was used as a standard nootropic agent. The learning and memory parameters were evaluated by using an elevated plus maze (EPM), passive avoidance and motor activity paradigms. The brain Ch E activity and the serum biochemical parameters like total cholesterol, total triglycerides and glucose were evaluated. It was observed that PA, at the above-mentioned doses, after 7 and 14 days of administration in the respective groups, significantly reversed scopolamine (1 mg/ kg i. p.)- induced amnesia, as was evidenced by a decrease in the transfer latency in the EPM task and in the step-down latency in the passive avoidance task. PA reduced the brain Ch E activity in rats. PA also exhibited a remarkable cholesterol and triglyceride lowering property and slight increase in the glucose levels in the present study. Kulkarni concluded that because the diminished cholinergic transmission and an increase in the cholesterol levels appeared to be responsible for the development of the amyloid plaques and the dementia in Alzheimer's patients, PA could be a useful memory- restorative agent. It would be worthwhile to explore the potential of this plant in the management of Alzheimer's disease [22] .

3.5. Pre-Biotic Potential

G. Mandalari et al demonstrated the prebiotic activity of almond seeds. Pre-biotics are non-digestible-food ingredients that stimulate the growth and activity of bacteria in the digestive system, in ways which are claimed to be beneficial to health. Typically, pre-biotics are carbohydrates (such as oligosaccharides). The most prevalent forms of pre biotics are nutritionally classified as soluble fibers. To some extent, many forms of dietary fibers exhibit some level of pre-biotic effects It has been shown that almonds altered the composition of gut bacteria by stimulating the growth of bifid bacteria and *Eubacterium rectale* [23] .

3.6. Anti-oxidant Activity

Ali Jahanban Isfahan, et al demonstrated that the ethanoic extracts of almonds possessed anti-oxidant and anti-radical activities and that their phenolic extract may be helpful in preventing or slowing the processes of various oxidative stress related diseases. On the basis of the comparison between the anti-oxidant and the anti-radical activity of wild almond hull and shell phenolic extracts, 4 almond species were selected. The fruits of these almonds were collected, their hulls and shells were dried and ground, and metabolic extracts were prepared from these hulls and shells. The total phenolic content was determined by using the Folin-Ciocalteu (F-C) method. The reducing power and the scavenging capacity of the extracts for radical nitrite, hydrogen peroxide, and superoxide were evaluated. The hull and shell extracts, respectively, had a range of 122.2 ± 3.11-75.9 ± 1.13, 46.6 ± 0.94-18.1 ± 0.15 mg/g gallic acid equivalents/g extract in total phenolic content, 0.667-0.343, 0.267-0.114 AU at 700 nm in reducing power, 94.9 ± 0.97 %-63.7 ± 1.14 %, 65.7 ± 0.64 %-24.2 ± 1.31% in hydrogen peroxide, 90.6 ± 1.11% -60.7 ±

2.13 %, 56.7 ± 1.33%-28.5 ± 1.65% in superoxide, and 85.2 ± 1.21%-53.4 ± 0.86 %-24.9 ± 1.63% in the nitrite radical scavenging percentage. The results showed that the anti-oxidant and the anti-radical activities of the almond hull were higher than those of its shell phenolic extract among correlated with the phenolic content and radical scavenging capacities of wild almond hull and shell extracts in different species were positively correlated with phenolic content and reducing power [24] .

3.7.Aphrodisiac Activity

Gopu Madhavan , et al, in their study with a polyherbal formulation (Tentex Royal) which contained *Prunus amygdalus* along with other herbal preparations, showed a significant improvement in all the parameters of the sexual indices. To assess the efficacy of Tentex royal, a polyherbal formulation, in enhancing the male sexual activity in an experimental model, the study involved virgin female rats which were in the oestrous state, which was induced by administering oestrogen, and male rats which were randomized into five groups and were classified into the control group, the sildenafil citrate reference standard group and the Tentex royal-treated group (125, 250 and 500 mg/kg) respectively, for 5 days. Parameters such as total sexual behaviour, mounting frequency, ejaculation frequency, ejaculation latency, serum testosterone levels and sperm count were carefully monitored. A significant improvement in all the parameters of the sexual indices was observed in the Tentex royal group. The treatment with Tentex royal also showed an increase in the sperm count and the testosterone levels. Histological evalu- ation of the anterior pituitary revealed an increase in the FSH-LH- producing basophils and a decrease in the ACTH producing cells. The study revealed that Tentex royal improved the erectile capacity. Considering the

limitations of sildenafil citrate in clinical practice, Tentex royal may be considered a safe and alternative treatment for the correction of erectile dysfunction [25] .

3.8. Hepatoprotective Activity

3.8.1. Effect of almond oil and CCl4 on hepatic antioxidant enzyme

The activities of liver SOD, CAT, and GPX in the CCl4-treated group were significantly decreased compared with the normal control group. There was a dramatic increase ($p < 0.05$) in SOD, CAT and GPX activity in the almond oil treated groups at the high dose (21 ml/kg b.w) compared to the CCl4-treated group. Moreover, the activity of almond oil at the dose of 21 ml/kg was comparable to that of the reference drug, bifendate [26] .

3.8.2. Effects of almond oil and CCl4 on lipid peroxidation level

The treatment with CCl4 had obviously higher MDA levels than the normal control group ($p < 0.01$). MDA levels in the almond oil-treated group at 14 and 21 ml/kg were remarkably lower than CCl4-treated group ($p < 0.01$). There was no significance difference in MDA levels in rats treated with bifendate and those treated with almond oil at 14 ml/kg.[26].

3.8.3. Effect of almond oil and CCl4 on histopathological examination

The histopathological changes were evaluated by hematoxylin and eosin stain in rat liver (Fig. 5A). Classical damage was caused by CCl4 administration in rat liver at 24 h, with severe hepatocyte necrosis, contraction of nucleus, cell debris in the cytoplasm, cell swelling, chromatin digestion, and disruption of the plasma membrane and organelle membranes (Fig. 5A, a). Treatment with almond oil

ameliorated the CCl4-induced liver injury and the typical histological changes were markedly alleviated in the liver sections (Fig. 5A, b). [26] .

3.8.4. Effect of almond oil and CCl4 on hepatocyte apoptosis

The number of apoptotic hepatocytes stained using the methyl green-pyronine method was significantly greater in the liver of CCl4-treated rats than in the normal control (Fig. 5B, a). Treatment with almond oil also dramatically reduced the number of apoptotic cells in the liver as compared with the CCl4-treated group (Fig. 5B, b). The number of living hepatocytes (Fig. 6) in the CCl4-treated group was significantly lower than that of the normal controls ($p < 0.01$). All the tested doses of almond oil resulted in a significant increase ($p < 0.05$) in the number of living hepatocytes as compared to the CCl4-treated group. Similar observations were also found in animals treated with bifendate. There was no significance difference between the number of living hepatocytes in rats treated with bifendate and those treated with almond oil at a dose of 21 ml/kg b.w. [26] .

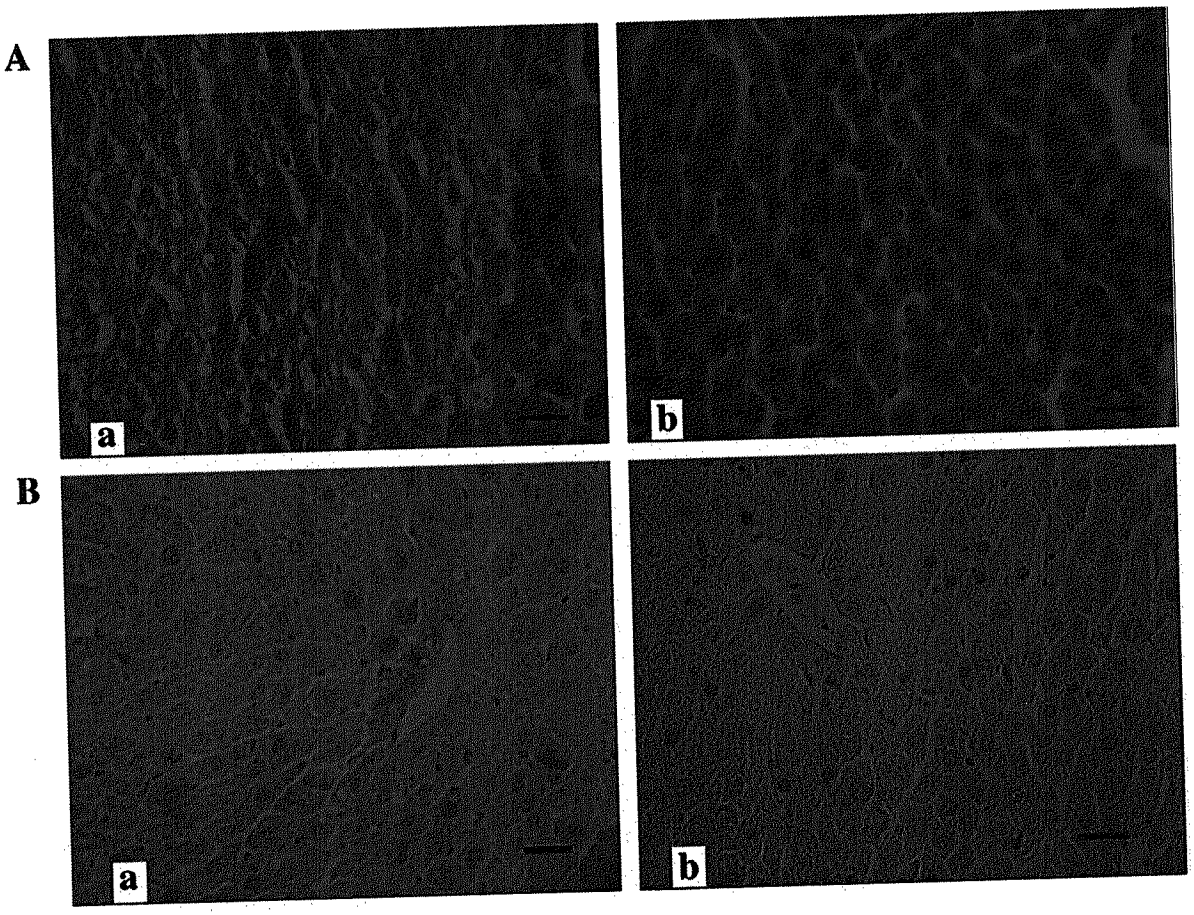

Fig. 5. Effect of almond oil on liver histopathological change and hepatocyte apoptosis in CCl4-treated rats. (A) Histopathological change stained with hematoxylin-eosin; (B) hepatocyte apoptosis evaluated with methyl green-pyronine methods. (a) Treated with CCl4; (b) pretreated with almond oil (21 ml/kg) before CCl4 treatment. Original magnifications, scale bar = 40 lm. (2010 Elsevier Ltd.)

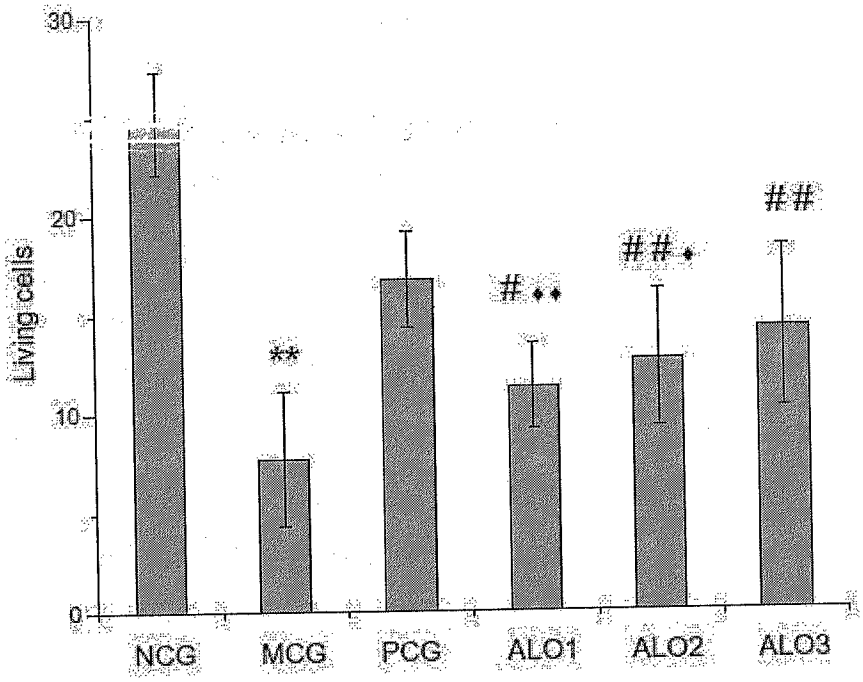

Fig. 6 . Effect of almond oil on the living cell number in CCl4-treated rats. Results are presented as the mean ± SD (n = 8). ALO1, ALO2 and ALO3, the groups administrated with almond oil at the dose of 7, 14 and 21 ml/kg, respectively; **p < 0.01, significant differences from the normal control group (NCG); #p < 0.05 and ##p < 0.01, significant differences from the CCl4 group (MCG); ♦p < 0.05 and ♦♦p < 0.01, significant differences from the bifendate + CCl4 group (PCG). (2010 Elsevier Ltd.)

The pre-treatment with almond oil was effective in the prevention of CCl4-induced hepatic damage in rats. Our thesis show that the hepatoprotective effects of almond oil may be due to several constituents with potential healthy biological properties, such as unsaturated fatty acids, tocopherols and phenolic compounds. The mechanisms o protection include the inhibition of lipid peroxidation processes and an increase in antioxidant enzyme activity, all of which resulted in the recovery of biological parameters and integrity of the tissues. The inhibitory

effects of dietary almond oil may be useful as a hepatoprotective agent against chemical-induced hepatotoxicity in vivo [26] .

3.9. Anticancer activity (column cancer)

The current study findings provide evidence that almond consumption may provide a measure of protection from the risk of colon cancer. Earlier epidemiological studies have indicated that higher nut consumption correlates with declines in heart disease morbidity and mortality and now the current study suggests that almonds have a potentially protective effect in colon cancer. These findings that almonds, a high fat food, might provide a measure of protection from the risk of chronic diseases suggests a need to reassess the current view that intake of high fat content foods invariably has deleterious health effects. In fact, have suggested that the statistical analyses that have shown a positive relationship between fat intake and cancer may actually show this association not due to increases in fat intake per se but rather as a result of the decline of fruit and vegetable intake in the face of fat intake increases. Almonds and other nuts represent a unique type of foodstuff whose consumption results in a higher fat intake more commonly associated with the meat-rich Western diet while providing a mix of other nutrients and on-nutrients associated with plant-derived foods. This unique combination may resolve the apparent contradiction between studies indicating that high fat consumption is unhealthy and those showing that elevated nut consumption, a high fat food, has protective, healthy effects. The results of the current study suggest that almonds might be an effective chemopreventive agent against colon carcinogenesis. Thus, further studies of the chemopreventive efficacy of almonds against colon cancer in other long-term in vivo models appear warranted.

4.Conclusion

In this thesis I describe some rule information about almond oil and I concluded The methods to obtaining of almond oil , there are Two main methods to obtain almond oil , by cold press and Supercritical CO2. Almond (*Prunus amygdalus*) seeds yield an important medicinal oil(Almond oil). The variations were found between almond genotypes collected from different locations in the Mediterranean regions in terms of fatty acids compositions. As a result, almond genotypes having essential properties such as high oleic acid and low linoleic acid or high value USFA were determined. It can contribute to the better quality almond production, cultivation and promotion by revealed to this study and new genotypes using similar studies will be carried out. Obtaining methods should be free from thermal and chemical processes. The best processes is Supercritical CO extraction or cold press are offered as the best methods. Analysis of almond oil are realized by using GC-MS.

The almond oil has The Cholesterol Lowering Activity , Hypoglycemic Activity , Immunostimulant Activity , Effect on amnesia , Pre-Biotic Potential , Anti-oxidant Activity , Aphrodisiac Action , Hepatoprotective activity and Anticancer activity (column cancer)

Almond oil is a unique substance with multifaceted properties, which if studied and harnessed appropriately, could offer better results postoperatively by minimising scars and contractures together with improving skin tone and complexion. Further study is suggested to explore its uses from this standpoint.

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