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Comparison of Chemical Composition of Pomegranate *(Punica Granatum L.)* and Grape *(Vitis Vinifera L.)* Seed Oils Obtained By Extraction

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Abstract

In this study, it was aimed to assess pomegranate (*punica granatum L.*) and grape (*vitis vinifera L.*) seed oil by comparing them physically and chemically. The fat output of the pomegranate (*punica granatum L.*) and grape (*vitis vinifera L.*) seeds was determined by the standard method of soxhlet extraction. Fatty acid composition was determined using refractive index, saponification number, iodine number, peroxide number and chromatographic method.

Mean of refraction index, saponification number, iodine number and peroxide number for pomegranate (*punica granatum L.*) seed were found to be 1.499; 172.2(mgKOH/g); 134.5(wijs) and 21.1(meqaktif O2/kg), respectively. Mean of refraction index, saponification number, iodine number and peroxide number for grape (*vitis vinifera L.*) seed oil were 1.462; 178.23(mgKOH/g); 117.2(wijs) and 23.8(meqaktif O2/kg), respectively. Fatty acid composition of oils was determined by gas chromatography. Gas chromographic analyses of the pomegranate (*punica granatum L.*) seed samples showed that the fatty acids compositions were consisted of 30.12% punicic asit(C18:3), 28.92 % linoleic acid(C18:2); 19.01 % oleic acid(C18:1); 10.12 % palmitic acid (C16:0); 3.92 % beheric acid (C20:0). The grape (*vitis vinifera L.*) seed samples showed that the mean of fatty acid composition were consisted of 66.88 % linoleic acid(C18:2); 27.53 % oleic acid (C18:1); 3.77 % stearic acid (C18:0); 0.31 % linolenic acid (C18:3). Pomegranate (*punica granatum L.*) and grape (*vitis vinifera L.*) seed oil are required to be studied for health and nutritional assessment opportunities. Thus, an important food product for human health will be obtained.

Keywords: Pomegranate (Punica granatum L.) Seed; Grape (Vitis vinifera L.) Seed; Oil; Fatty Acid

Introduction

The use of natural components for reducing cardiovascular diseases, cerebrovascular diseases and cancer mortality has gained considerable attention. Lipid components contribute large amounts to the nutritional and sensory value of almost all kinds of foods. Nature provides a large number of fats that differ in their chemical and functional properties. Four classes of lipids are habitually found in vegetable oils: triacylglycerols, diacylglycerols, polar lipids, and free fatty acids. The fatty acid composition determines the physical properties, stability, and nutritional value of lipids [1]. Most naturally occurring storage lipids are triacylglycerols. Triacylglycerols are natural compounds consisting of saturated and unsaturated fatty acids differing in their acyl chain lengths and the number and positions of double bonds: saturated, monoenoic, and polyunsaturated fatty acids that differ in detailed fatty acid composition.

Monoenoic fatty acids and polyunsaturated fatty acids are structurally distinguished by the presence of repeating methylene units. These units produce an extremely flexible chain rapidly reorienting through conformational states and constitute an influential group of molecules that promote health [2].

Pomegranate, an important member of the Punicaceae family (*Punica granatum L.*), is a fruit with mystical characteristics known since ancient times. Pomegranate was used for healing different diseases in ancient times. The therapeutic properties of pomegranate fruit, its peel, roots, and leaves have been reported in various studies [3]. Pomegranates are found throughout the Mediterranean Basin and eastward to India and China. Pomegranate is now cultivated throughout the tropics and subtropics. The edible oil found in pomegranates is quite rare. It is considered a powerful health-benefiting agent, due to its antioxidative, anticarcinogenic, and antilipidemic properties. Pomegranate seed lipid contents are 20%, with a punicic acid comprising the majority of fatty acids [4]. Elfalleh *et al.* (2011) reported the presence of linolenic (C18:3) acid as the major fatty acid in pomegranate leaves [5]. Pomegranate has been reported as a useful remedy to calm all forms of stomach trouble. Pomegranate has been also considered a natural source

of phenolic compounds, sugar, protein, and minerals. Pomegranate is quite rich in phenolic compounds, flavonoids, anthocyanins, catechins and other complex flavonoids (anthocyanins, catechins, and other complex flavonoids), tannins (punicalagin, punicic acid, punicalagin, gallic acid, ellagic acid), and other complex flavonoids. It has been reported that condensed tannins and proanthocyanidins two of the polyphenols contained in pomegranate seeds, pulp or peels reduce the absorption of intestinal cholesterol by increasing cholesterol transport and bile acid excretion. Pomegranate seed oil has been reported to play a role in delaying cancer spreading to other parts of the body by slowing down the formation of new blood vessels (angiogenesis) during the metastatic process. Pomegranate juice and seeds have been shown to induce programmed cell death (apoptosis) selectively in different cancer cells that are not hormone-dependent; and in prostate cancer cases, they have been shown to accelerate (pro-apoptotic) programmed cell deaths [6].

Grape (*Vitis vinifera L.*) is a temperate-zone plant and grows in the northern and southern latitudes between 30o and 40o [7]. Grape seed oil has a broad range of application, being used in various fields from cosmetics to cooking. Grape seed oil is gaining in popularity as culinary oil and has been studied as a possible source of specialty lipids. It is a rich source of linoleic acid, which is associated with the promotion of cardiovascular health by down-regulating low-density lipoprotein cholesterol. Grape seed oils have emerged as a product with the potential to be used in food and pharmaceutical applications. The benefits of grapes are associated with polyunsaturated fatty acids (PUFA) present mostly in seeds. Polyunsaturated acids such as linoleic and linolenic acids are essential for human metabolism due to the lack of enzymes responsible for their biosynthesis. PUFA are considered desirable compounds in the human diet because of their effectiveness in reducing the incidence of cardiovascular disease and cancer [8]. According to Assy *et al.* (2009), the ingestion of oleic acid is related to the reduction in the level of low-density lipoproteins and consequently, the prevention of arteriosclerosis. Due to dietetic habits, increased consumption of n-3 acids has been recommended in the diet [9]. Grape seed oil consists mainly of triglycerides, which are rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), compared with other oil-rich seeds. The oil contents were found to be different for each variety. Saturated fatty acids contents were lower than the values of MUFA and PUFA in all genotypes. Among the identified fatty acids, linoleic acid (C18:2) was the predominant fatty acid, followed by oleic acid (C18:1) and palmitic acid (C16:0) in all varieties.

The aim of the present study is to investigate and to compare chemical properties and fatty acid composition between pomegranate (*punica granatum*) and grape seed (*vitis vinifera L.*) oils. This study is also designed to achieve a comprehensive and detailed profile of the different components of pomegranate and grape seed oils, which may be of both industrial and nutritional interest.

Materials and Methods

Pomegranates and grapes were passed through a steel sieve, and after the residues on the seeds were removed the seeds were washed with distilled water. After the water residues on the seeds were removed with blotting paper they were ground down then dried in a drying oven at 60 °C. Then they were taken for oil analysis. Pomegranate and grape seeds were obtained after the fruits had been crushed, their stalks removed, and finally pressed. Oil was obtained via Soxhlet extraction, the standard method. Hexane was used as a solvent in the extraction process, and the extraction was performed for 10 hours. Oil samples obtained were stored at +4 °C [10].

Determination of the Properties of Oil Obtained from the Seeds

Physical Properties

Refractive index -- refractometer is sensitive to 20 ± 2 or 40±2°C, and can be calibrated using sodium D-light. The area between the two prisms was completely filled with the sample and the reading was performed at a temperature of 20°C [11].

Chemical Properties

Saponification number is the amount (in mg) of potassium hydroxide (KOH) that is required to saponify glycerides with free acids found in 1 g of oil [12]. Exactly 2 g of the sample was weighed. 25 mL of potassium hydroxide solution was added to the sample; then it was connected to the condenser. After boiling for 60 minutes, the phenolphthalein solution was added and the hot soap solution was titrated with a hydrochloric acid solution. A blank determination was also performed using ethanolic potassium hydroxide solution. Saponification number = V2 – V1/m x 28.05. Here: V1 is the 0.5 N hydrochloric acid solution spent for the experiment with the sample. V2 is the 0.5 N hydrochloric acid solution used for the witness test. m is sample weight, g. The determination of iodine index is the measure of unsaturation and is the mass of iodine in grams that is consumed by 100g of fat. A specified amount of sample was weighed. For the oil to dissolve 15 mL of carbon tetrachloride and 25 mL of Wijs reagent were added and the solution was left for one hour. At the end of this period, 20 mL of potassium iodide solution and 150 mL of water were added. With 0.1 N sodium thiosulfate solutions, it was titrated against the starch solution. At the same time, a blank determination was performed. Calculation of Iodine Value= V2 - V1/m x 1.269. Here; V2 is the 0.1 N sodium thiosulfate solution spent for the blank determination. V1 is the 0.1 N sodium thiosulfate solution spent on the experiment with the sample. m is sample weight, g [12]. Peroxide value is the measure of the amount of active oxygen in the oil and is the amount of peroxide oxygen

found in 1 kg of oil in milliequivalents. A specified amount of sample was weighed. After the addition of 10 mL of chloroform, 15 mL of acetic acid and 1 mL of potassium iodide solution were added successively. After adding 75 mL of water, the liberated iodine was titrated with 0.01 N sodium thiosulfate solutions against the starch solution. Peroxide Value (is found as) = V/m x 2 milliequivalent peroxide per kg of oil. V: 0.01 N sodium thiosulfate solution spent in titration, mL. m is the weight of the sample, g.

Determination of fatty acid composition by gas chromatography: Analysis of fatty acid methyl esters was carried out using DB-23 (Agilent) capillary column with flame ionization detector (FID) "Shimadzu 14B" brand gas chromatograph. The length of the column is 60 m and the inner diameter is 0.25 mm. The injection port temperature is 270 °C, the detector temperature is 280 °C and the split ratio is 1:50. After waiting for 1 minute at 130 °C, the column temperature was increased to 170 °C with a rise of 6.5 °C per minute and then to 215 °C with a rise of 2.75 °C per minute, and it was kept at this temperature for 12 minutes, then the temperature was increased to 230 °C by 40 °C per minute. And it was programmed to stay for 3 minutes at this temperature [13]. Helium was used as a carrier gas. The amount of sample injected into the device is 1 µl. Peak identification was performed with the help of standard compounds. The amount of fatty acids was calculated as % [13].

Results and Discussion

Refractive index, saponification number, iodine index, and peroxide value of pomegranate and grape seed oils obtained by extraction are given in (Table 1).

Sample	Refractive index (nD) (20 °C)	Saponification number (mgKOH/g)	Iodine index (Wijs)	Peroxide value (meqaktif O2/kg)
Pomegranate *(Punica granatum L.)* seed	1.499±0.03	172.2±0.02	134.5±0.05	21.1.±0.05
Grape (*vitis vinifera L.*) seed	1.462±0.04	178.23±0.03	117.2±0.04	23.8.±0.04

Table 1: Refractive index, saponification number, iodine index, and peroxide value of pomegranate and grape seed oils

Pomegranate seed oil index was found to be 1.499 while grape seed oil was found to be 1.462. In another study, the pomegranate seed oil refractive index was found to be 1.522. Saponification number (mgKOH/g), iodine value, and peroxides value (meq active O2/kg) determined in pomegranate seed oil were 172.2, 134.5, and 21.1, respectively, and in grape seed oil as 178.23, 117.2, and 23.8, respectively. According to the iodine value, seed oils can be said to be classified as semi-drying oils like cotton (108), rapeseed (100), corn (118), and sunflower (131) oils. These properties are also important for learning about the whole fatty acids composition of oils. If there are more molecules in a certain amount of fat, most of the fatty acids are short carbon chain fatty acids. In this case, the saponification number will be high. If the oil is mixed with iodine solution, the iodine value of the oil in which the double-bonded fatty acids are present will be high, since iodine will come to each double bond. The fatty acid composition of pomegranate seed oil and grape seed oil is given in (Table 2,3).

Fatty acid composition (m/m % methyl esters)		
Arachidonic acid	(C20:0)	1.56
Behenic acid	(C22:0)	3.92
Lignoseric acid	(C24:0)	1.58
Palmitic acid	(C16:0)	10.12
Stearic acid	(C18:0)	2.88
ΣSFA		20.06
cis-11-Ekosenoic(gadoleic) acid	(C20:1) w9	1.49
Erusic acid	(C22:1) w9	0.18
Margaric (heptadecanoic acid)	(C17:1) w8	0.06
Oleic acid	(C18:1) w9	19.01
Palmitoleic acid	(C16:1) w7	0.07
ΣMUFA		20.81
Linoleic acid	(C18:2) w6	28.92
Linolenic acid	(C18:3) w3	0.18
Punicic acid	(C18:3) w3	30.12
ΣPUFA		59.22

Table 2: Fatty acid composition of pomegranate seed oil

Lauric acid (12:0), mystic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), and beheric acid (C22:0) are the most important saturated fatty acids found in vegetable oils. Saturated fatty acids are synthesized in the human body [14]. In our study, the amount of palmitic acid (C16:0) is 10.12%, beheric acid (C22:0) is 3.92%, stearic acid (C18:0) is 2.88%, lignoceric acid (C24:0) is 1.58%, and arachidic acid (C20:0) in pomegranate seed oil is 1.56%. In the study conducted by Martin̕ez *et al.* (2006),

Fatty acid composition (m/m % methyl esters)		
Trichosanoic acid	(C23:0)	0.19
Arachidonic acid	(C20:0)	0.26
Stearic acid	(C18:0)	3.77
ΣSFA		4.22
Cis-11-Ekosenoic acid	(C20:1) w9	0.14
Oleic acid	(C18:1) w9	27.53
Heptadekanoic acid	(C17:1) w8	0.06
Palmitoleic acid	(C16:1) w7	0.18
Myrstoleic acid	(C14:1) w5	0.07
ΣMUFA		27.98
EPA	(C20:5) w3	0.10
Methyl-cis 11,14,17 echocatrienoic acid	(C20:3) w3	0.08
Methyl-cis 11,14 echocatrienoic acid	(C20:2) w6	0.08
Gama Linoleic acid	(C20:3) w6	0.25
Linolenic acid	(C18:3) w3	0.31
Linoleic acid	(C18:2) w6	66.88
ΣPUFA		67.80

Table 3: Fatty acid composition of grapeseed oil

palmitic acid content in pomegranate seed oil ranged from 3.26-5.25% and in another study, Fadavi *et al.* (2006) reported that palmitic acid was found in the range 3.7-16.7% in pomegranate seed oil. Unsaturated fatty acids are more reactive than saturated fatty acids [15,16]. Unsaturated fats are essential fatty acids that the body needs. They are liquid at room temperature and the vast majority is of plant origin. Fatty acids containing a single double bond in their structure are called monounsaturated fatty acids. The two most important members of this group are palmitoleic acid (C16: 1) and oleic acid (18: 1). Essential fatty acids include α-linolenic acid (omega 3) and linoleic acid (omega 6) fatty acids. Essential fatty acids, called prostaglandins, are synthesized hormones necessary for normal body functioning. It is reported that prostoglandins control blood pressure, blood clotting, blood lipid levels, and the immune and inflammatory responses associated with infection. Linoleic and alpha-linoleic acid derivatives are present in the brain. A correlation was found between the levels and ratios of these fatty acids resulting from the Western-type diet and depression and behavioral disorders. In our study, we found that pomegranate seed oil contained punicic acid (C18:3) at 30.12%, linoleic acid (C18:2) at 28.92%, oleic acid (C18:1) at 19.01%, cis-11-eicosenoic(gadoleic acid) (C20:1) at 1.49%, erucic acid(C22:1) at 0.18%, linolenic acid(C18:3) at 0.18% and palmitoleic acid(C16:1) at 0.07%. Fadavi *et al.* (2006) [16] found 24.4% linoleic acid, whereas Melgarejo and Artes (2000) found linoleic acid amount varying between 5.19% and 16.50% [17].

In our study, grape seed oil contains stearic acid (C18: 0) at 3.77%, arachidic acid (C20: 0) at 0.26%, tricosanoic acid (C23: 0) at 0.19%. The amounts of unsaturated fatty acids were found to be: linoleic acid (C18:2) 66.88%, oleic acid (C18:1) 27.53%, cis-11-eicosenoic (gadoleic) acid (C20:1) 0.14%, linolenic acid (C18:3) 0.31%, palmitoleic acid (C16:1) 0.18%, gamma-linoleic acid (C20:3) 0.25%, methyl-cis 11,14,17 eicosatrienoic acid (C20:3) 0.08%. Grape seeds contain a high amount of oil. Beveridge et al. (2005) reported in their study that linoleic acid is the most important fatty acid component of grape seed oil with 67.56-73.23% [18]. It was emphasized that with the utilization of this waste material in wineries, grape seeds can be used in the production of high-quality cooking oil.

α-Linoleic acid is used for the treatment of hyperlipidemia, reducing platelet aggregation, lowering high blood pressure, preventing heart attack and alleviating some symptoms of arthritis. In the meta-analysis of the clinical trials conducted, it has been stated that the risk of restenosis can be reduced significantly (13.9%) by the application of the EPA and DHA mixture between three months and one year after coronary angioplasty surgery. The anticarcinogenic, antiatherogenic, antidiabetic effects of linoleic acid have been experimentally demonstrated. In vivo/in vitro studies indicate that it may be useful in the treatment of certain cancers, cardiovascular diseases, hypercholesterolemia, triglyceridemia, and type-2 diabetes. It is suggested that it may help with losing weight by reducing fat mass in the body in some cases [19].

Linoleic acid was determined as 28.92% and 56.55% respectively in pomegranate seed oil and grape seed oil. When pomegranate and grape seed oils are compared with other oils in terms of linoleic acid content, it is observed that 50-54% of sunflower oil, 65-75% of tobacco seed oil, and 10-12% of palm oil contains linoleic acid [20]. The linoleic content of pomegranate seed oil and grape seed oil is observed to be close to that of sunflower, cotton, and tobacco seed oils. Functional fats have a special importance in the nutrition of the elderly and are particularly important in children's mental functions and bone development. Pomegranate oil, which has a strong antioxidant effect, helps keep your heart healthy. The acids it contains increase the body's resistance by activating the immune system. It has protective effects on the digestive system. In a study, it was determined that grape seed oil was rich in unsaturated fatty acids, linoleic acid, and oleic acid. High amounts of tannin allow the oil to withstand peroxidation [21].

In the study by Lachman et al. (2015), linoleic acid was the most abundant fatty acid in all analyzed grape seed oils, contributing between 68.10 g and 78.18 g per 100 g oil [22]. Linolenic acid was present only in small trace quantities ranging from 0.29 g to 0.77 g per 100 g oil. Oleic acid content conformed to MUFA content, which ranged from 8.82 g/100g-16.92 g/100g. SFA ranged between 9.04 g and 12.82 g per 100 g of total fatty acid. Rezanka and Rezankova (1999) found dominant FA linoleic acid (67.2 g/100g) and oleic acid (22.1 g/100 g), and in lesser amounts palmitic acid (70g/100g) and stearic acid (3.0 g/100 g). Seeds presented the largest FA concentrations, as reported by Orsanova *et al.* (2015) [23,24]. The main MUFA in seeds was oleic acid. Besides a high content of linoleic acid, which is a precursor of long-chain fatty acids of the n-6 series, the analyzed grapes also contained α-linolenic acid (18:3n-3), a precursor of the n-3 series. When considering the general classification of the fatty acids, it was found that the pomegranate and grape seed oils had the following order: PUFA>MUFA>SFA, which is agreement with other studies. In addition to these main fatty acids, we also identified α-linolenic acid in low quantities. However, low levels of linoleic acid may be desired in edible oils, while high levels of this fatty acid can produce oxidized unfavorable products due to having three double bonds. So, the pomegranate and grape seed oils possessing low amounts of linolenic acid can be advantageous in terms of human consumption and the shelf-life of the oil. Only a negligible contribution has been found for other fatty acids such as erusic, margaric (heptadecanoic acid), palmitoleic, arachidonic, trichosanoic, ecosenoic, echocatrienoic, and others presented in negligible amounts. Similar results were found in Turkish pomegranate and grape seed oils, where in some varieties eicosenoic acid content was similar to linolenic acid.

Numerous in vitro and in vivo studies suggest that pomegranate and grape seed oils have cardioprotective and anticancer effects. However, the amounts of lipophilic and hydrophilic pomegranate and grape seed oils constituents with cardioprotective, anti-inflammatory, and anticancer activities are small, requiring the consumption of a large amount of oil for beneficial effects to be achieved. With respect to clinical studies; most studies have an observational design and involve small sample sizes, and thus, caution must be exercised in the interpretation of results. Further studies are needed on the beneficial effects of grape seed oil on human health and its use as an adjuvant agent in the prevention and treatment of chronicle diseases [25].

The importance of substances such as grapes and pomegranate seeds does not only come from their oil content, but also from components such as phenolic compounds, vitamins and minerals.

In conclusion, while the large amount of residual resulting from the pressing and processing of grapes and pomegranates can be used as compost and animal feed, which are the current ways, it can also be used by prioritizing its grape and pomegranate seed oil aspects, which provides more income. These results make it necessary to investigate the possibilities of using pomegranate and grape seed oils for health and nutrition purposes. Thus, an important foodstuff would be obtained for human health.

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