

Comparison of Fatty Acid Composition of Red Grape Seeds (*Vitis vinifera* L. cvs. Öküzgözü and Boğazkere)

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Abstract

This study was carried out to determine the quality criteria of the seed oils of Öküzgözü and Boğazkere grape varieties grown in the Elazığ region, and to compare them in terms of oil amount and fatty acid levels. Grape seeds are rich in unsaturated fatty acids and phenolic content. It is thought that it can be used as cooking oil due to these properties. This study was carried out on Öküzgözü and Boğazkere, two black grapes grown locally. These two grape varieties are generally used as wine grapes. Interest in the use of grape seeds, a byproduct of wine production, has recently increased. Gas chromatography was used for fatty acid analysis. Amounts of defined fatty acids vary according to grape varieties. The present study, when the amounts of myristic acid (0.36 ± 0.02), palmitic acid (20.45 ± 0.67) and palmitoleic acid (5.80 ± 0.10) are compared, higher rates were detected in the seeds of Boğazkere grapes than those of Öküzgözü grapes. Stearic acid (1.15 ± 0.03), oleic acid (19.40 ± 0.4), linoleic acid (54.75 ± 0.69), α -linoleic acid (0.07 ± 0.02) and when the amount of gamma linoleic acid (0.24 ± 0.01) was compared, it was found at higher rates in the seeds of Öküzgözü grapes than those of Boğazkere grapes.

Keywords: Boğazkere, GC-MS, Öküzgözü, grape seed oil, fatty acids

Öküzgözü ve Boğazkere Üzüm Çeşitlerine Ait Çekirdeklerin Yağ Asit Kompozisyonu Bakımından Karşılaştırılması

Öz

Bu çalışmada, Elazığ bölgesinde yetiştiriciliği yapılan Öküzgözü ve Boğazkere üzüm çeşitlerinin çekirdek yağlarının kalite kriterlerinin belirlenmesi, yağ miktarı ve yağ asit kompozisyonu bakımından karşılaştırılması amaçlanmıştır. Üzüm çekirdekleri doymamış yağ asitleri ve fenolik madde içerikleri bakımından oldukça zengindir. Bu özelliklerinden dolayı yemeklik yağ olarak kullanılabilmesi düşünülmektedir. Çalışma, yöresel ticari olarak yetiştirilen iki kırmızı üzüm çeşidi olan Öküzgözü ve Boğazkere çekirdekleri kullanılarak gerçekleştirilmiştir. Bu iki üzüm çeşidi, genellikle şaraplık olarak kullanılmaktadır. Şarap üretiminin yan ürünü olan üzüm çekirdeklerinin değerlendirilmesine olan ilgi son zamanlarda giderek artmaktadır. Tanımlanan yağ asitlerinin miktarları üzüm çeşitlerine göre değişmektedir. Yaptığımız analizler sonucunda çekirdek yağlarının yağ asidi kompozisyonu; Miristik asit (0.36 ± 0.02), Palmitik asit (20.45 ± 0.67) ve Palmitoleik asit (5.80 ± 0.10) miktarları karşılaştırıldığında, Boğazkere üzümlerine ait çekirdeklere, Öküzgözü üzümlerine ait çekirdeklere göre daha yüksek oranlarda tespit edilmiştir. Stearik asit (1.15 ± 0.03), Oleik asit (19.40 ± 0.4), Linoleik asit (54.75 ± 0.69), α -linoleik asit (0.07 ± 0.02) ve Gama-linolenik asit (0.24 ± 0.01) miktarları karşılaştırıldığında ise Öküzgözü üzümlerine ait çekirdeklere, Boğazkere üzümlerine ait çekirdeklere göre daha yüksek oranlarda tespit edilmiştir.

Anahtar kelimeler: Boğazkere, GC-MS, Öküzgözü, üzüm çekirdeği yağı, yağ asitleri

INTRODUCTION

Grape is one of the most important fruit types grown in a wide area in the World. Cultivation, which has been done for thousands of years, has brought out the richness of vine forms, and suitable ecology and the history of viticulture has caused the

Anatolian soil to have a wide variety/type richness, and therefore a large vine gene potential in a long historical process (Karaca-Sanyürek, 2014). Even if there is an adverse effect of seasonal conditions that change annually and the possibility of a change in

consumer demands, it is estimated that along with production, exports will also increase on a yearly basis. 77.137.016 tons of grapes are produced in an area of 6.925.972 hectares in the world. 4.1 million tons of production in an area of 405.439 hectares takes place in Turkey (FAOSTAT, 2019), 94.463 tons in an area of 108.568 hectares takes place in Elazığ (Turkey Statistical Institute (TSI), 2020).

After grapes are processed for different purposes, the sugar pulp remaining as waste such as kernel, stem, shell after squeezing should be evaluated by featuring different ways of evaluation, such as grape seed oil and grape seed extract, which can provide more input.

The unsaturated fat content of about 90% in grape seeds is an indicator of the quality of the oil in grape seeds. These unsaturated fats contain linolenic (C18:3) and palmitoleic (C16:1) fatty acids in addition to linoleic (C18:2) and oleic (C18:1) fatty acids (Bail, 2008). Besides, about 10% of the fatty acids are comprised of saturated fat acids, these unsaturated fat acids are constituted by palmitic (C16:0) and stearic (C18:0) acids.

In addition to being very rich and valuable in fatty acids, grape seed oil contains phenolic compounds such as epicatechin, catechin, gallic acid, and much more tannins such as oligomeric proanthocyanidin resistant to peroxidation. It has been proven by various studies that radioprotective and antihyperglycemic effect contained in procyanidins is very important in terms of preventing cataracts, regulation of antioxidant enzyme systems, reduction of hypertriglyceridemia and insulin resistance (Thorsten *et al.*, 2009).

This study is planned in order to determine the quality criteria of the seed oils of Öküzgözü and Boğazkere grape varieties grown in Elazığ region and to compare them in terms of oil amount and fatty acid levels.

All products derived from grapes are influenced by genotype and their geographical origin (J. Pérez-Navarro, *et al.* 2019). Changes in sunlight, UV-B radiation levels have a direct effect on grape composition, and thus these changes will cause secondary metabolites such as flavonoids, amino acids and carotenoids to change (Schultz, 2000). For this reason, the varieties we work with reveal their difference. The data obtained as a result of our study will also provide guidance for the production of high quality grapes and grape-derived products.

The use of grape seeds and extracts, which make up 38-52% of the waste grape pulp on dry weight basis (Teixeira *et al.*, 2014), become increasingly popular for culinary, pharmaceutical, cosmetic, and medicinal purposes in terms of their rich compounds and applications. Especially, interest in grape oil used in the food industry and cosmetics is increasing every year. Unlike synthetic antioxidants, the use of natural antioxidants is of great importance for human health.

Grape seeds with rich oil content, constituting a significant part of grape pulp, which is the waste of wine and fruit juice enterprises, have been used as grape seed oil in many different areas in recent years. Many different extraction methods have been developed in the production of this oil from past to present, and these methods have been diversified with the effect of different factors (pressing, temperature, pressure, ultrasound, microwave and enzyme etc.) according to the usage area of the oil to be obtained. The extraction method to be used should be selected very carefully. The first of the two main factors to be considered in the selection of the method is the high extraction efficiency and the second is that the bioactive components of the seed oil are not damaged. With the rapid advancement of technology in recent years, it is expected that more sensitive methods will be developed and new methods will become widespread (Sevindik and Selli, 2016).

MATERIAL AND METHODS

Chemicals; Hexane, isopropanol, methanol, sulfuric acid, sodium chloride, KHC₃O₃, used in this study were purchased from Sigma Aldrich (Steinheim, Germany). Acetonitrile and acetic acid were purchased from Merck and VWR, respectively.

Collection of plant material

The seeds of the grape varieties of *V. vinifera* cv. Öküzgözü and *V. vinifera* cv. Boğazkere, cultivated in Elazığ region, were supplied from wine factories and grape producers in Elazığ. The seeds brought to the laboratory were removed of foreign substances.

Öküzgözü grape variety is a very good quality wine grape. Grains are large and round, dark black in colour. Their skins are of medium thickness and have plenty of must. It is also a good table grape. The colour of its wine is a beautiful red-violet. Its taste is full and aromatic.

The grains of Boğazkere grape variety have medium-sized round and thick-skinned grains. When processed alone, the wine is very sour, coarse, and heavy. For this reason, it is processed together with Öküzgözü grape. A quality and balanced blended wine is made with Öküzgözü.

Turkey's two most important grape varieties of *V. vinifera* cv. Öküzgözü and *V. vinifera* cv. Boğazkere grape varieties grown in Elazığ area were taken from the producers and the wine factory on the vineyard dates in the region and brought to the laboratory, and the foreign substances in them were removed.

Lipids extraction

Lipids were extracted by taking 1g from grape seeds. The extraction of lipids was done by the Hara and Radin method using 3:2 (v/v) hexane/isopropanol mixture. For this; 1g sample was homogenized in 10 mL hexane-isopropanol mixture at the ratio of 3:2 (v/v) for one minute. The homogenization vessel was taken to the centrifuge tubes. Then, from the samples that were centrifuged at 4.500 rpm for 10 minutes, the upper supernatant was taken and placed in capped test tubes.

Preparation of fatty acid methyl esters for gas chromatographic analysis

By adding 5 mL of 2% methanolic sulfuric acid to the sample reserved for fatty acid measurement, it was ensured to mix well with vortex. This mixture was left to be methylated in a 50 °C oven for 15 hours. At the end of the 15 hour period, the tubes were removed from the oven and cooled to room temperature and mixed thoroughly by adding 5 ml of 5% sodium chloride. The fatty acid methyl esters formed in the tubes were extracted with 5 mL of hexane and the hexane phase was pipetted on top and treated with 5 mL of 2% KHCO₃ and left for 4 hours to separate the phases. Then, the solvent of the mixture containing methyl esters was evaporated at 45 °C and under nitrogen flow, dissolved with 1 mL of n-hexane and taken into 2 mL capped autosampler vials and analyzed in gas chromatography.

Fatty acid methyl esters were analysed by SHIMADZU GC 17 gas chromatography. SP™ - 2380 capillary GC column (L× ID. 30 m × 0.25 mm, df 0.20 µm) was used for this analysis. And FID detector was used analysis of fatty acids. During the analysis, column temperature was kept as 120-220 °C, injection temperature as 240 °C and detector

temperature as 280 °C, and column temperature program was set from 120 °C to 220 °C. The temperature increase was determined as 5 °C/minute up to 200 °C and 4 °C/minute from 200 °C to 220 °C. Nitrogen gas was used as carrier gas. During the analysis, the retention times of each fatty acid were determined by injecting standard fatty acid methyl esters. Then, the analysis of fatty acid methyl esters of the samples was done. After this process, the amount of fatty acids was calculated according to the external standard method using the Class GC 10 program. Results are expressed in µg/g.

Statistical analysis

SPSS 18.0 program was used for statistical analysis. Comparison between species was made using Paired-Samples T Test. Results were given as mean±SEM. For differences between groups, p>0.05, p<0.05, p<0.01 and p<0.001 values were used.

RESULTS AND DISCUSSION

The findings we have obtained as a result of the studies we conducted in the R&D laboratories of our University and the laboratories of Fırat University to do oil analysis of dried seeds of Öküzgözü and Boğazkere grapes are given in Figure 1 and Figure 2.

As a result of our analysis, the amount of Myristic acid (C14:0) was determined at the rate of 0.36% in Boğazkere variety. In Öküzgözü variety, it was found at the rate of 0.26%. The myristic acid ratio in Boğazkere grape seeds was significantly higher than that of Öküzgözü grape seeds (p<0.001).

Palmitic acid is the most common saturated fat in plants and animals. As a result of our analysis, when the amount of palmitic acid (16:0) is examined; it was determined at the rate of 20.45% in Boğazkere variety. In Öküzgözü variety, it was found at the rate of 15.26%. It was determined that Boğazkere grape seeds were significantly higher than Öküzgözü grape seeds (p<0.001).

Palmitoleic acid, one of the unsaturated fatty acids, is known as Omega7. As a result of our analysis, when the amount of palmitoleic acid (C16:1, n-7) is examined; it was determined at the rate of 1,15% in Boğazkere variety. In Öküzgözü variety, it was 0,80%. Boğazkere grape seeds were found to be significantly higher than Öküzgözü grape seeds (p<0.001).

When the amount of stearic acid (C18:0) is examined; it was determined at the rate of 5.80% in

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Boğazkere variety. In Öküzgözü variety, it was found at the rate of 4.51%. It was determined that Boğazkere grape seeds showed a significant increase compared to Öküzgözü grape seeds ($p < 0.001$). In the fatty acid analysis made on 5 different grape varieties by Onhishi *et al.* (1990); it was determined to contain palmitic acid (6.7-8.9%), stearic acid (1.1%-5.3%), oleic acid (9.7% -17.5%), linoleic acid (69.2% -80.5%), palmitoleic acid (0.1%), and linolenic acid (0.1%).

As a result of our analysis, when the amount of 18:1, n-9 fatty acid is examined; it was determined at the rate of 17.90% in Boğazkere variety. In Öküzgözü variety, it is 19.40%. Unlike other fatty acids, Boğazkere variety showed a significant decrease compared to Öküzgözü ($p < 0.001$).

The amount of linoleic acid (C18:2, n-9) was determined to be 47.56% in Boğazkere variety. It is 54.75% in Öküzgözü variety. Unlike most other fatty acids, it was found to be more in Öküzgözü grape seed ($p < 0.001$). Kamel *et al.* (1985) examined the fatty acid profile of different grape seeds. They determined that the unsaturation rate of grape fatty acid was 88.6%, and stated that the dominant fatty acid in grape seed was linoleic acid. As a result of our analysis, it was determined that the dominant fatty acid in both Öküzgözü (54.75±0.69%) and Boğazkere (47.56 ± 0.01%) grape seeds was linoleic acid.

α -linolenic acid (C18:3, n-3) amount was determined as 0.07% in Boğazkere variety. In Öküzgözü variety, it was found at the rate of 0.04%. As a result of the comparison of the amount of α -linolenic acid (C18:3, n-3), it was found to be more in Boğazkere variety ($p < 0.001$).

When the amount of γ -linolenic acid (C18: 3, n-6) was examined; it was determined at the rate of 0.02% in Boğazkere variety. It was found in the Öküzgözü variety at the rate of 0.24%. It was determined that there is a significant increase in Boğazkere variety ($p < 0.001$).

In the study of Uslu and Dardeniz (2009) with 12 different grape varieties, it was determined that

grape seed oils have 8.40%-6.51% palmitic acid, 16.10%-11.62% oleic acid, 77.59%-72.50% linoleic acid, 3.86%-3.07% stearic acid, 0.46-0.11% linolenic acid, and 0.68-0.10% arachidic acid content. The rate of unsaturation of the oils varied between 88.10% and 90.12%. In this respect, grape fatty acid has a good edible oil quality.

When the data we have obtained as a result of our analyzes (Barron *et al.*, 1988; Onhishi *et al.*, 1990; Schuste, 1992; Baydar and Akkurt, 1999; Uslu and Dardeniz, 2009; Sevindik and Selli, 2016; İşlek, 2018) and the fatty acid ratios of grape seeds in previous studies are compared, the first three fatty acid rankings appear to be the similar. The differences between the number and amount of grape seed fatty acids obtained between varieties were found to be statistically significant. It was determined that differences in fatty acids in grape seeds (Rubio *et al.*, 2009, Podolyan *et al.*, 2010) may be affected by ecological differences such as temperature, sunbathing, humidity, etc. caused by locations. In the study of İşlek (2018) on the determination of suitable varieties and altitudes for fatty acid amounts and ratios by looking at the effect of different altitudes on the fatty acid amounts and ratios of grape seeds, it was determined that altitude affects the fatty acid ratio.

In the study of Aljuhaimi and Özcan (2017) obtained 17 grape seed oils by the cold press method and determined the amounts of mainly linoleic and then oleic, palmitic and stearic acid, which are the main components of grape seed oils. The rate of tocopherol varied between varieties. Fatty acids and tocopherol contents of cold pressed oil were found to be higher than grape seed oil extracted with soxhlet.

The quality of the basic bioactive components of all the seeds and the amount of isolation may differ according to the various methods applied, and many different studies have been carried out on this.

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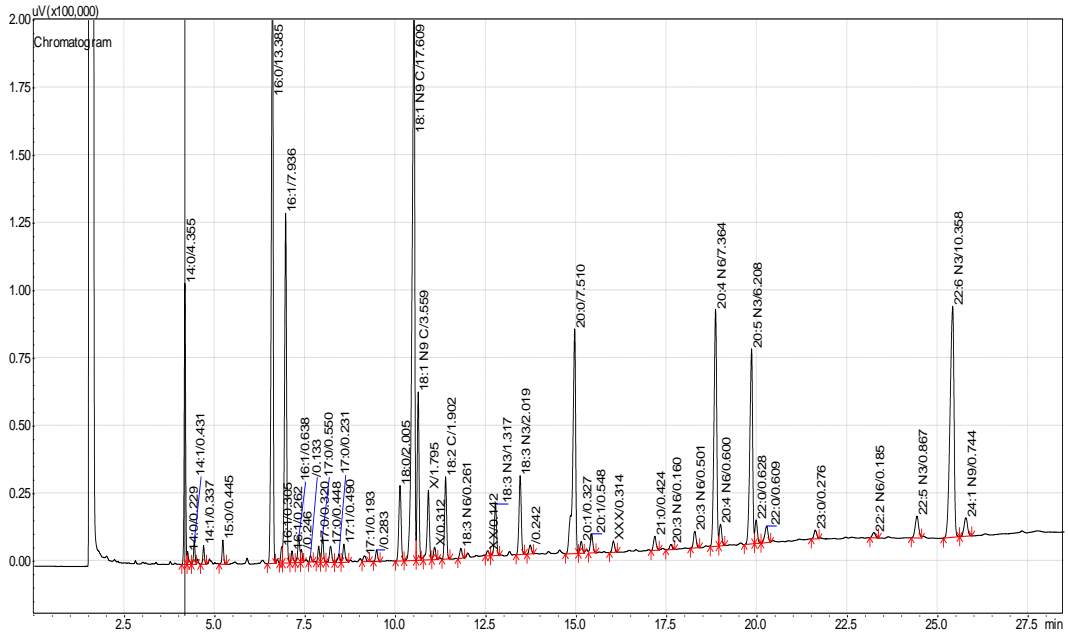


Figure 1. Öküzgözü fatty acid chromatogram (GC)

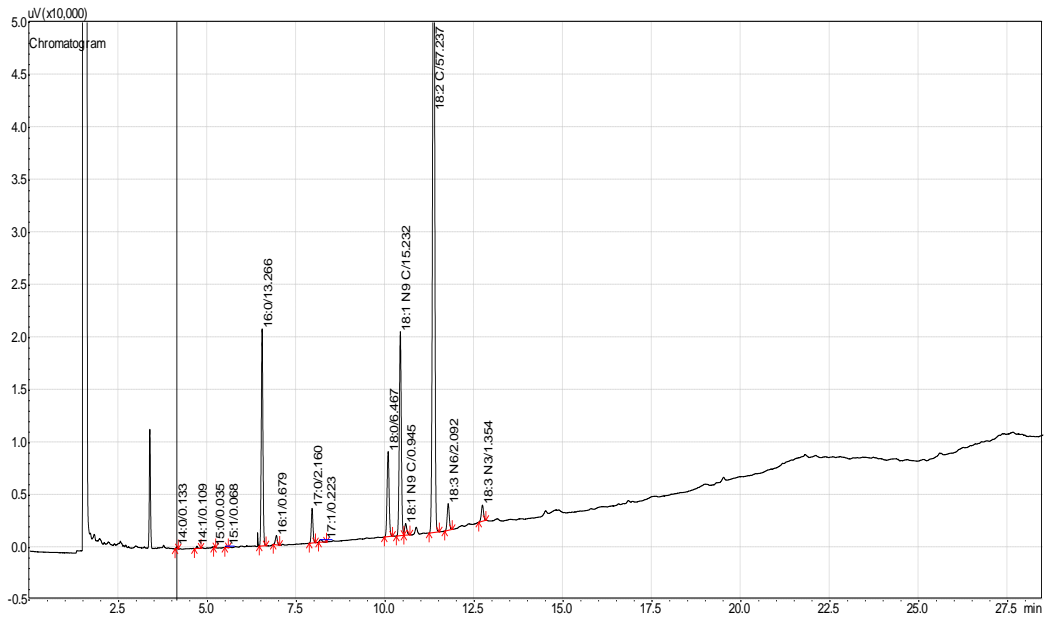


Figure 2. Boğazkere fatty acid chromatogram (GC)

Table 1. Fatty acid content of Öküzgözü and Boğazkere grape varieties ($\mu\text{g/g}$)

Fatty acids	Öküzgözü	Boğazkere
Myristic acid (C14:0)	0.26 \pm 0.02	0.36 \pm 0.02 ^d
Palmitic acid (C16:0)	15.26 \pm 0.8	20.45 \pm 0.67 ^d
Stearic acid (C18:0)	4.51 \pm 0.07	5.80 \pm 0.10 ^d
Palmitoleic acid (C16:1, n-7)	0.80 \pm 0.01	1.15 \pm 0.03 ^d
Oleic acid (C18:1, n-9)	19.40 \pm 0.4 ^c	17.90 \pm 0.01
Linoleic acid (C18:2, n-6)	54.75 \pm 0.69 ^d	47.56 \pm 0.01
α -linolenic acid (C18:3 n-3)	0.04 \pm 0.04	0.07 \pm 0.02 ^d
γ - linolenic acid (C18:n-6)	0.02 \pm 0.01	0.24 \pm 0.01 ^d

c: $p < 0,01$, d: $p < 0,001$ (Boğazkere variety was compared according to Öküzgözü variety using Paired-Samples T Test, each fatty acid was compared individually)

CONCLUSION

This study was carried out on Öküzgözü and Boğazkere, two black grape varieties, which are grown especially in Elazığ and Tunceli regions in Turkey. Analysis studies of grape seeds were carried out in Munzur University R&D (Research and Development) laboratories and in the application and research laboratories of Fırat University, Faculty of Arts and Sciences.

Grapes that are used in different ways is the main source of raw materials especially for the wine industry. A large amount of grape pulp is produced as the main waste as a result of wine production. Around 77.137.016 tons of grapes can be produced in an area of about 6.925.972 hectares in the world (FAOSTAT, 2019). Nearly half of the grapes produced are processed into grape juice and wine. As a result of processing, this means approximately 38 million tons of grape pulp on average. In addition to valuable bioactive components such as fatty acids, tocopherols, proanthocyanidins, and sterols in grape pulp, it becomes a very efficient and profitable raw material for pharmaceutical and cosmetic industries (Demirtaş *et al.*, 2013; Barba *et al.*, 2016).

Turkey, one of the biggest grape producers in the world, sets new strategies to increase production potential. The search for different consumption ways for grape produced as table, dried, wine, and must is increasing and studies continue to determine new strategies. Different consumption alternatives must

be used to achieve Turkey's real production potential. In this context, it is important to present different ways of using the seeds separated from the pulp formed as a result of the processing of grapes.

Today, when every waste is tried to be used both economically and environmentally with the depletion of natural resources, grape seeds, which are taking their place in the ever-growing recycling chain, are thought to gain great importance for our country day by day.

These two grape varieties are generally used for wine. Interest in the utilization of grape seeds, which are by-products of wine production, has been increasing in recent years.

Including the bioactive compounds containing grape seed oil, phytosterols, tocopherols, tocotrienols, flavonoids, and phenolic acids are of biological importance, recognized for their antioxidant activity, as they contribute to the beneficial effects of the oil. Due to its powerful antioxidant properties, its benefit to human health has become indisputable (Shinagawa *et al.* 2015).

Grape seeds are rich in unsaturated fatty acids and phenolic substance contents. Therefore, their use is increasing, especially in obtaining edible oil. It is quite important to know the amount of oil in grape seeds and the types of oil in the seeds in terms of their usage area.

The oil content of grape seed varies according to the variety of grape (Luque-Rodríguez *et al.*,

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2005). For this reason, knowing the fatty acid components of the oils belonging to the seeds of the existing grape varieties will enable production according to the use of intent.

The results obtained by determining the fatty acid amount and fatty acid ratios in different grape seeds with this study will shed light on the studies to be carried out in their field, the food sector, and other sectors according to the area of use.

Firstly, it will be possible to bring waste and unusable raw materials to the industry by determining and presenting the proportions, composition, and nutritional properties of grape seed oil and evaluating it on variety basis.

Grape seeds, which are very rich in unsaturated fatty acids and phenolic substance contents, are aimed to be a good food source in human nutrition, to increase their use in the food industry, and also to contribute to the reduction of product costs in grape processing factories.

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CONFLICT OF INTEREST

The Author report no conflict of interest relevant to this article.

RESEARCH AND PUBLICATION ETHICS STATEMENT

The research was not involve human participants and animals so it does not require an ethics committee.

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