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**Research Article** 

## Üzüm Çekirdeği Tozu ve Ununun Lipofilik Vitamin ve Fitosterol İçeriklerinin Belirlenmesi

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Öz

Bu çalışmada üzüm çekirdeği tozu ve ununun lipofilik (ADEK) vitaminler, fitosteroller ayrıca besin madde kompozisyonlarının belirlenmesi amaçlanmıştır. Üzüm çekirdeği ve unu (yağı alınmış ve peletlenmiş) öğütüldükten ve toz haline getirildikten sonra analiz edilmiştir. Analiz sonuçlarına göre üzüm çekirdeği tozu ve ununda kuru madde, ham protein, ham yağ, ham selüloz ve ham kül değerleri sırasıyla % 91.48 ve 91.39; 19.11 ve 18.83; 6.80 ve 6.45; 4.43 ve 4.34; 6.71 ve 6.45 olarak belirlenmiştir. Üzüm çekirdeği tozu ve ununun vitamin içeriklerinde sırasıyla D3 vitamini 7.89 ve 7.63  $\mu$ g/g,  $\alpha$ -Tokoferol ise 28.26 ve 11.03 olarak tespit edilmiştir. Ergosterol, stigmasterol ve  $\beta$ -sitosterol gibi fitosterol içerikleri sırayla 2.76 ve 36.03; 2757.76 ve 214.03; 2951.33 ve 1631.10  $\mu$ g/g olarak belirlenmiştir. Analiz sonuçlarına göre üzüm çekirdeğinin besin madde bileşimleri, lipofilik vitamin (ADEK) ve fitosterol değerlerinin aynı ürünün (çekirdek) farklı kullanımlarına bağlı olarak değişiklik gösterebileceği kanısına varılmıştır.

Anahtar kelimeler: : Yem Katkısı, Üzüm Çekirdeği Tozu, Üzüm Çekirdeği Unu, Lipofilik Vitamin, Fitosterol

## Determination of Lipophilic Vitamins and Phytosteroles Contents of Grape Seed Powder and Meal

#### Abstract

The objective of this study was determine the content of lipophilic (ADEK) vitamins, phytosterols of grape seed powder and meal and also determine their nutritional compositions. Grape seed and meal (defatted and pelletted seed) were analysed after grinded and powdered. According to the analysis results, dry matter, crude protein, crude oil, crude fiber and crude ash values in grape seed powder and meal were determined 91.48% and 91.39%; 19.11 and 18.83; 6.80 and 6.45; 4.43 and 4.34; 6.71 and 6.45 respectively. Vitamin D3 content of grape seed powder and meal were determined as 7.89  $\mu$ g/g and 7.63  $\mu$ g/g, and  $\alpha$ -Tocopherol 28.26  $\mu$ g/g and 11.03  $\mu$ g/g respectively. The content of physterols such as ergosterol, stigmasterol and  $\beta$ -sitosterol of grape seed powder and meal were determined 2.76 and 36.03; 2757.76 and 214.03 and 2951.33 and 1631.10  $\mu$ g/g respectively. According to this results it was concluded that the nutritional composition, lipophilic vitamin (ADEK) and phytosterol content of grape seed may vary depending on different uses of the same product (seed).

Key words: Feed additive, Grape Seed Powder, Grape Seed Meal, Lipophilic Vitamin, Phytosterol

## Introduction

Vitamins are identified as a group of complex organic compounds that are necessary for normal metabolism, are found in very small

amounts in natural foodstuffs, and their deficiency in the diet can cause diseases. Classification of vitamins is based on their function not their chemical properties. Due to their organic structure vitamins are separated from trace elements, which are found in small amounts in the diet. Vitamins are necessary in small amounts (micrograms to milligrams per day) in the diet for health, growth and reproduction. Most vitamins act as coenzymes (metabolic catalysis). However, some do not have such a role, but perform some basic functions (Mc Dowell, 2012). Phytosterols are plant sterols and stanols that are commonly found in various vegetable oils, nuts, and plant seeds. The most common phytosterols in nature are campesterol, βstigmasterol sitosterol, and brassicasterol. Phytosterols behave as hypocholesterolemic, immunomodulatory, anti-inflammatory and antidiabetic agents in animals and humans (Feng et al. 2020). Phytosterols are added to the poultry diet to reduce cholesterol in plasma or product (egg, muscle) due to their regulatory effects on blood lipid profile and total cholesterol by inhibiting cholesterol absorption in the small intestine (Luo et al., 2015; Feng et al., 2020). It has also been reported that phytosterols additive to diets reduce oxidative stress by increasing superoxide dismutase activity and decreasing xanthine oxidase and malondialdehyde (Song et al., 2017) improve antioxidant status and quality of meat in chickens (Zhao et al., 2019).

Grape seed meal is the residue after the grape seed oil has been removed (Grosu et al., 2019; Marin et al., 2020) and this waste product can be used to enrich the diets of farm animals (Marin et al., 2020). Grape seed powder is obtained by drying and grinding the seed after it is separated from the peel (Kadri et al., 2020). Grape seed powder is a recoverable by-product that is increasingly used worldwide as a healthy nutritional supplement (Kadri et al., 2019). Grape seed powder contains high concentrations of vitamin E (Abu Hafsa et al., 2018). Besides determining the content of vitamins and physterols of grape seed powder and meal it was also aimed to determined the nutritional contents in this study.

## **Material And Method**

The grape seed meal and powder used in this study were obtained from a company (Lermonos Natural Products) in Çal/DENİZLİ province where wine grapes are grown. The grape seed meal and powder were obtained was Vitis vinifera Linné subsp. Vinifera. The oil of the seeds was extracted by cold pressing method and then grape seed meal obtained.

The analysis of ADEK and Phytosterols of feeds by HPLC device: Samples were homogenized by mixing n-hexane/isopropyl at 3/2 (v/v) ratio (Hara and Radin 1978).The hydrolysis was performed with 5% KOH at 85°C for 15 minute and

then the extraction of phytosterols was obtained by adding hexane. After vortexing, test tubes were kept at 85°C for 15 minutes. The tubes were removed later, the tubes were added 5 mL of distilled water and mixture. The hexane phase was evaporated by nitrogen stream; later it was dissolved in 1 mL of acetonitrile/ methanol (50% + 50%, v/v) mix put into auto-sampler vials and analysed. The analyzes was done on Shimadzu fully equipment HPLC equipment. Calculations were done by Class VP 6.27 program (Shimadzu, Kyota Japan). The quantity of ADEK vitamins with phytosterols were analysed at 202 nm and 326 nm by a UV detector on a HPLC equipment(Katsanidis and Addis, 1999).

Calculation of nutritional composition of feed ingredients: Nutritional ingredients of feed (dry matter, crude protein, crude ash, and ether extract) were analyzed according to the AOAC (1990) procedures and crude fiber was determined by the methods of Van Soest (1991).The metabolisable energy of feeds calculated acording to the MAFF (1984).

### **Results And Discussion**

The nutritional ingredients of the grape seed meal and powder are given in Table 1. It was observed that the dry matter, crude protein, crude oil, crude fiber and crude ash values of grape seed powder were numerically higher than meal. The lipophilic vitamin and phytosterol values of the grape seed meal and powder are given in Table 2. Retinol Acetate and Vitamin K2 could not be detected in grape seed meal. Vitamin D2 could not be detected in grape seed powder. Vitamin D3, Σtocopherol,  $\alpha$ -tocopherol and vitamin K1 values were found to be higher in grape seed powder compared to meal. When the phytosterol values of the grape seed meal and powder were examined, it was determined that the stigmasterol and  $\beta$ sitosterol values of grape seed powder were higher than meal, while the ergosterol ratio was found to be low. Poultry breeding is developing rapidly all over the world. Therefore, the use of agricultural by-products as feed provides an advantage for the development of poultry breeding (Yang et al., 2021). Likewise lack of quality roughage is encountered in many parts of the world in ruminant nutrition and alternative feed sources are being researched. For this reason, it is very important to determine alternative roughage sources and their nutritional composition for farms that need roughage (Kiliç and Abdiwali, 2016). Grape seeds are known as important agricultural and industrial waste of wineries (Sotiropoulou et al., 2015). Wine industry waste has also attracted attention recently in terms of its potential as an alternative source of roughage

(Kılıç and Abdiwali, 2016). For this reason it was aimed to compare the seed powder and meal, which are grape by-products in terms of composition of nutrients, vitamins and phytosterol in this study. When Table 1 and 2 are examined, it is seen that the nutritional, vitamin and phytosterol contents of powder and meal are mathematically different. The oil rate obtained from grape may vary according to the grape variety since the grape is a fruit with different varieties. While the oil rate obtained from white grape seeds is 20%, the oil rate obtained from some black grape seeds can be around 6% (Marin et al., 2020). When the oil rate of the grape seed meal and powder used in this study is examined, it is seen that the oil rate of the seed (6.80%) is higher than the meal (6.45%). Cold pressed method was used to obtain the oil from the seed that used in this study. Oil obtained from pressing is much safer source of health-promoting phytochemicals. It may even have a higher content of fatty acids and tocopherols in the final composition (Tobar et al., 2005; Karaman et al., 2015; Marin et al., 2020). Vitamin A is essential for animals health and supporting growth. Vitamin A deficiency in animals can cause growth arrest and eventual death. Lack of vitamin A effects immun functions and can cause loss of vision, flaw in bone growth and reproduction. It is necessary for maintenance of epithelial cells (Mc Dowell, 2012). Vitamin D have important roles such as regulating calcium metabolism and development of skeletal health of animals (Clarke et al., 2021). Vitamin E is necessary for integrity and optimum function of the reproductive, muscular, circulatory, nervous, and immune systems. In addition it has important effects on the prevention of free radical injuries, cancer, heart, cataracts, Parkinson's and a number of other diseases. It is an essential vitamin for all species of animals. Vitamin K is required for maintaining the function of the blood coagulation system in animals (Mc Dowell, 2012). It was reported that grape seed oil contain lipophilic compounds such as vitamin E and phytosterols (Garavaglia et al., 2016). When ADEK vitamins and phytosterol contents of the samples are examined, it is seen that the powder has high oil, vitamin E and phytosterol content, as reported by Garavaglia et al (2016).

Many scientific studies on grape byproducts (Abu Hafsa and İbrahim., 2018; Munoz Gonzalez et al., 2019; Romero et al., 2021) have focused on their antioxidant effects due to their high polyphenol content. No studies have been found that determine the content of vitamin and phytosterols of grape powder and meal. In this study, it was aimed to evaluate the form of the same product (seed), which is both with oil (powder) and defatted (meal) in terms of these components especially. However, it has been reported that the chemical composition may vary mainly depending on the degree of maturity of the seed, the grape variety and many environmental growing factors and less depending on the extraction method of the seed (Marin et al., 2020). For this reason, the values in Table 1 and Table 2 belong to the grape specie used in this study and it should be taken into consideration that the compositions of different species may vary.

The lipid ratio in the diets of pets may vary between 5%-40%. Dietary lipids sources are vegetable, animal or both of them. Recently, fatrelated disorders and diseases have attracted attention. The requirements omega-3 and 6 fatty acids for dogs are not indicated clearly but they necessary at certain stages of their life cycle (Bauer, 2007; Glodde et al., 2018). High levels of unsaturated fatty acids are more sensitive to oxidative damage which causes major sensory changes during storage (Brewer, 2011; Glodde et al., 2018). The oxidation of polyunsaturated fatty acids negatively affects the flavor, texture, color, odor and nutritional composition of food during storage. It has been defined that dietary lipid oxidation inhibits growth, damage antioxidant status and weaken some immune functions in growing dogs. Synthetic antioxidants such as hydroxytoluene (BHT), butylated butylated hydroxyanisole and ethoxyquin frequently inhibit the oxidation. The probable toxicity of synthetic antioxidants has been considered in recent years. Therefore, there is increasing interest in natural antioxidants (Glodde et al., 2018). Glodde et al (2018) investigated the effect of grape seed extract as a natural antioxidant on the stability of omega-3 in dog feeds and reported that it could be added to feed instead of Butilated Hydroxyanisol, which is used as a synthetic antioxidant. Grape seed includes phenolic compounds such as gallic acid, ellagic acid, resveratrol, catechin, epicatechin, anthocyanins and procyanidins (Maier et al., 2009; Glodde et al., 2018). It has been reported that the antioxidant property of grape seed extract is because of its radical scavenging, potential of chelation with metals and synergistic interaction with other antioxidants (Adamez et al., 2012; Glodde et al., 2018).

Nutritional composition	Grape seed powder	Grape seed meal
Dry matter	91.48	91.39
Crude protein	19.11	18.83
Crude oil	6.80	6.45
Crude fiber	4.43	4.34
Crude ash	6.71	6.45
*ME, Mcal/kg	2.64	2.63

Table 1: Nutritional composition of grape seed meal and powder (%).

Table 2: Contents of ADEK vitamins and phytosterol in grape seed powder and meal ( $\mu g/g$ ).

Ingredients	Grape seed powder	Grape seed meal
Vitamins		
Retinol asetat	0.06	-
Vitamin D <sub>2</sub>	-	47.73
Vitamin D₃	7.89	7.63
Σ-Tocopherol	50.86	1.16
Vitamin K₃	28.26	11.03
Vitamin K1	7.86	1.46
Vitamin K <sub>2</sub>	3.66	-
Phytosterols		
Ergosterol	2.76	36.03
Stigmasterol	2757.76	214.03
β-sitosterol	2951.33	1631.10

As a result, it can be said that the powder and meal obtained from the same product (seed) contain ADEK vitamins and phytosterols, which have important functions, in different proportions, and this difference may be due to the defatted of seed. Because of the average of 19% crude protein and the low crude cellulose level (4.40%) contents of the seed thoughted that it can be added to ruminant feeds as well as cat-dog foods and poultry mixed feeds at certain rates. The grape seed contains an average of 19% crude protein and low crude fiber such as 4.40% suggests that it can be added to ruminant feeds, cat-dog feeds and poultry mixed feeds at certain rates.

**Conflict of Interest Statement:** There is no conflict of interest between the authors of the article.

**Researchers' Contribution Rate Statement Summary:** The authors contributed equally to the article.

#### Kaynaklar

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