ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2021, 6(3): 155-165 doi: 10.28978/nesciences.1036844

# Determination of Vitamin K2 Content of Dairy Products Produced in Hatay Region in Turkey

Şana Sungur[1*], Mustafa Çömez[1], Muaz Köroğlu[2]

[1]Mustafa Kemal University, Science and Letters Faculty, Department of Chemistry, 31024 Hatay, Turkey

[2]Mustafa Kemal University, Altınözü Vocational School of Agricultural Sciences, Department of Food Processing, 31024 Hatay, Turkey

## Abstract

In the past, vitamin K was only known as a vitamin that plays a role in the production of coagulation factors and has been a neglected vitamin because its deficiency was rarely encountered. However, recent studies have shown that vitamin K2 plays an important role in building healthy bones, preventing bone resorption, protecting heart health, and even having a protective effect against various cancers such as lung, liver and prostate. More importantly, studies have shown that vitamin K2 is effective even on COVID-19, which causes mass deaths by affecting the whole world. In this study, vitamin K2 content of dairy products such as cheese, milk, yogurt, eggs, kefir, butter and margarine was determined by high performance liquid chromatography (HPLC). The highest total vitamin K2 contents were found in weave (503.40 ng $g^{-1}$), cara (487.94 ng $g^{-1}$) and crushing (439.19 ng $g^{-1}$) cheese. Then, optimum conditions were determined to increase the vitamin K2 content of cheeses.

**Keywords:**
Vitamin K2, menaquinones, dairy products, cheese, HPLC
**Article history:**
Received 25 June 2021, Accepted 11 September 2021, Available online 15 December 2021

## Introduction

All K vitamins have 2-methyl-1,4 naphthoquinone (menadione) structure. They differ only in the length and saturation of the side chain (Suttie & Booth, 2011). Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) is called vitamin K1 and is found in dark green leafy vegetables such as spinach, kale, broccoli, sprouts and vegetable oils such as soybean, olive, canola (Shearer et al.,, 2012). The menaquinone is called vitamin K2 and is most commonly found in fermented foods and

[*] *Corresponding Author: Şana SUNGUR, E-mail: sungur@mku.edu.tr*

animal products (Hubicka et al., 2020). All forms of vitamin K are fat soluble (Harsman & Shea, 2016). The menaquinones are shown as MK-n, where n is the length of the isoprenoid side chain. There are different forms from MK-4 to MK-13. Although menaquinones are formed as a result of microbial fermentation, MK-4 can be converted from phylloquinone or menadione in animal tissues (Finnan et al.,, 2017).

The most widely known function of vitamin K is allow to blood coagulation (Booth 2009). However, studies in recent years have revealed that it strengthens bone health and prevents bone resorption (Capozzi et al., 2020; Sato et al., 2020; Zhang et al., 2020). Furthermore, there are studies showing that vitamin K lowers inflammation and blood lipids, improves insulin resistance and glucose tolerance (Yoshida et al., 2008; Manna and Kalita, 2016; Ozdogan et al., 2017). Regular use of vitamin K2 has been shown to reduce inflammation in rheumatoid arthritis patients (Ebina et al., 2013; Xu et al., 2021). Studies have shown that even a small amount of K2 keeps calcium away from the vessels, prevents the formation of dangerous calcified plaques, and combats heart disease by controlling proteins that regulate calcium in vascular tissue (Palmer et al., 2020). In one of the first studies conducted to examine the effects of vitamin K on cancer, 61 patients diagnosed with liver cancer were given 45 mg of MK-4 per day after surgical intervention, and it was found that the cancer-free survival rate increased significantly (Mizuta et al., 2006). In addition, there are studies showing that the use of vitamin K2 prevents the growth of lung (Yoshida et al., 2003), pancreatic (Showalter et al., 2010), ovarian (Shibayama-Imazu et al., 2003, 2006, 2008), stomach (Tokita et al., 2006), breast (Wu et al., 1993), and leukocyte (Yaguchi 1997) cancer cells. In a study of 113 patients, a reduction in advanced prostate cancer was noted with increasing vitamin K2 intake (Nimptsch et al., 2008). Recent studies have shown that vitamin K2 exhibits a remarkable anticancer activity (Duan et al., 2020). There are studies showing that vitamin K also plays an important role in sphingolipid synthesis in the brain and in the peripheral and central nervous systems (Ferland, 2012; Ramazani et al., 2019). In addition, vitamin K2 has been shown to slow the progression of Alzheimer's disease (Hadipour et al., 2020). More importantly, it has been shown that the use of D3, K2 and magnesium supplements together reduces the mortality rate in COVID-19 patients (Goddek, 2020). In another study, it was stated that among the diagnosed COVID-19 patients with vitamin K deficiency, the disease was more severe (Anastasi et al., 2020).

In order to benefit from vitamin K, which is extremely beneficial for health, it is of great importance to know which foods and in what quantities. In recent years, the number of studies conducted to determine the vitamin K content of foods has increased. Vitamin K contents of cereals, legumes, fruits and vegetables, fish, meat, animal foods such as cheese, milk, eggs, butter, sauces and spices were examined (Schurgers & Vermeer 2000; Manoury et al., 2013; Finnan et al., 2017; Vermeer et al., 2018; Tarvainen et al., 2019; Fu et al., 2020). Turkey, a country that cheese is extremely rich in terms of diversity. The aim of this study was to determine the menaquinone content of various animal foods such as milk, eggs, yogurt, margarine and butter, especially cheeses, and to determine the optimum conditions to increase the vitamin K2 content of cheeses.

## Materials and Method

### *Reagents and Standards*

MK-4 and MK-7 (100 µg mL-1 in acetonitrile, certified reference materials) were purchased from Sigma-Aldrich (Gillingham, UK). All chemicals used were of analytical reagent grade and were at least 99.5% pure. All of the animal food samples were taken from different local markets in Turkey.

***Extraction of Animal Foods***

One gram of samples were taken and extracted by adding 4 ml of 2-propanol, 20 ng of internal standard (2,3-dihydrophylloquinone) and 2 ml of distilled water. After the mixture was homogenized, it was heated to 60 ° C and then extracted with 8 ml of hexane. The hexane phase was evaporated and the residue was taken up with 2 ml of hexane. After passing through silica Sep-Pak cartridges, vitamin K analyzes were performed by giving to HPLC device (Schurgers & Vermeer, 2000).

***MK-4 and MK-7 Analysis***

The dilution solution was prepared by mixing 255 ml of methanol containing 10 mM $ZnCl_2$, 5 mM acetic acid, 5 mM sodium acetate with 45 ml dichloromethane. Six different standard solutions of MK-4 and MK-7 between 5 - 100 ng ml-1 concentrations in this dilution solution were prepared. Calibration lines were established using these solutions. In all cases, the correlation coefficients of linear functions were > 0.995.

HPLC analysis was performed on a Shimadzu LC20 A (Kyoto, Japan) instrument. The C-18 reverse phase column (5µm, 250 x 4.6 mm) was used. Mobile phase was prepared by mixing 900 ml solution of 2 mM ZnCl2, 1 mM acetic acid, 1 mM sodium acetate in methanol with 100 ml methylene chloride solution. The column temperature was set to 30 ° C and the flow rate to 1 ml min-1. MK-4 and MK-7 were determined using the method developed by Gijsbers et al., (1996) by fluorometric detection after post column electrochemical reduction. The excitation and emission wavelengths were 243 nm and 430 nm, respectively. All analyzes were repeated three times.

Under the applied HPLC conditions, the retention times of MK-4 and MK-7 were 4.868 and 9.313 min, respectively (Figure 1). Ten blank cheese samples (previously analyzed and found to be not vitamin K) were fortified with an amount of standard MK-4 and MK-7 able to produce signal-to-noise ratios ranging from 2.5 to 5. All of the blank values were averaged, and the average value was subtracted from the detected MK-4 and MK-7 values. The limit of detection (LOD) was determined to be three times the standard deviation of the blank test values. The limit of quantification (LOQ) was taken as three times the LOD. The values of retention time, correlation coefficient, and recovery, LOD and LOQ of MK-4 and MK-7 are listed in Table 1.

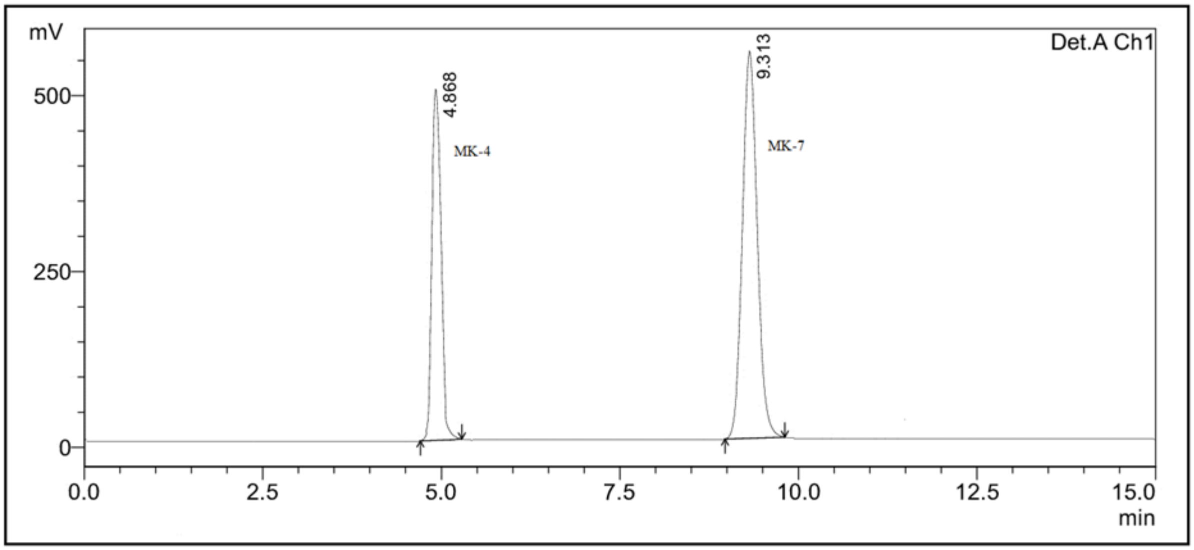


Figure 1. The chromatogram of MK-4 and MK-7

Table 1. The values of retention time, correlation coefficient, recovery, LOD and LOQ of MK-4 and MK-7

| Vitamin K compound | Retention time (min) | Correlation coefficient ($R^2$) | Recovery (%) | LOD (ng $ml^{-1}$) | LOQ (ng $ml^{-1}$) |
|---|---|---|---|---|---|
| MK-4 | 4.868 | 0.9998 | 95.4 | 1.65 | 5.44 |
| MK-7 | 9.313 | 0.9998 | 93.5 | 1.79 | 5.91 |

### *The Making of Cheese*

5 L of milk was heated up to 75 ℃ in a beaker and kept at this temperature for 15 seconds. By soaking the beaker in ice water, the temperature of the milk was quickly reduced to 34 ° C. 10 ml of milk coagulating enzyme was added and mixed gently. The beaker was capped, wrapped in a thick towel and left to ferment for 1.5 hours. The cheese that became curd was cut into small squares. Cheese pieces were drained through cheesecloth. It was kept for 3-4 hours to release its water. 6 % saline solution was added on the cheese pieces in the jar and left to stand (Ünsal, 1997).

## Results

MK-4 and MK-7 contents of the milk and their products examined are shown in Table 2. MK-4 content in cows' milk was detected higher than goats' and ewes' milk. MK-7 contents were found to be almost the same. Among the cheese samples examined, the highest MK-4 concentrations were detected in weave cheese (470.30 ± 1.58 ng $g^{-1}$), cara cheese (464.54 ± 1.53 ng $g^{-1}$) and crushing cheese (414.46 ± 1.28 ng $g^{-1}$). Low MK-4 contents were found in cottage cheese (57.73 ± 0.17 ng $g^{-1}$) and white cheese (129.13 ± 0.35 ng $g^{-1}$). MK-7 contents of the cheese samples examined did not change much and were determined between 18.45 and 33.10 ng $g^{-1}$. Both MK-4 and MK-7 content of butter was higher than that of margarine. While MK-4 concentrations of the analyzed egg samples varied as egg < village egg < farm egg, no major differences were detected between MK-7 concentrations. The MK-4 content of yogurt (169.80 ± 0.43 ng $g^{-1}$) was found to be quite high compared to that of kefir (90.68 ± 0.16 ng $g^{-1}$).

Table 2. MK-4 and MK-7 contents of the milk and their products

| Sample | MK-4 (ng $g^{-1}$) | MK-7 (ng $g^{-1}$) | Total Vitamin K2 (ng $g^{-1}$) |
|---|---|---|---|
| **Cow milk** | 274.76 ± 1.02 | 24.72 ± 0.11 | 299.48 ± 1.02 |
| **Goat milk** | 182.35 ± 0.58 | 24.68 ± 0.10 | 207.03 ± 0.59 |
| **Sheep milk** | 129.58 ± 0.36 | 24.84 ± 0.11 | 154.42 ± 0.38 |
| **Cottage cheese** | 57.73 ± 0.17 | 18.45 ± 0.06 | 76.18 ± 0.18 |
| **Weave cheese** | 470.30 ± 1.58 | 33.10 ± 0.12 | 503.40 ± 1.58 |
| **Cheddar cheese** | 186.84 ± 0.59 | 26.78 ± 0.11 | 213.62 ± 0.60 |
| **Cubed cheese** | 208.42 ± 0.62 | 24.11 ± 0.10 | 232.53 ± 0.63 |
| **Cara cheese** | 464.54 ± 1.53 | 23.40 ± 0.09 | 487.94 ± 1.53 |
| **Creep cheese** | 187.96 ± 0.59 | 26.18 ± 0.10 | 214.14 ± 0.60 |
| **White cheese** | 129.13 ± 0.35 | 23.41 ± 0.09 | 152.54 ± 0.36 |
| **Spiced skim-milk cheese** | 170.29 ± 0.44 | 23.39 ± 0.08 | 193.68 ± 0.45 |
| **String cheese** | 241.28 ± 0.72 | 25.64 ± 0.10 | 266.92 ± 0.73 |
| **Crushing cheese** | 414.46 ± 1.28 | 24.73 ± 0.11 | 439.19 ± 1.28 |
| **Lavash cheese** | 331.35 ± 0.88 | 27.20 ± 0.11 | 358.55 ± 0.89 |
| **Moldy cheese** | 158.19 ± 0.40 | 30.17 ± 0.11 | 188.36 ± 0.41 |
| **Goat cheese** | 299.39 ± 1.02 | 26.13 ± 0.10 | 325.52 ± 1.02 |
| **Tulum cheese** | 272.47 ± 0.72 | 25.44 ± 0.09 | 297.91 ± 0.72 |
| **Farm cheese** | 170.32 ± 0.44 | 32.27 ± 0.11 | 202.59 ± 0.45 |
| **Cottage cheese** | 380.30 ± 1.05 | 23.41 ± 0.08 | 403.71 ± 1.05 |
| **Yogurt** | 169.80 ± 0.43 | 24.00 ± 0.10 | 193.80 ± 0.44 |
| **Butter** | 100.25 ± 0.20 | 41.64 ± 0.12 | 141.89 ± 0.23 |
| **Margarine** | 75.01 ± 0.15 | 39.05 ± 0.11 | 114.06 ± 0.19 |
| **Egg** | 147.97 ± 0.36 | 23.89 ± 0.08 | 171.86 ± 0.37 |
| **Farm egg** | 210.96 ± 0.63 | 28.22 ± 0.11 | 239.18 ± 0.64 |
| **Village egg** | 195.32 ± 0.59 | 24.32 ± 0.08 | 219.64 ± 0.59 |
| **Kefir** | 90.68 ± 0.16 | 26.96 ± 0.10 | 117.64 ± 0.19 |

In order to see the effect of the milk coagulating enzyme used in cheese making on the vitamin K2 content, cheeses were produced using both commercial and vegetable milk coagulating enzymes. The vitamin K2 contents of cheeses fermented with different milk coagulating enzymes are shown in Table 3. It was observed that the cheese fermented with chymosin had the highest MK-4 content (288.20 ± 0.98 ng $g^{-1}$) among the cheeses produced using commercial milk coagulating enzymes (chymosin, M.miehei, M.pusillus, shirdan). Among the cheeses produced using plant-based coagulants, it was determined that cheeses fermented with garlic (403.09 ± 1.05 ng $g^{-1}$) and fig milk (306.10 ± 0.97 ng $g^{-1}$) had the highest MK-4 content. However, when fig milk was mixed with milk coagulating enzymes such as chickpea and M.miehei, there was a decrease in MK-4 content of the cheeses obtained. As can be seen in Table 3, the milk coagulating enzyme variety had no significant effect on the MK-7 content of the cheeses.

Table 3. The vitamin K2 contents of cheeses fermented with different milk coagulating enzymes

| Type of milk coagulating enzymes | MK-4 (ng $g^{-1}$) | MK-7 (ng $g^{-1}$) | Total Vitamin K2 (ng $g^{-1}$) |
|---|---|---|---|
| **Chymosin** | 288.20 ± 0.98 | 23.75 ± 0.07 | 311.95 ± 0.98 |
| **M.miehei** | 265.37 ± 0.80 | 24.05 ± 0.07 | 289.42 ± 0.80 |
| **M.pusillus** | 228.67 ± 0.62 | 23.98 ± 0.06 | 252.65 ± 0.62 |
| **Shirdan** | 192.62 ± 0.56 | 23.83 ± 0.06 | 216.45 ± 0.56 |
| **Wild artichoke** | 169.43 ± 0.44 | 24.68 ± 0.08 | 194.11 ± 0.45 |
| **Vinegar** | 168.23 ± 0.42 | 27.46 ± 0.09 | 195.69 ± 0.43 |
| **Garlic** | 403.09 ± 1.05 | 23.69 ± 0.06 | 426.78 ± 1.05 |
| **Chickpea** | 182.13 ± 0.56 | 41.34 ± 0.10 | 223.47 ± 0.57 |
| **Fig milk** | 306.10 ± 0.97 | 33.97 ± 0.11 | 340.07 ± 0.98 |
| **Chickpea + fig milk** | 140.70 ± 0.42 | 29.85 ± 0.09 | 170.55 ± 0.43 |
| **M.miehei+ fig milk** | 170.60 ± 0.43 | 24.05 ± 0.07 | 194.65 ± 0.43 |

Although the vitamin K2 content of cheeses fermented with vegetable milk coagulating enzymes such as fig milk and garlic were found to be higher, such cheeses could not be molded. These cheeses are obtained in curd form. Therefore, commercial milk coagulating enzymes of M.miehei and chymosin were used for the rest of the study.

In order to see the effect of the fermentation time on the vitamin K content, cheeses were produced by fermenting at different times. The results obtained are given in Table 4. MK-4 content of the cheeses reached its maximum after 2 hours of fermentation, and then MK-4 content decreased with the increase of the fermentation time. MK7 content of the cheeses was affected neither by the milk coagulating enzyme type nor by the fermentation time.

Table 4. The vitamin K2 content of cheeses fermented for different periods

| Type of milk coagulating enzymes | Time (hours) | MK-4 (ng $g^{-1}$) | MK-7 (ng $g^{-1}$) | Total Vitamin K2 (ng $g^{-1}$) |
|---|---|---|---|---|
| **M.miehei** | 1 | 211.56 ± 0.60 | 25.31 ± 0.06 | 236.87 ± 0.60 |
| | 2 | 263.79 ± 0.78 | 25.16 ± 0.06 | 288.95 ± 0.78 |
| | 3 | 233.61 ± 0.62 | 25.33 ± 0.06 | 258.94 ± 0.62 |
| | 4 | 191.64 ± 0.57 | 24.68 ± 0.05 | 216.32 ± 0.57 |
| | 5 | 184.31 ± 0.45 | 25.00 ± 0.05 | 209.31 ± 0.45 |
| **Chymosin** | 1 | 197.91 ± 0.58 | 24.22 ± 0.04 | 222.13 ± 0.58 |
| | 2 | 291.44 ± 0.80 | 25.94 ± 0.06 | 317.38 ± 0.80 |
| | 3 | 197.83 ± 0.58 | 22.15 ± 0.04 | 219.98 ± 0.58 |
| | 4 | 172.54 ± 0.42 | 25.97 ± 0.06 | 198.51 ± 0.42 |
| | 5 | 168.15 ± 0.38 | 26.17 ± 0.07 | 194.32 ± 0.39 |

In order to examine the effect of waiting time on vitamin K content, cheeses were kept for 3 months and then their vitamin K content was determined. The vitamin K2 content of the kept cheeses is shown in Table 5. It was determined that MK-4 content of cheeses kept for two months increased, however, MK-4 content decreased after three months. No significant difference was observed in MK-7 contents.

Table 5. Vitamin K2 content of kept cheeses

| Type of milk coagulating enzymes | Holding period (month) | MK-4 (ng $g^{-1}$) | MK-7 (ng $g^{-1}$) | Total Vitamin K2 (ng $g^{-1}$) |
|---|---|---|---|---|
| | 1 | 268.89 ± 0.78 | 25.74 ± 0.06 | 294.63 ± 0.78 |
| **M.miehei** | 2 | 297.76 ± 0.80 | 23.47 ± 0.06 | 321.23 ± 0.80 |
| | 3 | 272.21 ± 0.78 | 21.59 ± 0.05 | 293.80 ± 0.78 |
| | 1 | 288.51 ± 0.62 | 24.77 ± 0.06 | 313.28 ± 0.62 |
| **Chymosin** | 2 | 323.17 ± 0.79 | 23.55 ± 0.06 | 346.72 ± 0.79 |
| | 3 | 295.66 ± 0.76 | 25.12 ± 0.06 | 320.78 ± 0.76 |

The total vitamin K2 contents of cheeses produced under laboratory conditions were lower than that of some cheeses sold in the market. However, studies have shown that the vitamin K2 content of cheeses can be increased when optimum conditions are obtained.

## Discussion

There are few studies in the literature that determine the vitamin K2 content of various cheeses and dairy products from different countries such as France, Italy, Norway, Greece, Sweden, Britain, Finland, and the Netherlands (Schurgers & Vermeer, 2000; Koivu-Tikkanen et al., 2000; Manoury et al., 2013; Fu et al., 2017; Vermeer et al., 2018). However, no studies have examined the animal products in Turkey. Total vitamin K2 contents were between 111 - 801 ng $g^{-1}$ for French cheeses, 235-494 ng $g^{-1}$ for British cheeses, 3-153 ng $g^{-1}$ for Italian cheeses, 65.3-433 ng $g^{-1}$ Swiss cheeses, 415-542 ng $g^{-1}$ Norwegian cheeses, 103-587 ng $g^{-1}$ for Greek cheeses, 248-763 ng $g^{-1}$ for Dutch cheese, 78-494 ng $g^{-1}$ for Finnish cheese. In our study, the total vitamin K2 content of the examined cheeses was determined between 76.18 and 503.40 ng $g^{-1}$. The total vitamin K2 content of Finnish (7 ng $g^{-1}$) and Dutch (9 ng $g^{-1}$) yogurts is quite low, while the total vitamin K2 content of Greek yogurt (282 ng $g^{-}$1) and our yogurt (193.80 ng $g^{-1}$) is much higher. Schurgers and Vermeer found the total vitamin K2 content of eggs and butter consumed in Finland as 330 and 150 ng $g^{-1}$, respectively. In margarine samples, they could not detect vitamin K2. In our study, the total vitamin K2 contents of egg, butter and margarine samples were found as 210.23, 141.89 and 114.06 ng $g^{-1}$, respectively. The difference between the results may be due to different sources of animal products.

In order to increase the vitamin K2 content of cheeses, the effects of milk coagulating enzymes, fermentation and holding times on vitamin K2 content were investigated by producing cheese in laboratory conditions. It was determined that the total vitamin K2 content of cheeses produced using commercial milk coagulating enzymes (chymosin, M.miehei, M.pusillus, shirdan) ranged between 216.45 and 311.95 ng $g^{-1}$, and the highest value was determined in cheese fermented with chymosin. The total vitamin K2 content of cheeses produced using plant-based milk coagulants (wild artichoke, vinegar, chickpea, garlic, fig milk) was found to be between 194.11 and 426.78 ng $g^{-1}$, and the highest value was found in cheese fermented with garlic. When milk coagulants were mixed, there was a decrease in the total vitamin K2 content of the cheeses. The total vitamin K2 content of cheeses produced using both M.miehei and chymosin reached a maximum after 2 hours of fermentation, and then began to decrease with the increase of fermentation time.

Fatal diseases such as cardiovascular diseases and cancer are increasing day by day. The preservatives, additives and dyes contained in the consumed foods contain chemicals that are harmful to health. Undoubtedly, at such a time, it is very important to determine which foods contain a vitamin that is both extremely beneficial and natural for health. As a result, this study revealed that cheeses are rich in vitamin K2 and this content can be further increased by adjusting the optimum conditions.

**Acknowledgment**

This research (19.M.021) was supported by the Scientific Research Projects Coordination Unit of Mustafa Kemal University. The authors would like to thank the Scientific Research Projects Coordination Unit of Mustafa Kemal University for financial support.

**Author Contributions**

All author contributions are equal for the preparation research in the manuscript.

**Conflict of Interest**

The authors declare that they have no competing interests.

**References**

Anastasi, E., Ialongo, C., Labriola, R., Ferraguti, G., Lucarelli, M., & Angeloni, A. (2020). Vitamin K deficiency and covid-19. *Scandinavian Journal of Clinical and Laboratory Investigation*, 80(7), 525-527. https://doi.org/10.1080/00365513.2020.1805122.

Booth, S.L. (2009). Roles for vitamin K beyond coagulation. *Annual Review of Nutrition*., 29, 89–110.

Capozzi, A., Giovanni, S., Stefano, L. (2020). Calcium, vitamin D, vitamin K2, and magnesium supplementation and skeletal health. *Maturitas*, 140, 55-63. https://doi.org/10.1016/j.maturitas.2020.05.020.

Duan, F., Mei, C., Yang, L., Zheng, J., Lu, H., Xia, Y., Hsu, S., Liang, H., Hong, L. (2020). Vitamin K2 promotes PI3K/AKT/HIF-1α-mediated glycolysis that leads to AMPK-dependent autophagic cell death in bladder cancer cells. *Scientific Reports*, 10(1), 7714. https://doi.org/10.1038/s41598-020-64880-x.

Ebina, K.., Shi, K., Hirao M. (2013). Vitamin K2 administration is associated with decreased disease activity in patients with rheumatoid arthritis. *Modern Rheumatology*, 23(5), 1001-1007. https://doi.org/10.1007/s10165-012-0789-4.

Ferland, G. (2012). Vitamin K and the nervous system: An overview of its actions. *Advances in Nutrition*, 3(2), 204–212. https://doi.org/10.3945/an.111.001784.

Finnan, E. G., Harshman, S. G., Haytowitz, D. B., Booth, S. L. (2017). Mixed dishes are an unexpected source of dietary vitamin K. *Journal of Food Composition and Analysis*, 64, 127-131. https://doi.org/10.1016/j.jfca.2017.04.002.

Fu, X., Harshman, S. G., Shen, X., Haytowitz, D. B., Karl, J. P., Wolfe, B. E., Booth, S. L. (2017). Multiple vitamin K forms exist in dairy foods. *Current Developments in Nutrition*, 1, e000638. https://doi.org/10.3945/cdn.117.000638.

Gijsbers, B.L.M.G., Jie, K.S.G., Vermeer, C. (1996). Effect of food composition on vitamin K absorption in human volunteers. *British Journal of Nutrition*, 76, 223-229. https://doi.org/10.1079/BJN19960027.

Goddek, S. (2020). Vitamin D3 and K2 and their potential contribution to reducing the COVID-19 mortality rate. *International Journal of Infectious Diseases*, 99, 286–290. https://doi.org/10.1016/j.ijid.2020.07.080.

Hadipour, E.,Tayarani-Najaran, Z., Fereidoni, M. (2020). Vitamin K2 protects PC12 cells against Aβ (1-42) and H2O2-induced apoptosis via p38 MAP kinase pathway. *Nutritional Neuroscience*, 23(5), 343-352. https://doi.org/10.1080/1028415X.2018.1504428.

Harsman, S. G., Shea, M. K. (2016). The role of vitamin K in chronic aging diseases: Inflammation, cardiovascular disease, and osteoarthritis. *Current Nutrition Reports*, 5, 90–98. https://doi.org/10.1007/s13668-016-0162-x.

Hubicka, U., Padiasek, A., Zuromska-Witek, B., Szlosarczyk, M. (2020). Determination of vitamins K1, K2 MK-4, MK-7, MK-9 and D3 in pharmaceutical products and dietary supplements by TLC-densitometry. *Processes*, 8(7), 870. https://doi.org/10.3390/pr8070870.

Koivu-Tikkanen, T.J., Ollilainen, V., Piironen, V.I. (2000). Determination of phylloquinone and menaquinones in animal products with fluorescence detection after postcolumn reduction with metallic zinc. *Journal of Agricultural and Food Chemistry*, 48, 6325-6331. https://doi.org/10.1021/jf000638u.

Manna, P., Kalita, J. (2016). Beneficial role of vitamin K supplementation on insulin sensitivity, glucose metabolism, and the reduced risk of type 2 diabetes: A review. *Nutrition*, 32(7-8), 732–739. https://doi.org/10.1016/j.nut.2016.01.011.

Manoury, E., Jourdon, K., Boyaval, P., Fourcassie, P. (2013). Quantitative measurement of vitamin K2 (menaquinones) in various fermented dairy products using a reliable high-performance liquid chromatography method. *Journal of Dairy Science*, 96(3), 1335-1346. https://doi.org/10.3168/jds.2012-5494.

Mizuta, T., Ozaki, I., Eguchi, Y., Yasutake, T., Kawazoe, S., Fujimoto, K., Yamamoto, K. (2006). The effect of menatetrenone, a vitamin K2 analog, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment: a pilot study. *Cancer*, 106, 867–872. https://doi.org/10.1002/cncr.21667.

Nimptsch, K.,Rohrmann, S., Linseisen, J. (2008). Dietary intake of vitamin K and risk of prostate cancer in the Heidelberg cohort of the European prospective investigation into cancer and nutrition. *American Journal of Clinical Nutrition*, 87(4), 985-992. https://doi.org/10.1093/ajcn/87.4.985.

Ozdogan, Y., Gokustun, K. K., Turk, O. P. (2017). Effect of vitamin K on insulin resistance. *Ankara Journal of Health Sciences*, (1-2-3), 37-47.

Palmer, C.R., Blekkenhorst, L.C., Lewis, J.R., Ward, N.C., Schultz, C.J., Hodgson, J.M., Croft, K. D., Sim, M. (2020). Quantifying dietary vitamin K and its link to cardiovascular health: a narrative review. *Food & Function*, 11(4), 2826-2837. https://doi.org/10.1039/C9FO02321F.

Ramazani, E., Fereidoni, M., Tayarani-Najaran, Z. (2019). Protective effects of vitamin K2 on 6 OHDA-induced apoptosis in PC12 cells through modulation bax and caspase-3 activation. *Molecular Biology Reports*, 46, 5777–5783. https://doi.org/10.1007/s11033-019-05011-2.

Sato, T., Inaba, N., Yamashita, T. (2020). MK-7 and its effects on bone quality and strength. *Nutrients*, 12, 965. https://doi.org/10.3390/nu12040965.

Schurgers, L.J., Vermeer, C. (2000). Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations. *Haemostasis*, 30, 298-307. https://doi.org/10.1159/000054147.

Shearer, M.J., Fu, X., Booth, S.L. (2012). Vitamin K nutrition, metabolism, and requirements: current concepts and future research. *Advances in Nutrition*, (Bethesda, MD), 3 (2), 182–195. https://doi.org/10.3945/an.111.001800.

Shibayama-Imazu, T., Sakairi, S., Watanabe, A., Aiuchi, T., Nakajo, S., Nakaya, K. (2003). Vitamin K2 selectively induced apoptosis in ovarian TYK-nu and pancreatic MIA PaCa-2 cells out of eight solid tumor cell lines through a mechanism different from geranylgeraniol. *Journal of Cancer Research and Clinical Oncology*, 129(1), 1-11. https://doi.org/10.1007/s00432-002-0393-7.

Shibayama-Imazu, T., Sonoda, I., Sakairi, S., Aiuchi, T., Ann, W.W., Nakajo, S., Itabe, H., Nakaya, K. (2006). Production of superoxide and dissipation of mitochondrial transmembrane potential by vitamin K2 trigger apoptosis in human ovarian cancer TYK-nu cells. *Apoptosis*, 11(9), 1535-1543. https://doi.org/10.1007/s10495-006-7979-5.

Shibayama-Imazu, T., Fujisawa, Y., Masuda, Y., Aiuchi, T., Nakajo, S., Itabe, H., Nakaya, K. (2008). Induction of apoptosis in PA-1 ovarian cancer cells by vitamin K2 is associated with an increase in the level of TR3/Nur77 and its accumulation in mitochondria and nuclei. *Journal of Cancer Research and Clinical Oncology*, 134(7), 803-812. https://doi.org/10.1007/s00432-007-0349-z.

Showalter, S.L., Wang, Z., Costantino, C.I. (2010). Naturally occurring K vitamins inhibit pancreatic cancer cell survival through a caspase-dependent pathway. *Journal of*

*Gastroenterology and Hepatology*, 25(4), 738-744. https://doi.org/10.1111/j.1440-1746.2009.06085.x.

Suttie, J.W., Booth, S.L. (2011). Vitamin K. *Advances in Nutrition*, 2, 440–441. https://doi.org/10.3945/an.111.000786.

Tarvainen, M., Fabritius, M., Yang, B. (2019). Determination of vitamin K composition of fermented food. *Food Chemistry*, 275, 515-522. https://doi.org/10.1016/j.foodchem.2018.09.136.

Tokita, H., Tsuchida, A., Miyazawa, K., Ohyashiki, K., Katayanagi, S., Sudo, H., Enomoto, M., Takagi, Y., Aoki, T. (2006). Vitamin K2-induced antitumor effects via cell-cycle arrest and apoptosis in gastric cancer cell lines. *International Journal of Molecular Medicine*, 17(2), 235-243. https://doi.org/10.3892/ijmm.17.2.235.

Ünsal, A. (1997). *Süt uyuyunca*. YKY Basımevi, İstanbul.

Vermeer, C., Raes, J., Hoofd, C.V.,Knapen, M.H.J., Xanthoulea, S. (2018). Menaquinone content of Cheese. *Nutrients*, 10, 1-9. https://doi.org/10.3390/nu10040446.

Xu, W., Chen, S., Wang, X., Tahara, K., Wu, H., Tanaka, S., Sugiyama, K., Sawada, T., Hirano, T. (2021). Suppressive effect of vitamin K2 against mitogen-activated peripheral blood mononuclear cells of rheumatoid arthritis patients. *Internatioanl Journal of Clinical Pharmacology and Therapeutics*, 59(1), 55-62. https://doi.org/10.5414/cp203827.

Wu, F. Y., Liao, W. C., Chang, H. M. (1993). Comparison of antitumor activity of vitamins K1, K2 and K3 on human tumor cells by two (MTT and SRB) cell viability assays. *Life Sciences*, 52(22), 1797-1804. https://doi.org/10.1016/0024-3205(93)90469-J.

Yaguchi, M., Miyazawa, K., Katagiri, T., Nishimaki, J., Kizaki, M., Tohyama, K., Toyama, K. (1997). Vitamin K2 and its derivatives induce apoptosis in leukemia cells and enhance the effect of all-trans retinoic acid. *Leukemia*, 11(6), 779-787. https://doi.org/10.1038/sj.leu.2400667.

Yoshida, T., Miyazawa, K., Kasuga, I. (2003). Apoptosis induction of vitamin K2 in lung carcinoma cell lines: the possibility of vitamin K2 therapy for lung cancer. *International Journal of Oncology*, 23(3), 627-632. https://doi.org/10.3892/ijo.23.3.627.

Yoshida, M., Jacques, P. F., Meigs, J. B., Saltzman, E., Shea, M. K., Gundberg, C., Dawson Hughes, B., Dallal, G., Booth, S.L. (2008). Effect of vitamin K supplementation on insulin resistance in older men and women. *Diabetes Care*, 31(11), 2092–2096. https://doi.org/10.2337/dc08-1204.

Zhang, Y. F., Liu, Z. P., Duan, L.L., Ji, Y.Y., Yang, S., Zhang, Y., Li, H.Y., Wang, Y., Wang, P., Chen, J.P., Li, Y. (2020). Effect of low-dose vitamin K2 supplementation on bone mineral density in middle-aged and elderly Chinese: A randomized controlled study. *Calcified Tissue International*, 106(5), 476-485. https://doi.org/10.1007/s00223-020-00669-4.