Dr n. wet. inż. Magdalena POLAK-ŚLIWIŃSKA[1]
Inż. Jolanta REMISZEWSKA[2],
Mgr inż. Mariusz ŚLIWIŃSKI[3]
[1]Department of Commodity Science and Food Analysis, Faculty of Food Science
University of Warmia and Mazury in Olsztyn, Poland
Katedra Towaroznawstwa i Badań Żywności, Wydział Nauki o Żywności
Uniwersytet Warmińsko – Mazurski w Olsztynie, Polska
[2]Student Scientific Circle of Food Hygiene and Toxicology
Department of Commodity Science and Food Analysis, Faculty of Food Science
University of Warmia and Mazury in Olsztyn, Poland
Studenckie Koło Higieny Żywności i Toksykologii
Katedra Towaroznawstwa i Badań Żywności, Wydział Nauki o Żywności
Uniwersytet Warmińsko – Mazurski w Olsztynie, Polska
[3]Instytut Innowacji Przemysłu Mleczarskiego Sp. z.o.o., Kormoranów 1, 11-700 Mrągowo, Polska
Dairy Industry Innovation Institute Ltd., Kormoranów 1, 11-700 Mrągowo, Poland

EFFECT OF RIPENING PROCESS AND FAT CONTENT ON CHANGES IN VITAMIN K LEVEL OF CHEESE®

Wpływ procesu dojrzewania i zawartości tłuszczu na zmiany poziomu witaminy K w serach®

Key words: vitamin K_1, vitamin K_2, cheese ripening, HPLC method.

I*n this study, the vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinone) contents of a selected range of cheeses were measured by high-performance liquid chromatography (HPLC) and compared with the fat content, maturation time and origin of the cheeses.*

In our study, the highest vitamin K_2 content was recorded in Gouda (678.12 ng/g), Edam (712.70 ng/g) and Emmentaler (733.10 ng/g) cheeses with comparable levels of vitamin K1 in the analysed products (31.60 ng/g, 34.63 ng/g and 24.35 ng/g, respectively) and fat content (27%, 28% and 30%, respectively), as well as in Gouda cheese with a fat content of 48% after 48 weeks of maturation (756.50 ng/g). The fat content of the cheese was a factor that influenced the vitamin K content, with products with lower fat content having lower total vitamin K content compared to the other products analysed.

Given the reports that poor vitamin K status is one of the risk factors for cardiovascular disease in the absence of conclusive evidence of adverse cardiovascular effects of dairy fats, cheese should be considered as an important dietary component for those concerned about heart health.

Słowa kluczowe: witamina K_1, witamina K_2, dojrzewanie sera, metoda HPLC.

W tym badaniu oznaczono zawartość witaminy K_1 (filochinonu) i witaminy K_2 (menachinonu) w wybranej gamie serów metodą wysokosprawnej chromatografii cieczowej (HPLC) i porównano z zawartością tłuszczu, czasem dojrzewania i pochodzeniem serów.

W badaniach własnych największą zawartość witaminy K_2 odnotowano w serach Gouda (678,12 ng/g), Edam (712,70 ng/g) i Emmentaler (733,10 ng/g) przy porównywalnych poziomach witaminy K_1 w analizowanych produktach (odpowiednio 31,60 ng/g, 34,63 ng/g i 24,35 ng/g) i zawartości tłuszczu (odpowiednio 27%, 28% i 30%), jak również w serze Gouda o zawartości tłuszczu 48% po 48 tygodniach dojrzewania (756.50 ng/g). Zawartość tłuszczu w serze była czynnikiem, który wpływał na zawartość witaminy K, przy czym produkty o niższej zawartości tłuszczu miały niższą całkowitą zawartość witaminy K w porównaniu z pozostałymi analizowanymi produktami.

Biorąc pod uwagę doniesienia, że słaby status witaminy K jest jednym z czynników ryzyka chorób sercowo-naczyniowych przy braku jednoznacznych dowodów na niekorzystny wpływ tłuszczów mlecznych na układ krążenia, sery powinny być traktowane jako ważny składnik diety osób, które dbają o zdrowie serca.

Corresponding author – Adres do korespondencji: Magdalena Polak-Śliwińska, Department of Commodity Science and Food Analysis, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, Pl. Cieszyński 1, 10-957 Olsztyn; e-mail:m.polak@uwm.edu.pl

INTRODUCTION

Dairy products have historically been linked to cardiovascular disease (CVD) risk due to their relatively high saturated fatty acids (SFAs) content [3], which has changed with research from the last decade showing that the association between SFAs and CVD is highly dependent on the type and chain length of SFAs present in a given food, as well as the type of food and other nutrients in it, which is known as the food matrix effect [11, 16, 33, 48]. While some foods high in SFA are associated with negative health consequences, others, such as dairy products, have a positive effect on CVD risk markers in multiple meta-analyses [1, 16]. A systematic review and meta-analysis on individual dairy products showed that the consumption of different dairy products has an individual impact on CVD risk markers [16]. Many of these studies specifically point to cheese as either a positively positive or neutral product in terms of CVD risk, despite its saturated fat content [12]. The specific matrix of cheese and the structure of the protein and other nutrients it contains may partly explain these benefits [19]. Cheese and curd are the most important sources of long-chain menaquinones (MK) in the Western diet [49]. A number of recent reviews highlight the need to understand the interactions between vitamin K_2, food structure and other chemical components to fully elucidate the health effects of the dairy matrix [19, 48].

During the cheese ripening process, a number of transformations take place, including physicochemical transformations (curd formation, whey syneresis, NaCl diffusion), microbiological transformations (autolysis of the sourdough starter, growth of secondary microflora such as non-sourdough *Lactobacillus* bacilli and technologically harmful microflora), and consequently all biochemical reactions take place under the influence of milk native, coagulating and bacterial enzymes [13, 44]. The growth of lactic fermentation bacteria is influenced by free amino acids, low-molecular-weight peptides, nucleotides, glutathione and vitamins present in milk. The opposite effect is exerted by the presence of fatty acids, immunoglobulins, lactoperoxidases, bacteriocins, residues of cleaning and disinfecting agents [13, 14, 44]. Dairy fat is relatively high in SFA content and has been associated with cardiovascular disease, yet evidence exists for either neutral or beneficial effects from cheese consumption, due to the specific food matrix [16, 19].

Vitamin K is an essential bioactive compound required for optimal body function [23]. The term vitamin K, or naphthoquinone, refers to a family of fat-soluble molecules which have a similar structure made by a 2-methyl-1,4-naphthoquinone ring but with different origin and function [24]. Currently, three primary forms are known, which differ in the side chains linked to the 2-methyl-1,4-naphthoquinone ring at the position 3 [21]; namely, these are vitamin K_1 (phylloquinone), vitamin K_2 (menaquinone), and vitamin K_3 (menadione). The main known biological function of vitamin K_1 is played in blood clotting, since it acts as a cofactor for the enzymatic conversion of glutamic acid (Glu) residues to gamma-carboxyglutamic acid (Gla) in vitamin K-dependent proteins (VKDPs), through vitamin K-dependent gamma-glutamyl carboxylase, localized in the endoplasmic reticulum of the cells of all mammalian tissues [10, 42], and for the conversion of protein-bound glutamate in carboxy-glutamate, needed for II, VII, IX, and X coagulation cascade factors, and for the natural anticoagulants proteins S and C [35, 40]. The source of vitamin K_1 is mainly represented by leafy or flowering vegetables (spinach, lettuce, broccoli, cabbage, Brussels sprouts, turnip greens), but chickpeas, peas, soya, green tea, eggs, pork, and beef liver also contain vitamin K_1 [37, 39, 54, 55]. Vitamin K_2 is synthetized essentially by intestinal microbiota and is denoted as menaquinone (MK); according to the length of the isoprene chain attached to the methylated naphthoquinone ring, several different forms could be identified, as numbered from 4 to 13 [29, 31]. Vitamin K_2 MK-4 is obtained from the conversion of phylloquinone or menadione and is found mainly in meat and animal by-products such as eggs, cow's milk and yoghurt [7, 23, 49]. On the other hand, K_2 MK-7 is a long-chain form also produced by intestinal bacteria and it is found in fermented food, such as cheese and soya [15, 49, 51]. The K_2 MK-4 and K_2 MK-7 are two of the most common menaquinones in the human diet, along with K_2 MK-8, K_2 MK-9, and K_2 MK-10 [7]. Considerable variability in values was also reported across cheese types and in different studies. Potential reasons include different starter cultures used, fermentation conditions, the fat content and milk source [57]. The difference in structure between K_1 and K_2 can be seen in their different rates of absorption, tissue distribution and bioavailability. Despite the differences in structure, both act as a cofactor for the enzyme gamma-glutamylcarboxylase, involving both hepatic and extrahepatic activity. Only the carboxylated proteins are active and promote the health profile of haemostasis [23]. Vitamin K_3, also known as menadione, was formerly considered to be a synthetic form of vitamin K. However, it has been demonstrated that vitamin K_3 could also originate in the intestine as the intermediate product of oral vitamin K_1 conversion to vitamin K_2, namely MK-4 [9, 56]. Vitamin K absorption occurs in different tracts of the intestine: vitamin K_1 is absorbed in the ileum; vitamins K_2 in the colonic portions. Efficient biliary and pancreatic function is essential for its adequate absorption [5]. Vitamin K molecules are incorporated into chylomicrons and then released to very low-density lipoprotein (VLDL) and lowdensity lipoprotein (LDL), with subsequent release to tissues. Vitamin K_1 and K_2 should be continually synthetized and supplied by intestinal bacteria, due to their relatively short half-life (17 h) [5]. The intake recommendations for vitamin K by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) are 65 µg /day for men and 55 µg /day for women, based on a calculated requirement of 1 µg /day/kg body weight [5].

Chronic kidney disease (CKD) is commonly associated with vitamin K deficiency. CKD is characterized by a secondary hyperparathyroidism: the progressive reduction of GFR leads to an increase of serum phosphate levels, a progressive hypocalcemia, and augmented fibroblast growth factor 23 (FGF-23) production [5]. Meanwhile, the reduced activity of the enzyme 1 alpha-hydroxylase induces a decrease of 1,25-dihydroxyvitamin D_3, further determining a parathyroid hormone (PTH) rising. High serum phosphate and FGF-23 levels also stimulate an increase of sclerostin production by osteocytes [5]. Sclerostin and FGF-23 are involved in the progression of vascular calcification (VC). Some of the serious complications of CKD are represented by cardiovascular disease (CVD) and skeletal fragility with

an increased risk of morbidity and mortality [5]. A complex pathogenetic link between hormonal and ionic disturbances, bone tissue and metabolism alterations, and vascular calcification (VC) exists and has been defined as chronic kidney disease-mineral and bone disorder (CKD-MBD). Poor vitamin K status seems to have a key role in the progression of CKD, but also in the onset and advance of both bone and cardiovascular complications [5, 58].

Vitamin K plays different roles, including in activating vitamin K-dependent proteins (VKDPs) and in modulating bone metabolism and contributing to the inhibition of VC [5]. Vitamin K is considered a possible marker of kidney, CV and bone damage in the CKD population and its potential use to promote health in this clinical setting is being investigated. Treatment strategies for osteoporosis and CV disease associated with CKD should include vitamin K supplementation [5]. According to a Scientific Opinion provided by the European Food Safety Authority (EFSA), the vitamin K dietary reference values (DRVs) for the European population are estimated to be 1 µg/kg body weight per day of phylloquinone, which corresponds to an amount of 70 µg phylloquinone/day for adults, both women and men. Since data about menaquinones absorption, function and content in the body or organs are limited, EFSA released adequate intake recommendations for phylloquinone only [17]. This amount of phylloquinone could play a role in reduction of CVD progression, especially in Arterial Hypertension acting on arterial calcification activity and arterial stiffening [4, 41, 46]. Intravascular thrombosis and pulmonary fibrosis in COVID-19 patients with pneumonia are significantly associated with disease severity [45]. Vitamin K is known to balance clotting mechanisms and prevent calcification and fibrosis of extrahepatic soft tissues. The paper by Souparnika et al [45] presented reports collected from WHO, PubMed, Scopus and Clinical Trial Registry databases searched using relevant keywords. Among the original articles were the few observational studies that showed reduced levels of vitamin K as well as activated extrahepatic vitamin K-dependent proteins (VKDP) in COVID-19 patients compared to healthy controls [28, 45]. Chronic treatment with vitamin K antagonists did not reduce the risk of in-hospital death. A docking study was conducted using Swiss docking and showed a significant interaction between menaquinone and SARS-CoV-2 major protease (SARS-CoV-2 Mpro). The studies presented show that vitamin K deficiency in COVID-19 may be caused by overuse of antagonists or faulty ingestion or absorption [45]. This causes an imbalance in the normal coagulation-anticoagulation mechanism by diverting available vitamin K to the liver, thereby causing a deficiency of the same in extrahepatic tissues, ultimately leading to thrombosis. This also prevents carboxylation and activation of extrahepatic VKDP required to prevent soft tissue calcification, leading to pulmonary fibrosis. The authors concluded that vitamin K supplementation should be considered as a potentially modifiable risk factor in severe COVID-19. Randomized controlled trials are recommended to provide more conclusive evidence in this regard [45].

Alzheimer's disease is defined as a progressive brain disorder that affects memory, thinking and language skills and the ability to perform the simplest of tasks [2, 27]. In a study by Huy et al. [26], serum levels of vitamin K_2 were reduced in Alzheimer's disease (AD) patients. Vitamin K_2 has the potential to slow the progression of AD and contribute to its prevention. Hadipour et al. [22] investigated the effects of vitamin K_2 at concentrations ranging from 5 to 200 µM in rat pheochromocytoma PC-12 cells to provide protection against hydrogen peroxide and β-amyloid-induced toxicity. In Alzheimer's disease, β-amyloid led to neuronal death through direct toxicity and by promoting apoptosis, which is prevented by vitamin K_2 [22]. Thus, the study showed, among other things, that pretreatment with vitamin K_2 decreased apoptosis signalling proteins (β-amyloid, caspase 3, etc.), attenuated ROS levels and increased glutathione levels. The study confirmed the protective role of vitamin K_2 mediated by its antioxidant and anti-apoptotic properties [22].

With the growing interest in the health benefits of long-chain menaquinones [50], it is important to have up-to-date data on vitamin K concentrations in the human diet. In the present study, we focused on the vitamin K content of different forms of cheese. We compared the vitamin K content of the most popular Dutch cheese taking into account the influence of ripening period and fat content, and also examined popular cheeses available in Poland and cheeses from different geographical areas in Europe and their relative vitamin K content. The data obtained can be used to calculate vitamin K intake from food frequency questionnaires in population-based studies.

MATERIALS AND METHODS

Reagents and standards

All solvents were of high purity: n-hexane and 2-propanol for vitamin extraction and acetonitrile, methanol and orthophosphoric acid for chromatographic analyses were purchased from Merck Life Science Sp.z.o.o. (Poland). Certified analytical standards for vitamin K_1 (Supelco, ≥99.0% purity) and vitamin K_2 (Supelco, 99.9% purity) were purchased from Sigma Aldrich. Vitamins K_1 and K_2 were identified by retention time and quantification was performed using the standard curve method. For this purpose, six dilutions of the analytical standard of vitamin K_1 and K_2 were prepared in acetonitrile with concentrations ranging from 5.15 to 250 µg/ml.

Origin of cheese samples for study

Most of the products mentioned in this paper were purchased in Polish supermarkets (industrial Gouda, Edam and Emmentaler cheeses) and specialty grocery stores (foreign cheeses), as well as from a Dutch manufacturer (Gouda cheese after different ripering time) for their analysis for vitamin K determination. Popular cheeses on the Polish market, differing in fat content, were selected. These cheeses were compared with those from different European countries (Greek, Italian and French cheeses). The fat content reported in this study refers to the content reported on the labels of the products selected for the study (Table 1). Immediately after transporting the samples from the place of purchase to the testing laboratory, all samples were stored frozen at -20□, protected from light and oxygen to prevent loss of vitamin K during the storage period until analysis.

Table 1. Study material

Tabela 1. Materiał badawczy

Sample description	Primary samples, n	Purchase location/Country of origin
Gouda cheese	2	Polish supermarkets /Poland
Edam cheese	2	Polish supermarkets /Poland
Emmentaler cheese	2	Polish supermarkets /Poland
Gouda cheese, 8 weeks of ripening	4	Dutch manufacturer / The Netherlands
Gouda cheese, 48 weeks of ripening	4	Dutch manufacturer / The Netherlands
Feta cheese	2	Polish specialty grocery stores/ Greece
Mozzarella cheese	2	Polish specialty grocery stores/ Italy
Grana Padano cheese	2	Polish specialty grocery stores/ Italy
Camembert cheese	2	Polish specialty grocery stores/ France

Source: Own study

Źródło: Opracowanie własne

Stages of the production of Dutch gouda cheese

The gouda cheese used in the study was produced from milk from Holstein-Friesian cows in their third and fourth lactation periods, kept at the Farm„De Bitenhoeve" located in the village of Haaften, the Netherlands. The average annual yield of cows was 12 000 kg of milk, while the daily average was 34 liters of milk, with 4.2% of fat and 3.3% of protein. The production of gouda cheese used raw milk obtained from evening milking using a Lely Astronaut automatic milking robot, which was pasteurized at 72°C for 15 seconds, then pumped from the pasteurizer to the cheese boiler and cooled to 32°C. After reaching the required temperature, deep-frozen concentrated starter cultures were added to the milk. After the initial fermentation period, the enzyme that causes coagulation of milk- rennet and calcium chloride were added to the processed milk. The milk was then subjected to thorough mixing. Subsequently, sodium (V) nitrate ($NaNO_3$) was added to the raw material. The milk thus treated was left until curdling (about 35 minutes). Once the curd was formed, it was evaluated using a cheese knife to see if the curd was ready for slicing. The curd was then sliced into 3-6 mm grains for about 10 min. After slicing the curd and pre-drying the resulting grains, the excess whey that had formed, in the amount of 30-40%, was drained off. The next step was grain drying, which involved removing excess whey from the grain by stirring the curd with warm process water. This was followed by reheating the grain to 38°C and re-drying it by intense stirring. After that, a grain compression test was carried out, namely, it was checked whether the grain was dry and did not stick together. After this step, the cheese grains were transferred to their respective molds and were subjected to turning after about 15 minutes of molding. The 10 kg cheeses formed in this way were pressed for 3 h. After this procedure, they were transferred to brine with a concentration of 22% and left for about 24 h. After this process, the cheeses were placed on appropriately prepared shelves and re-dried for another 24 h. In the next stage, the cheeses were covered with paraffin and turned daily for several consecutive days. After this time, the cheeses were transferred to a ripening room with 85% humidity and 12°C, where they were subjected to the ripening process. Care of the cheeses during ripening consisted of turning them (once a week) and wiping their surfaces and shelves with a chlorine solution. In order to obtain the appropriate sensory qualities, short- and long-matured gouda cheeses were aged in the ripening room for 8 weeks and 48 weeks, respectively. After reaching the required maturity, a total of 8 samples (4 of each type of cheese), with an average weight of 240 g, were taken from 4 pieces of short-matured gouda cheese and 4 pieces of long-matured gouda cheese. The cheese samples prepared in this way were then vacuum-packed (Figure 1) and transported in a portable isothermal container at a temperature of 4±1°C to the laboratory for quantitative and qualitative analyses including analysis of vitamin K content (Figure 2). The tests were carried out within the shelf life declared by the manufacturer.

Fig. 1. Samples of long and short matured Gouda cheese – vacuum packed.

Rys. 1. Próbki sera Gouda długo- i krótkodojrzewającego – pakowane próżniowo.

Source: (photo. J. Remiszewska)

Źródło: (fot. J. Remiszewska)

Extraction of vitamins from cheese samples

Sample preparation followed the procedure described by Schurgers and Vermeer [39].

The cheese sample was crushed, then an analytical portion of 1 g was taken, followed by the addition of 4 mL of 2-propanol and 2 mL of distilled water. After homogenisation, the mixture was heated to 60°C and then extracted with 8 mL of hexane. The hexane was evaporated and the sample was then re-dissolved in 2 mL of hexane. After prepurification over silica Sep-Pak cartridges, to allow for the final determination of vitamin K, the procedure was modified by replacing the solvent with acetonitrile and filtering through a 0.45 µm membrane filter immediately before analysis by high-performance liquid chromatography (HPLC) after prior evaporation of the solvent under a stream of nitrogen [47].

Analysis of HPLC

All analyses were performed on a Shimadzu HPLC system equipped with a photodiode array spectrophotometric detector (DAD) (Kyoto, Japan). Data were processed using LCSolution software. Chromatography was carried out at

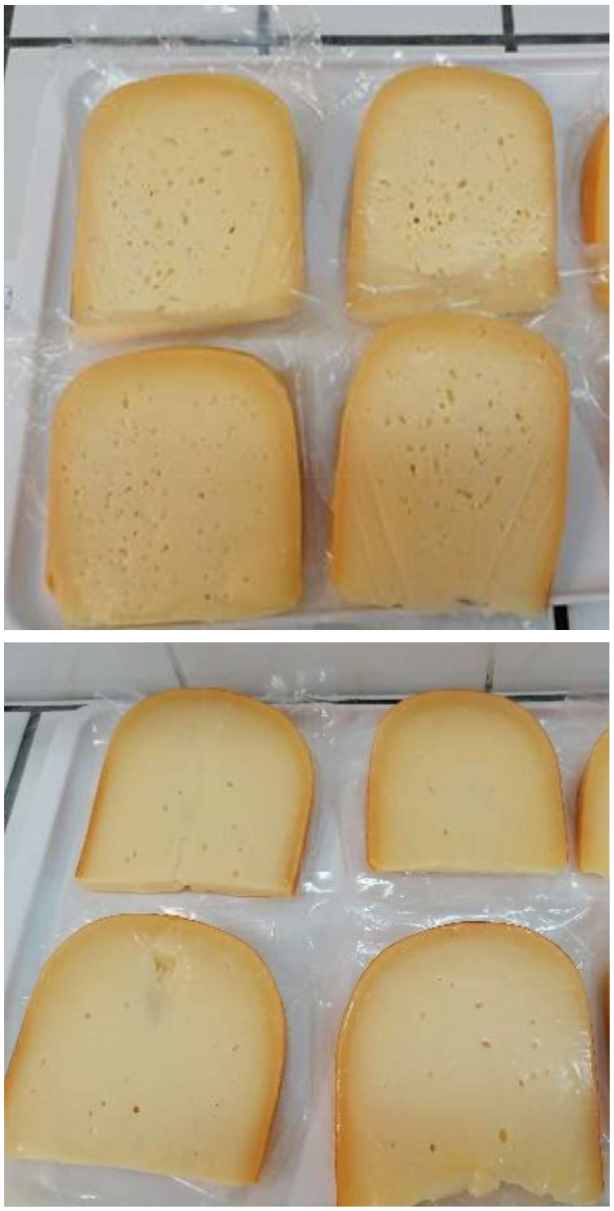

Fig. 2. Samples of long (a) and short (b) matured Gouda cheese - cross-sectional area.
Rys. 2. Próbki sera Gouda długo- (a) i krótkodojrzewającego (b) – powierzchnia w przekroju.
Source: (photo. J. Remiszewska)
Źródło: (fot. J. Remiszewska)

25°C. Sample separation was performed on a Phenomenex Kinetex C18 column (150 x 2.1 mm) packed with 1.7 µm particles. The chromatographic conditions were as follows [43]: in gradient elution mode, a mixture of water and methanol (1:1, v/v) acidified to pH 3.0 with orthophosphoric acid (A) and acetonitrile (B) was used as the mobile phase, the eluent flow rate was 0.8 mL. The sample injection volume was 20 µL. Vitamins K_1 and K_2 were recorded in the same run. All data given are means of duplicate samples that were analyzed separately.

The limits of detection (LOD) and limits of quantification (LOQ) were checked experimentally using an established standard curve for a series of standard solutions [32].

DATA ANALYSIS

All analyses were conducted in triplicate and the data expressed as mean ± standard deviation.

RESULTS AND DISCUTION

Analyses of selected cheese samples for vitamin K determination were preceded using an HPLC/UV-DAD system (270 nm). To improve selectivity and ensure high accuracy, the evaluation focused on linearity, LOD and LOQ as validation parameters. Six different analytical standard solutions of vitamin K_1 and K_2 were analysed three times (each separately) by chromatography and regression equations were obtained (Table 2). The linearity of the detector response was established using the squared correlation coefficients (R^2) determined for the calibration curves and, at the same time, the regression line was shown to be linear over the range of all concentrations tested (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the formulae: $3.3\times\sigma$ / S, and $10\times\sigma$ / S, respectively [32], where σ is the standard deviation of the response/signal and S is the slope of the calibration curve. In our study, the LOD and LOQ values for vitamins K_1 and K_2, respectively, are presented in Table 2.

Cheese is an important source of this microbiologically synthesised vitamin [52, 53]. Several studies have reported high levels of total and individual MKs in cheese and fermented dairy [20, 25, 30, 36, 38, 49, 53].

Selected cheeses commercially available in Poland with different fat contents and cheeses produced in the Netherlands with different ripening times were analysed for vitamin K_1 and vitamin K_2 content (Table 3). Dairy products are a relatively low source of K_1, but a good source of K_2, which was also confirmed in the conducted study. The presence of both types of vitamin K was found in all analysed cheeses, with lower vitamin K_2 content (468.20 ng/g) determined in the young cheese samples with a shorter ripening time than in the older cheese samples (756.50 ng/g), similar to the study by Vermeer et al. [49]. A study by Veermer et al. [49] found that in the most popular full-fat cheese (Gouda 13 weeks, 50% fat in dry matter), the

Table 2. Values of correlation coefficient, recovery, LOD and LOQ and regression formulas for K1 and K2 vitamins
Tabela 2. Wartości współczynnika korelacji, odzysku, LOD i LOQ oraz równania regresji dla witamin K1 i K2

Compound	Calibration Curve	Correlation coefficient (R^2)	Recovery (%)	LOD (µg/ml)	LOQ (µg/ml)
K_1	y = 1.4831x − 0.1171	0.9987	85.50	0.25	1.94
K_2	y = 0.4115x + 0.3638	0.9998	91.30	0.30	2.15

Source: The own study
Source: Badania własne

content of menaquinones was around 650 ng/g, while in the very young cheese (Gouda 4 weeks) it was significantly lower (473 ng/g). No significant differences were found between freshly cut cheeses and vacuum-packed products. Most likely, this difference is mainly due to increased levels of long-chain menaquinones in more mature cheeses, which come from bacterial growth during fermentation. The main factors influencing vitamin K_2 formation are the bacterial strains used and the scalding temperature, which has a direct influence on the bacterial species [53]. Mesophilic lactic acid bacteria such as *Lactococcus* and *Leuconostoc* species are known for their menaquinone formation potential. Thermophilic bacterial strains as well as high scalding temperatures lead to reduced amounts of menaquinones. In Emmentaler, 'eye'-forming propionic acid bacteria are responsible for the formation of tetrahydromenachinon-9 [MK-9(H_4)], which is typical of this variety [53]. The contribution of selected cheese types to vitamin K_2 supply can be very significant. The large differences between varieties can largely be explained by the cultures and processing procedures used, especially for the stages that have a large impact on the growth of the cultures, where the temperature chosen determines the survival of the different bacterial species [53]. Differences within a given variety, both between seasons (winter/summer) and between different cheese producers, can be very large, but cannot be reconstructed with the data collected. Further detailed research is required for this purpose [53].

Table 3. K_1 and K_2 content of cheeses purchased in Poland and the Netherlands

Tabela 3. Zawartość K_1 i K_2 w serach zakupionych w Polsce i w Holandii

Sample description	Fat content [g/100 g]	K_1 [ng/g]	K_2 [ng/g]
Gouda cheese	27	31.60±1.55	678.12±3.51
Edam cheese	28	34.63±1.77	712.70±4.10
Emmentaler cheese	30	24.35±1.80	733.10±2.89
Gouda cheese, 8 weeks of ripening	48	36.71±1.82	468.20±3.91
Gouda cheese, 48 weeks of ripening	48	39.45±1.46	756.50±5.40
Feta cheese	21	14.50±0.38	114.80±1.60
Mozzarella cheese	22	15.24±0.81	60.50±2.21
Grana Padano cheese	29	20.64±1.15	2.94±0.56
Camembert cheese	24	48.15±2.20	121.25±1.76

Concentrations are presented as the mean of duplicate analyses ± standard deviation with a variation coefficient < 15%.

Source: The own study

Source: Badania własne

The study Bertola at al. [6] analysed the effects of ripening temperature, type of packaging film and storage period before packaging on the degree of proteolysis and texture of Gouda cheese in order to determine the optimal ripening conditions. Gouda cheeses from a local plant were subjected to different ripening conditions. Only ripening time and temperature had a significant effect on water content, non-protein nitrogen concentration and rheological parameters. The results indicated that the texture characteristics of Gouda cheese ripened in low gas permeability plastic films were similar to those of traditionally ripened Gouda cheese. Texture development was accelerated by increasing the storage temperature [6].

When comparing cheeses from different countries in the study, significant differences in vitamin K_2 content were observed (Table 3). In particular, cheeses produced in Mediterranean countries (feta, mozzarella) were characterised by low vitamin K_2 content (114.80 ng/g and 60.50 ng/g, respectively), and Grana Padano cheese had negligible levels of K_2 compared to the products tested (2.94 ng/g). French Camembert cheese was characterised by higher levels of vitamin K_1 (48.15 ng/g) and K_2 (121.25 ng/g) compared to the other cheeses tested. Vermeer et al. [49] conducted a study of vitamin K_2 content in cheese and other foods. In a study by Vermeer et al. [49] the highest vitamin K_2 content in French cheeses was measured in Münster cheese (made from raw, unpasteurised milk) with 801 ng/g of total vitamin K_2. The two British cheeses measured (Cheddar and Stilton) contained medium to high amounts of vitamin K_2 (235 ng/g and 494 ng/g, respectively), as did the Swiss cheeses, Emmental (433 ng/g) and Raclette (323 ng/g), while Gruyère contained low amounts (65.30 ng/g). The study authors highlight the fact that the Swiss Emmental cheese contained almost exclusively K_2 MK-10, which is produced by the probiotic *Propionibacterium freudenreichii*, which is also thought to be responsible for the large holes and typical taste of this cheese. In contrast, the two Norwegian cheeses measured, Norvegia and Gamalost, contained relatively high amounts of vitamin K_2, 415 ng/g and 542 ng/g respectively Vermeer et al. [49]. Based on the global state of K_2 deficiency and the strong evidence indicating that it is a cardio-protective nutrient, the researchers recommend cheese as a component of a heart-healthy diet due to its vitamin K_2 content. However, the actual content of menaquinones varies considerably and depends on the type of cheese, maturation time, fat content and the geographical area where the cheeses are produced Vermeer et al. [49]. The results of the study showed that the total amount of K_2 in cheese ranged from 3 to 802 ng/g. Therefore, the researchers' recommendation for K_2 is in the range of 180–360 µg/day [49]. In the last decade, more and more attention has been paid to the health benefits of vitamin K_2, especially the long-chain menaquinones K_2 MK-6 through K_2 MK-9. This research complements important population-based studies that have shown that food-derived vitamin K (including cheese) improves long-term cardiovascular health outcomes by more specifically providing a spectrum of long-chain menaquinones, from menaquinones 5 to 10, while menaquinones 6, 7 and 9 are among the most bioactive, representing the best dietary source of menaquinones in Western countries [7, 8]. Nevertheless, it is important to be aware that the majority of the population cannot consume enough of these daily to obtain optimal amounts of vitamin K_2, nor is cheese a practical source for extracting menaquinones [7, 8, 19].

Recent study in the USA found that vitamin K_2 was present in higher amounts in the higher-fat dairy and yoghurt products tested compared to lower-fat and non-fat products [20, 57].

Vitamin K_2 is found in much higher amounts in cheese, but noteworthy is the significant variation in the same cheese varieties from different studies. For example, Fu et al. [20] reported values of 281 µg/100 g for total vitamin K in cheddar cheese, while Vermeer et al. [49] reported a much lower value for total vitamin K (25.66 µg/ 100 g) in the cheddar cheese analysed in their study. Values reported for blue cheeses are similarly variable, with total vitamin K values between 5.05 and 37.2 µg/ 100 g reported by Manoury et al. [36] from some French blue cheeses, while US blue cheeses ranged from 399.30 to 480.70 µg/ 100 g [20]. As MK content is mainly derived from bacteria, some specific fermentation factors in the cheese may influence these levels. Starter cultures are one potential influencing factor and are of particular importance in cheese production. Lactic acid bacteria (LAB) are commonly used as primary starter cultures in cheese production [18]. This is confirmed by several studies reporting high levels of total vitamin K_2 in Gouda cheese from the Netherlands (47.30-72.90 µg/ 100 g), Edam cheese from the Netherlands (64.70 µg/ 100 g), cheddar cheese from the USA (266.80–290.40 µg/ 100 g) and 4% fat Cottage cheese from the USA (49.10–55.70 µg/ 100 g) [20, 49]. Thermophilic fermented cheeses show much lower levels of MK compared to cheeses fermented by mesophilic bacteria. [57]. Manoury et al. [36] found no detectable levels of total vitamin K_2 in Comté cheese from France and Mozzarella cheese from Germany. Other studies have reported relatively low levels of total vitamin K_2 in Comté cheese (11.50–13.60 µg/ 100 g) and Mozzarella cheese (6.22 µg/ 100 g), and the main MK recorded in these cheeses is actually K_2 MK-4, which is not bacterially synthesised [25, 49]. Hojo et al. [25], Vermeer et al. [49] and Walther et al. [53] reported significantly higher levels of total vitamin K_2 in Emmental cheese of 30.80–39.50 µg/ 100 g, 43.30 µg/ 100 g and 13.30–60.40 µg/100 g, respectively, compared to studies by Koivu-Tikkanen et al [30] and Manoury et al [36], who found that these were relatively low at 5.13–6.61 µg/ 100 g and only 3.19–5.12 µg/ 100 g, respectively. Temperature, carbon source, aeration and metabolic mode were found to have an effect on the growth of lactic acid bacterial strains and MK content [34]. Therefore, ripening time alone cannot predict levels of K_2 vitamin, and further research is also needed in this area to clarify the optimal conditions for different fermented products [57]. In our study, high amounts of total vitamin K_2 were reported in Gouda (678.12 ng/g), Edam (712.70 ng/g) and Emmentaler (733.10 ng/g) cheeses with comparable levels of vitamin K_1 in these products (respectively, 31.60 ng/g, 34.63 ng/g and 24.35 ng/g) with the fat content of the analysed products (respectively, 27%, 28% and 30%). The fat content of cheese is another factor that may affect vitamin K content, with lower-fat products such as reduced-fat quark, cheddar cheese and cream having lower total vitamin K content compared to full-fat cheese or cream [20]. This may be due to the fact that vitamin K is a fat-soluble vitamin. Walther et al. [53] observed a positive correlation between fat content and K_2 MK-4 in Swiss-type cheese. However, they also noted a negative correlation between fat content and K_2 MK-6, K_2 MK-7, K_2 MK-8 and K_2 MK-9, and more in-depth studies are needed to clarify these reports.

Deadly diseases such as cardiovascular diseases or cancer are increasing day by day. Undoubtedly, at such a time, it is very important to identify which foods contain substances that support the body, and at the same time are beneficial and natural for health, such as vitamin K. Cheese, which is available to the Polish consumer and consumers in European countries, can be a good source of vitamin K_2 and a dietary ingredient so crucial in the fight against many dangerous diseases.

CONCLUSION

This study provided data on vitamin K levels in cheeses available in Poland and other European countries. All samples contained both forms of vitamin K. This study has contributed to a growing number of reports indicating that forms of vitamin K and their concentrations in foods, including cheese, may vary according to ripening time and regional differences, as these dictate not only the type of cheese, but also the fat and nutrient content. A larger project would be warranted to develop a comprehensive analytical database of food composition in the context of the occurrence of vitamin K (PKs and MKs) in a broad spectrum of foods commonly consumed in EU countries so that vitamin K intake can be estimated in a given population and the knowledge on the subject has become comprehensive.

PODSUMOWANIE

Badanie dostarczyło danych dotyczących poziomu witaminy K w serach dostępnych w Polsce i innych państwach europejskich. Wszystkie próbki zawierały obie formy witaminy K. Badanie to przyczyniło się do rosnącej liczby doniesień wskazujących, że formy witaminy K i ich stężenia w żywności, w tym w serze, mogą się różnić czasem dojrzewania i różnicami regionalnymi, gdyż dyktują one nie tylko rodzaj sera, ale także zawartość tłuszczu i składników odżywczych. Zasadny byłby większy projekt w celu rozwinięcia kompleksowej analitycznej bazy danych składu żywności w kontekście występowania witaminy K (PK i MK) w szerokim spektrum żywności powszechnie spożywanej w krajach UE tak, aby spożycie witaminy K mogło być oszacowane w danej populacji i wiedza na ten temat stała się kompleksowa.

REFERENCES

[1] **ALEXANDER D.D., L.C. BYLSMA, A.J. VARGAS, S.S. COHEN, A. DOUCETTE, M. MOHAMED, S.R. IRVIN, P.E. MILLER, H. WATSON, J.P. FRYZEK. 2016.** "Dairy consumption and CVD: a systematic review and metaanalysis". British J. Nutr. 115: 737–750.

[2] **ALZHEIMER'S FOUNDATION OF AMERICA. 2022.** Alzheimer's Foundation of America. Available at: https://alzfdn.org/caregiving-resources/about-alzheimers-diseaseand-dementia/ (accessed on 10 November 2022)

[3] **ARTAUD-WILD S.M., S.L. CONNOR, G. SEXTON, W.E. CONNOR. 1993.** "Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox". Circulation 88: 2771–2779.

[4] **BELLINGE J.W., R.J. FRANCIS, S.C. LEE, A. VICKERY, W. MACDONALD, S.K. GAN, G.T. CHEW, M. PHILLIPS, J.R. LEWIS, G.F. WATTS, C.J. SCHULTZ. 2021.** "The effect of Vitamin-K_1 and Colchicine on Vascular Calcification Activity in subjects with Diabetes Mellitus (ViKCoVaC): A double-blind 2x2 factorial randomized controlled trial". J. Nucl. Cardiol. 29(4): 1855–1866. doi: 10.1007/s12350-021-02589-8.

[5] **BELLONE F., M. CINQUEGRANI, R. NICOTERA, N. CARULLO, A. CASARELLA, P. PRESTA, M. ANDREUCCI, G. SQUADRITO, G. MANDRAFFINO, M. PRUNESTÌ, C. VOCCA, G. DE SARRO, D. BOLIGNANO, G. COPPOLINO. 2022.** "Role of Vitamin K in Chronic Kidney Disease: A Focus on Bone and Cardiovascular Health". Int J Mol Sci. 23(9): 5282. doi: 10.3390/ijms23095282.

[6] **BERTOLA N.C., A.N. CALIFANO, A.E. BEVILACQUA, N.E. ZARITZKY. 2000.** "Effects of ripening conditions on the texture of Gouda cheese". Int. J. Food Sci. Tech. 35: 207–214. doi: 10.1046/j.1365-2621.2000.00347.x

[7] **BEULENS J.W., S.L. BOOTH, E.G. VAN DEN HEUVEL, E. STOECKLIN, A. BAKA, C. VERMEER. 2013.** "The role of menaquinones (vitamin K_2) in human health". Br. J. Nutr. 110: 1357–1368.

[8] **BEULENS J.W., M.L. BOTS, F. ATSMA, M.L. BARTELINK, M. PROKOP, J.M. GELEIJNSE, J.C. WITTEMAN, D.E. GROBBEE, Y.T. VAN DER SCHOUW. 2009.** "High dietary menaquinone intake is associated with reduced coronary calcification". Atherosclerosis 203(2): 489–93. doi: 10.1016/j.atherosclerosis.2008.07.010.

[9] **BOOTH S.L. 2012.** "Vitamin K: Food composition and dietary intakes". Food Nutr. Res. 56: 5505.

REFERENCES

[1] **ALEXANDER D.D., L.C. BYLSMA, A.J. VARGAS, S.S. COHEN, A. DOUCETTE, M. MOHAMED, S.R. IRVIN, P.E. MILLER, H. WATSON, J.P. FRYZEK. 2016.** "Dairy consumption and CVD: a systematic review and metaanalysis". British J. Nutr. 115: 737–750.

[2] **ALZHEIMER'S FOUNDATION OF AMERICA. 2022.** Alzheimer's Foundation of America. Available at: https://alzfdn.org/caregiving-resources/about-alzheimers-diseaseand-dementia/ (accessed on 10 November 2022)

[3] **ARTAUD-WILD S.M., S.L. CONNOR, G. SEXTON, W.E. CONNOR. 1993.** "Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox". Circulation 88: 2771–2779.

[4] **BELLINGE J.W., R.J. FRANCIS, S.C. LEE, A. VICKERY, W. MACDONALD, S.K. GAN, G.T. CHEW, M. PHILLIPS, J.R. LEWIS, G.F. WATTS, C.J. SCHULTZ. 2021.** "The effect of Vitamin-K1 and Colchicine on Vascular Calcification Activity in subjects with Diabetes Mellitus (ViKCoVaC): A double-blind 2x2 factorial randomized controlled trial". J. Nucl. Cardiol. 29(4): 1855–1866. doi: 10.1007/s12350-021-02589-8.

[5] **BELLONE F., M. CINQUEGRANI, R. NICOTERA, N. CARULLO, A. CASARELLA, P. PRESTA, M. ANDREUCCI, G. SQUADRITO, G. MANDRAFFINO, M. PRUNESTI, C. VOCCA, G. DE SARRO, D. BOLIGNANO, G. COPPOLINO. 2022.** "Role of Vitamin K in Chronic Kidney Disease: A Focus on Bone and Cardiovascular Health". Int J Mol Sci. 23(9): 5282. doi: 10.3390/ijms23095282.

[6] **BERTOLA N.C., A.N. CALIFANO, A.E. BEVILACQUA, N.E. ZARITZKY. 2000.** "Effects of ripening conditions on the texture of Gouda cheese". Int. J. Food Sci. Tech. 35: 207–214. doi: 10.1046/j.1365-2621.2000.00347.x

[7] **BEULENS J.W., S.L. BOOTH, E.G. VAN DEN HEUVEL, E. STOECKLIN, A. BAKA, C. VERMEER. 2013.** "The role of menaquinones (vitamin K2) in human health". Br. J. Nutr. 110: 1357–1368.

[8] **BEULENS J.W., M.L. BOTS, F. ATSMA, M.L. BARTELINK, M. PROKOP, J.M. GELEIJNSE, J.C. WITTEMAN, D.E. GROBBEE, Y.T. VAN DER SCHOUW. 2009.** "High dietary menaquinone intake is associated with reduced coronary calcification". Atherosclerosis 203(2): 489–93. doi: 10.1016/j.atherosclerosis.2008.07.010.

[9] **BOOTH S.L. 2012.** "Vitamin K: Food composition and dietary intakes". Food Nutr. Res. 56: 5505.

[10] **BOXMA P.Y., E.V.D. BERG, J.M. GELEIJNSE, G.D. LAVERMAN, L.J. SCHURGERS, C. VERMEER, I.P. KEMA, F.A. MUSKIET, G. NAVIS, S.J.L. BAKKER, ET AL. 2012.** "Vitamin K Intake and Plasma Desphospho-Uncarboxylated Matrix Gla-Protein Levels in Kidney Transplant Recipients". PLoS ONE. 7: e47991.

[11] **CHATELAN A., S. BEER-BORST, A. RANDRIAMIHARISOA, J. PASQUIER, J.M. BLANCO, S. SIEGENTHALER, F. PACCAUD, N. SLIMANI, G. NICOLAS, E. CAMENZIND-FREY, C.A. ZUBERBUEHLER, M. BOCHUD. 2017.** "Major Differences in Diet across Three Linguistic Regions of Switzerland: Results from the First National Nutrition Survey menuCH". Nutrients. 9(11): 1163. doi: 10.3390/nu9111163.

[12] **CHEN Z., M. AHMED, V. HA, K. JEFFERSON, V. MALIK, P. RIBEIRO, P. ZUCHINALI, J-P. DROUIN-CHARTIER. 2021.** "Dairy product consumption and cardiovascular health: a systematic review and meta-analysis of prospective cohort studies". Adv. Nutr. 13: 439–454.

[13] **CICHOSZ G. 2005.** „Jakość sera gouda w zależności od formy kultur starterowych". Przegl. Mlecz., 2: 4–8.

[14] **DEREWIAKA D., M. OBIEDZIŃSKI. 2011.** „Charakterystyka frakcji sterolowej wybranych serów dojrzewających". Bromat. Chem. Toksykol. XLIV(3): 508–511.

[15] **DISMORE M.L., D.B. HAYTOWITZ, S.E. GEBHARDT, J.W. PETERSON, S.L. BOOTH. 2003.** "Vitamin K content of nuts and fruits in the US diet". J. Am. Diet. Assoc. 103: 1650–1652.

[16] **DROUIN-CHARTIER J-P., P. HERNÁNDEZ-ALONSO, M. GUASCH-FERRÉ, M. RUIZ-CANELA, J. LI, C. WITTENBECHER, C. RAZQUIN, E. TOLEDO, C. DENNIS, D. CORELLA, R. ESTRUCH, M. FITÓ, A.H. ELIASSEN, D.K. TOBIAS, A. ASCHERIO, L.A. MUCCI, K.M. REXRODE, E.W. KARLSON, K.H. COSTENBADER, C.S. FUCHS, L. LIANG, C.B. CLISH, M.A. MARTÍNEZ-GONZÁLEZ, J. SALAS-SALVADÓ, F.B. HU. 2021.** "Dairy consumption, plasma metabolites, and risk of type 2 diabetes". Am. J. Clin. Nutr. 114(1): 163–174. doi: 10.1093/ajcn/nqab047

[17] **EFSA PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES (NDA), TURCK D., J.L. BRESSON, B. BURLINGAME, T. DEAN, S. FAIRWEATHER-TAIT, M. HEINONEN, K.I. HIRSCH-ERNST, L. MANGELSDORF, H.J. MCARDLE, A. NASKA, G. NOWICKA, K. PENTIEVA, Y. SANZ, A. SIANI, A. SJÖDIN, M. STERN, D. TOMÉ, H. VAN LOVEREN, M. VINCETI, P. WILLATTS, C. LAMBERG-ALLARDT, H. PRZYREMBEL, I. TETENS, C. DUMAS, L. FABIANI, S. IOANNIDOU, M. NEUHÄUSER-BERTHOLD. 2017.**

[10] **BOXMA P.Y., E.V.D. BERG, J.M. GELEIJNSE, G.D. LAVERMAN, L.J. SCHURGERS, C. VERMEER, I.P. KEMA, F.A. MUSKIET, G. NAVIS, S.J.L. BAKKER, ET AL. 2012.** "Vitamin K Intake and Plasma Desphospho-Uncarboxylated Matrix Gla-Protein Levels in Kidney Transplant Recipients". PLoS ONE. 7: e47991.

[11] **CHATELAN A., S. BEER-BORST, A. RANDRIAMIHARISOA, J. PASQUIER, J.M. BLANCO, S. SIEGENTHALER, F. PACCAUD, N. SLIMANI, G. NICOLAS, E. CAMENZIND-FREY, C.A. ZUBERBUEHLER, M. BOCHUD. 2017.** "Major Differences in Diet across Three Linguistic Regions of Switzerland: Results from the First National Nutrition Survey menuCH". Nutrients. 9(11): 1163. doi: 10.3390/nu9111163.

[12] **CHEN Z., M. AHMED, V. HA, K. JEFFERSON, V. MALIK, P. RIBEIRO, P. ZUCHINALI, J-P. DROUIN-CHARTIER. 2021.** "Dairy product consumption and cardiovascular health: a systematic review and meta-analysis of prospective cohort studies". Adv. Nutr. 13: 439–454.

[13] **CICHOSZ G. 2005.** „Jakosc sera gouda w zaleznosci od formy kultur starterowych". Przegl. Mlecz., 2: 4–8.

[14] **DEREWIAKA D., M. OBIEDZINSKI. 2011.** „Charakterystyka frakcji sterolowej wybranych serow dojrzewajacych". Bromat. Chem. Toksykol. XLIV(3): 508–511.

[15] **DISMORE M.L., D.B. HAYTOWITZ, S.E. GEBHARDT, J.W. PETERSON, S.L. BOOTH. 2003.** "Vitamin K content of nuts and fruits in the US diet". J. Am. Diet. Assoc. 103: 1650–1652.

[16] **DROUIN-CHARTIER J-P., P. HERNANDEZ-ALONSO, M. GUASCH-FERRE, M. RUIZ-CANELA, J. LI, C. WITTENBECHER, C. RAZQUIN, E. TOLEDO, C. DENNIS, D. CORELLA, R. ESTRUCH, M. FITO, A.H. ELIASSEN, D.K. TOBIAS, A. ASCHERIO, L.A. MUCCI, K.M. REXRODE, E.W. KARLSON, K.H. COSTENBADER, C.S. FUCHS, L. LIANG, C.B. CLISH, M.A. MARTINEZ-GONZALEZ, J. SALAS-SALVADO, F.B. HU. 2021.** "Dairy consumption, plasma metabolites, and risk of type 2 diabetes". Am. J. Clin. Nutr. 114(1): 163–174. doi: 10.1093/ajcn/nqab047

[17] **EFSA PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES (NDA), TURCK D., J.L. BRESSON, B. BURLINGAME, T. DEAN, S. FAIRWEATHER-TAIT, M. HEINONEN, K.I. HIRSCH-ERNST, L. MANGELSDORF, H.J. MCARDLE, A. NASKA, G. NOWICKA, K. PENTIEVA, Y. SANZ, A. SIANI, A. SJODIN, M. STERN, D. TOME, H. VAN LOVEREN, M. VINCETI, P. WILLATTS, C. LAMBERG-ALLARDT, H. PRZYREMBEL, I. TETENS, C. DUMAS, L. FABIANI, S. IOANNIDOU, M. NEUHAUSER-BERTHOLD. 2017.**

"Dietary reference values for vitamin K". EFSA J. 15(5): e04780. doi: 10.2903/j.efsa.2017.4780.

[18] **ENGELS W., E.-M. DÜSTERHÖFT. 2022.** "Starter cultures for cheese manufacture". In Encyclopedia of Dairy Sciences, 3rd edn, pp. 352–357. McSweeney P. L. H., McNamara J. P., eds. Oxford: Academic Press.

[19] **FEENEY E. L., P. LAMICHHANE, J.J. SHEEHAN. 2021.** "The cheese matrix: understanding the impact of cheese structure on aspects of cardiovascular health–a food science and a human nutrition perspective". Int. J. Dairy Technol. 74(4): 656-670.

[20] **FU X., S.G. HARSHMAN, X. SHEN, D.B. HAYTOWITZ, J.P. KARL, B.E. WOLFE, S.L. BOOTH. 2017.** "Multiple Vitamin K Forms Exist in Dairy Foods". *Curr. Dev. Nutr*. 1(6): e000638. doi: 10.3945/cdn.117.000638

[21] **FUSARO M., G. CIANCIOLO, P. EVENEPOEL, L. SCHURGERS, M. PLEBANI. 2021.** "Vitamin K in CKD Bone Disorders". Calcif. Tissue Int. 108: 476–485.

[22] **HADIPOUR E., Z. TAYARANI-NAJARAN, M. FEREIDONI. 2020.** "Vitamin K_2 Protects PC12 Cells against Aβ (1-42) and H_2O_2-Induced Apoptosis via P38 MAP Kinase Pathway". Nutr. Neurosci. 23: 343–352. doi:10.1080/1028415X.2018.1504428

[23] **HALDER M., P. PETSOPHONSAKUL, A.C. AKBULUT, A. PAVLIC, F. BOHAN, E. ANDERSON, K. MARESZ, R. KRAMANN, L. SCHURGERS. 2019.** "Vitamin K: double bonds beyond coagulation insights into differences between vitamin K_1 and K_2 in health and disease". Int. J. Mol. Sci. 20(4): 896.

[24] **HARIRI E., N. KASSIS, J.P. ISKANDAR, L.J. SCHURGERS, A. SAAD, O. ABDELFATTAH, A. BANSAL, T. ISOGAI, S.C. HARB, S. KAPADIA. 2021.** "Vitamin K_2-a neglected player in cardiovascular health: a narrative review". Open Heart. 8(2): e001715. doi: 10.1136/openhrt-2021-001715.

[25] **HOJO K., R. WATANABE, T. MORI, N. TAKETOMO. 2007.** "Quantitative measurement of tetrahydromenaquinone-9 in cheese fermented by propionibacteria". J. Dairy Sci. 90: 4078–4083.

[26] **HUY P. D., Y.C. YU, S.T. NGO, T.V. THAO, C.P. CHEN, M.S. LI, ET AL. 2013.** "In Silico and In Vitro Characterization of Anti-amyloidogenic Activity of Vitamin K_3 Analogues for Alzheimer's Disease". Biochim. Biophys. Acta 1830: 2960–2969. doi:10.1016/j.bbagen.2012.12.026.

[27] **JADHAV N., S. AJGAONKAR, P. SAHA, P. GURAV, A. PANDEY, V. BASUDKAR, Y. GADA, S. PANDA, S. JADHAV, D. MEHTA, S. NAIR. 2022.** "Molecular Pathways and Roles for Vitamin K_2-7 as a Health Beneficial Nutraceutical: Challenges and Opportunities". Front. Pharmacol. 13: 896920. doi: 10.3389/fphar.2022.896920.

"Dietary reference values for vitamin K". EFSA J. 15(5): e04780. doi: 10.2903/j.efsa.2017.4780.

[18] **ENGELS W., E.-M. DUSTERHOFT. 2022.** "Starter cultures for cheese manufacture". In Encyclopedia of Dairy Sciences, 3rd edn, pp. 352–357. McSweeney P. L. H., McNamara J. P., eds. Oxford: Academic Press.

[19] **FEENEY E. L., P. LAMICHHANE, J.J. SHEEHAN. 2021.** "The cheese matrix: understanding the impact of cheese structure on aspects of cardiovascular health-a food science and a human nutrition perspective". Int. J. Dairy Technol. 74(4): 656–670.

[20] **FU X., S.G. HARSHMAN, X. SHEN, D.B. HAYTOWITZ, J.P. KARL, B.E. WOLFE, S.L. BOOTH. 2017.** "Multiple Vitamin K Forms Exist in Dairy Foods". Curr. Dev. Nutr. 1(6): e000638. doi: 10.3945/cdn.117.000638

[21] **FUSARO M., G. CIANCIOLO, P. EVENEPOEL, L. SCHURGERS, M. PLEBANI. 2021.** "Vitamin K in CKD Bone Disorders". Calcif. Tissue Int. 108: 476–485.

[22] **HADIPOUR E., Z. TAYARANI-NAJARAN, M. FEREIDONI. 2020.** "Vitamin K2 Protects PC12 Cells against A? (1-42) and H2O2-Induced Apoptosis via P38 MAP Kinase Pathway". Nutr. Neurosci. 23: 343–352. doi:10.1080/1028415X.2018.1504428

[23] **HALDER M., P. PETSOPHONSAKUL, A.C. AKBULUT, A. PAVLIC, F. BOHAN, E. ANDERSON, K. MARESZ, R. KRAMANN, L. SCHURGERS. 2019.** "Vitamin K: double bonds beyond coagulation insights into differences between vitamin K1 and K2 in health and disease". Int. J. Mol. Sci. 20(4): 896.

[24] **HARIRI E., N. KASSIS, J.P. ISKANDAR, L.J. SCHURGERS, A. SAAD, O. ABDELFATTAH, A. BANSAL, T. ISOGAI, S.C. HARB, S. KAPADIA. 2021.** "Vitamin K2-a neglected player in cardiovascular health: a narrative review". Open Heart. 8(2): e001715. doi: 10.1136/openhrt-2021-001715.

[25] **HOJO K., R. WATANABE, T. MORI, N. TAKETOMO. 2007.** "Quantitative measurement of tetrahydromenaquinone-9 in cheese fermented by propionibacteria". J. Dairy Sci. 90: 4078–4083.

[26] **HUY P. D., Y.C. YU, S.T. NGO, T.V. THAO, C.P. CHEN, M.S. LI, ET AL. 2013.** "In Silico and In Vitro Characterization of Anti-amyloidogenic Activity of Vitamin K3 Analogues for Alzheimer's Disease". Biochim. Biophys. Acta 1830: 2960–2969. doi:10.1016/j.bbagen.2012.12.026.

[27] **JADHAV N., S. AJGAONKAR, P. SAHA, P. GURAV, A. PANDEY, V. BASUDKAR, Y. GADA, S. PANDA, S. JADHAV, D. MEHTA, S. NAIR. 2022.** "Molecular Pathways and Roles for Vitamin K2-7 as a Health Beneficial Nutraceutical: Challenges and Opportunities". Front. Pharmacol. 13: 896920. doi: 10.3389/fphar.2022.896920.

[28] **JANSSEN R., M. VISSER, A. DOFFERHOFF, C. VERMEER, W. JANSSENS, J. WALK. 2021.** "Vitamin K metabolism as the potential missing link between lung damage and thromboembolism in Coronavirus disease 2019". British J. Nutr. 126(2): 191–198. doi: 10.1017/S0007114520003979.

[29] **JENSEN M.B., K.E. RØD, P.L. ŠVARC, E. OVELAND, J. JAKOBSEN. 2022.** "Vitamin K (phylloquinone and menaquinones) in foods – Cost-effective quantification by LC-ESI-MS/MS". Food Chemistry 385: 132672. doi: 10.1016/j.foodchem.2022.132672.

[30] **KOIVU-TIKKANEN T. J., V. OLLILAINEN, V.I. PIIRONEN. 2000.** "Determination of phylloquinone and menaquinones in animal products with fluorescence detection after post column reduction with metallic zinc". J Agri Food Chem. 48: 6325–6331.

[31] **KONG F., Q. GUO, S. BAI, J. XU, D. LI, Z. WANG, X. ZHANG, Y. CUI. 2021.** "Determination of Vitamin K_1 and Vitamin K_2 in Foods by HPLC-FLD". Sci. Techn. Food Industry. 42(4): 226–231.doi: 10.13386/j.issn1002-0306.2020040359.

[32] **KONIECZKA P. 2003.** „Walidacja metodyk analitycznych". Ecol. Chem. Eng. 10(10): 1071–1100.

[33] **KRÓL J., A. WAWRYNIUK, A. BRODZIAK, J. BARŁOWSKA, B. KUCZYŃSKA. 2020.** "The Effect of Selected Factors on the Content of Fat-Soluble Vitamins and Macro-Elements in Raw Milk from Holstein-Friesian and Simmental Cows and Acid Curd Cheese (Tvarog)". Animals (Basel). 10(10): 1800. doi: 10.3390/ani10101800.

[34] **LIU Y., E.O. VAN BENNEKOM, Y. ZHANG, T. ABEE, E.J. SMID. 2019.** "Long-chain vitamin K_2 production in *Lactococcus lactis* is influenced by temperature, carbon source, aeration and mode of energy metabolism". Microbial. Cell Factories. 18: 129.

[35] **MAJUMDER R., T. NGUYEN. 2021.** "Protein S: Function, regulation, and clinical perspectives". Curr. Opin. Hematol. 28: 339–344.

[36] **MANOURY E., K. JOURDON, P. BOYAVAL, P. FOURCASSIÉ. 2013.** "Quantitative measurement of vitamin K_2 (menaquinones) in various fermented dairy products using a reliable high-performance liquid chromatography method". J. Dairy Sci. 96(3): 1335–46. doi: 10.3168/jds.2012-5494.

[37] **OFFICE OF DIETARY SUPPLEMENTS-NATIONAL INSTITUTE OF HEALTH. VITAMIN K FACT SHEETS FOR HEALTH PROFESSIONALS. 2021.** Available online: https://ods.od.nih.gov/factsheets/vitaminK-HealthProfessional/#en3 (accessed on 3 November 2022).

[38] **Qureshi T., C. Vermeer, G. Vegarud, R. Abrahamsen, S. Skeie. 2013.** "Formation of biogenic amines and vitamin K contents in the Norwegian autochthonous cheese Gamalost during ripening. Dairy Sci. Technol. 93 (3): 303–314.

[28] **JANSSEN R., M. VISSER, A. DOFFERHOFF, C. VERMEER, W. JANSSENS, J. WALK. 2021.** "Vitamin K metabolism as the potential missing link between lung damage and thromboembolism in Coronavirus disease 2019". British J. Nutr. 126(2): 191-198. doi: 10.1017/S0007114520003979.

[29] **JENSEN M.B., K.E. ROD, P.L. SVARC, E. OVELAND, J. JAKOBSEN. 2022.** "Vitamin K (phylloquinone and menaquinones) in foods - Cost-effective quantification by LC-ESI-MS/MS". Food Chemistry 385: 132672. doi: 10.1016/j.foodchem.2022.132672.

[30] **KOIVU-TIKKANEN T. J., V. OLLILAINEN, V.I. PIIRONEN. 2000.** "Determination of phylloquinone and menaquinones in animal products with fluorescence detection after post column reduction with metallic zinc". J Agri Food Chem. 48: 6325–6331.

[31] **KONG F., Q. GUO, S. BAI, J. XU, D. LI, Z. WANG, X. ZHANG, Y. CUI. 2021.** "Determination of Vitamin K1 and Vitamin K2 in Foods by HPLC-FLD". Sci. Techn. Food Industry. 42(4): 226–231.doi: 10.13386/j.issn1002-0306.2020040359.

[32] **KONIECZKA P. 2003.** „Walidacja metodyk analitycznych". Ecol. Chem. Eng. 10(10): 1071-1100.

[33] **KROL J., A. WAWRYNIUK, A. BRODZIAK, J. BARLOWSKA, B. KUCZYNSKA. 2020.** "The Effect of Selected Factors on the Content of Fat-Soluble Vitamins and Macro-Elements in Raw Milk from Holstein-Friesian and Simmental Cows and Acid Curd Cheese (Tvarog)". Animals (Basel). 10(10): 1800. doi: 10.3390/ani10101800.

[34] **LIU Y., E.O. VAN BENNEKOM, Y. ZHANG, T. ABEE, E.J. SMID. 2019.** "Long-chain vitamin K2 production in Lactococcus lactis is influenced by temperature, carbon source, aeration and mode of energy metabolism". Microbial. Cell Factories. 18: 129.

[35] **MAJUMDER R., T. NGUYEN. 2021.** "Protein S: Function, regulation, and clinical perspectives". Curr. Opin. Hematol. 28: 339–344.

[36] **MANOURY E., K. JOURDON, P. BOYAVAL, P. FOURCASSIE. 2013.** "Quantitative measurement of vitamin K2 (menaquinones) in various fermented dairy products using a reliable high-performance liquid chromatography method". J. Dairy Sci. 96(3): 1335–46. doi: 10.3168/jds.2012–5494.

[37] **OFFICE OF DIETARY SUPPLEMENTS-NATIONAL INSTITUTE OF HEALTH. VITAMIN K FACT SHEETS FOR HEALTH PROFESSIONALS. 2021.** Available online: https://ods.od.nih.gov/factsheets/vitaminK-HealthProfessional/#en3 (accessed on 3 November 2022).

[38] **QURESHI T., C. VERMEER, G. VEGARUD, R. ABRAHAMSEN, S. SKEIE. 2013.** "Formation of biogenic amines and vitamin K contents in the Norwegian autochthonous cheese Gamalost during ripening. Dairy Sci. Technol. 93 (3): 303–314.

[39] **SCHURGERS L.J., C. VERMEER. 2000.** "Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations". Haemostasis, 30: 298–307. doi: 10.1159/000054147.

[40] **SCHWALFENBERG G.K. 2017.** "Vitamins K_1 and K_2: The Emerging Group of Vitamins Required for Human Health". J. Nutr. Metab. 2017: 6254836.

[41] **SHEA M.K., S.L. BOOTH, D.E. WEINER, T.E. BRINKLEY, A.M. KANAYA, R.A. MURPHY, E.M. SIMONSICK, C.I. WASSEL, C. VERMEER, S.B. KRITCHEVSKY, ET AL. 2017.** "Circulating Vitamin K is Inversely Associated with Incident Cardiovascular Disease Risk among Those Treated for Hypertension in the Health, Aging, and Body Composition Study (Health ABC)". J. Nutr. 147: 888–895.

[42] **SHEARER M.J., A. BACH, M. KOHLMEIER. 1996.** "Chemistry, Nutritional Sources, Tissue Distribution and Metabolism of Vitamin K with Special Reference to Bone Health". J. Nutr. 126 (Suppl. S4): 1181S–1186S.

[43] **SŁOWIK-BOROWIEC M., L. POTOCKI, B. OKLEJEWICZ, D. BRODA, M. PODBIELSKA, E. SZPYRKA. 2021.** „Preparation of vitamin K_2 MK-7 in a process of fermentation of different seeds and cereals by bacteria *Bacillus subtilis*". Acta Universitatis Cibiniensis Series E: Food Technology 25(1): 93–104.

[44] **ŚMIETANA Z., K. BOHDANIEWICZ, W. DERENGIEWICZ. 2006.** „Czynniki technologiczne warunkujące cechy serów dojrzewających". Przegl. Mlecz. 4: 4–8.

[45] **SOUPARNIKA S., D. NAYANA, M. ARADHANA, M. 2022.** "Vitamin K as a Simple but Efficient Add-on to the Current Treatment Regime of COVID-19: A Narrative Review". Cur. Nutr. Food Sci. 18(7). doi: 10.2174/1573401318666220405134759.

[46] **STĘPIEŃ A., M. KOZIARSKA-ROŚCISZEWSKA, J. RYSZ, M. STĘPIEŃ. 2022.** "Biological Role of Vitamin K – With Particular Emphasis on Cardiovascular and Renal Aspects". Nutrients 14: 262.

[47] **SUNGUR Ş., M. ÇÖMEZ, M. KÖROĞLU. 2021.** "Determination of Vitamin K_2 Content of Dairy Products Produced in Hatay Region in Turkey". NESciences. 6(3): 155–165 doi: 10.28978/nesciences. 1036844.

[48] **THORNING T.K., H.C. BERTRAM, J.-P. BONJOUR ET AL. 2017.** "Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps". Am. J. Clin. Nutr. 105: 1033–1045.

[49] **VERMEER C., J. RAES, C. VAN'T HOOFD, M.H.J. KNAPEN, S. XANTHOULEA. 2018.** "Menaquinone Content of Cheese". Nutrients 10(4): 446. doi: 10.3390/nu10040446.

[39] **SCHURGERS L.J., C. VERMEER. 2000.** "Determination of phylloquinone and menaquinones in food. Effect of food matrix on circulating vitamin K concentrations". Haemostasis, 30: 298-307. doi: 10.1159/000054147.

[40] **SCHWALFENBERG G.K. 2017.** "Vitamins K1 and K2: The Emerging Group of Vitamins Required for Human Health". J. Nutr. Metab. 2017: 6254836.

[41] **SHEA M.K., S.L. BOOTH, D.E. WEINER, T.E. BRINKLEY, A.M. KANAYA, R.A. MURPHY, E.M. SIMONSICK, C.I. WASSEL, C. VERMEER, S.B. KRITCHEVSKY, ET AL. 2017.** "Circulating Vitamin K is Inversely Associated with Incident Cardiovascular Disease Risk among Those Treated for Hypertension in the Health, Aging, and Body Composition Study (Health ABC)". J. Nutr. 147: 888–895.

[42] **SHEARER M.J., A. BACH, M. KOHLMEIER. 1996.** "Chemistry, Nutritional Sources, Tissue Distribution and Metabolism of Vitamin K with Special Reference to Bone Health". J. Nutr. 126 (Suppl. S4): 1181S–1186S.

[43] **SLOWIK-BOROWIEC M., L. POTOCKI, B. OKLEJEWICZ, D. BRODA, M. PODBIELSKA, E. SZPYRKA. 2021.** "Preparation of vitamin K2 MK-7 in a process of fermentation of different seeds and cereals by bacteria Bacillus subtilis". Acta Universitatis Cibiniensis Series E: Food Technology 25(1): 93–104.

[44] **SMIETANA Z., K. BOHDANIEWICZ, W. DERENGIEWICZ. 2006.** „Czynniki technologiczne warunkujace cechy serow dojrzewajacych". Przegl. Mlecz. 4: 4–8.

[45] **SOUPARNIKA S., D. NAYANA, M. ARADHANA, M. 2022.** "Vitamin K as a Simple but Efficient Add-on to the Current Treatment Regime of COVID-19: A Narrative Review". Cur. Nutr. Food Sci. 18(7). doi: 10.2174/1573401318666220405134759.

[46] **STEPIEN A., M. KOZIARSKA-ROSCISZEWSKA, J. RYSZ, M. STEPIEN. 2022.** "Biological Role of Vitamin K – With Particular Emphasis on Cardiovascular and Renal Aspects". Nutrients 14: 262.

[47] **SUNGUR S., M. COMEZ, M. KOROGLU. 2021.** "Determination of Vitamin K2 Content of Dairy Products Produced in Hatay Region in Turkey". NESciences. 6(3): 155–165 doi: 10.28978/nesciences. 1036844.

[48] **THORNING T.K., H.C. BERTRAM, J.-P. BONJOUR ET AL. 2017.** "Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps". Am. J. Clin. Nutr. 105: 1033–1045.

[49] **VERMEER C., J. RAES, C. VAN'T HOOFD, M.H.J. KNAPEN, S. XANTHOULEA. 2018.** "Menaquinone Content of Cheese". Nutrients 10(4): 446. doi: 10.3390/nu10040446.

[50] **VISSERS L.E., G.W. DALMEIJER, J.M. BOER, W.M. VERSCHUREN, Y.T. VAN DER SCHOUW, J.W. BEULENS. 2016.** "The relationship between vitamin K and peripheral arterial disease". Atherosclerosis 252: 15–20.

[51] **WALTHER B., M. CHOLLET. 2017.** "Menaquinones, Bacteria, and Foods: Vitamin K_2 in the Diet". In: Gordeladze J.O. (Ed.) "Vitamin K_2 – Vital for Health and Wellbeing" (pp. Ch. 04). Rijeka, InTech.

[52] **WALTHER B., D. GUGGISBERG, R.S. SCHMIDT, R. PORTMANN, M.-C. RISSE, R. BADERTSCHER, M. CHOLLET. 2020.** "Quantitative analysis of menaquinones (vitamin K_2) in various types of cheese from Switzerland". Int. Dairy J. 104853.

[53] **WALTHER B., R. SCHMIDT. 2021.** "Swiss cheeses are in important dietary source of vitamin K_2". Swiss Agricultural Research 12: 73–77. doi: 10.34776/afs12-73. Available online.

[54] **WEIZMANN N., J.W. PETERSON, D. HAYTOWITZ, P.R. PEHRSSON, V.P. JESUS, S.L. BOOTH. 2004.** "Vitamin K content of fast foods and snack foods in the US diet". J. Food Comp. Anal. 17(3–4): 379–384. doi: 10.1016/J.JFCA.2004.02.005.

[55] **XU Y., L. ZHANG, R. YANG, X. YU, L. YU, F. MA, H. LI, X. WANG, P. LI. 2020.** "Extraction and Determination of Vitamin K_1 in Foods by Ultrasound-Assisted Extraction, SPE, and LC-MS/MS". Molecules 25(4): 839. doi: 10.3390/molecules25040839.

[56] **ZHENG X., Y. HOU, H. HE, Y. CHEN, R. ZHOU, X. WANG, T. GONG, W. JIANG. 2021.** "Synthetic vitamin K analogs inhibit inflammation by targeting the NLRP3 inflammasome". Cell. Mol. Immunol. 18: 2422–2430.

[57] **ZHOU S., E. FEENEY. 2022.** "An overview of vitamin K in cheese and the potential role on cardiovascular disease". Proc. Nutr. Soc. 81(OCE4): E112. doi:10.1017/S0029665122001410.

[58] **ZIEMIŃSKA M., B. SIEKLUCKA, K. PAWLAK. 2021.** "Vitamin K and D Supplementation and Bone Health in Chronic Kidney Disease-Apart or Together?" Nutrients 13: 809.

[50] **VISSERS L.E., G.W. DALMEIJER, J.M. BOER, W.M. VERSCHUREN, Y.T. VAN DER SCHOUW, J.W. BEULENS. 2016.** "The relationship between vitamin K and peripheral arterial disease". Atherosclerosis 252: 15–20.

[51] **WALTHER B., M. CHOLLET. 2017**. "Menaquinones, Bacteria, and Foods: Vitamin K2 in the Diet". In: Gordeladze J.O. (Ed.) "Vitamin K2 – Vital for Health and Wellbeing" (pp. Ch. 04). Rijeka, InTech.

[52] **WALTHER B., D. GUGGISBERG, R.S. SCHMIDT, R. PORTMANN, M.-C. RISSE, R. BADERTSCHER, M. CHOLLET. 2020.** "Quantitative analysis of menaquinones (vitamin K2) in various types of cheese from Switzerland". Int. Dairy J. 104853.

[53] **WALTHER B., R. SCHMIDT. 2021.** "Swiss cheeses are in important dietary source of vitamin K2". Swiss Agricultural Research 12: 73–77. doi: 10.34776/afs12–73. Available online.

[54] **WEIZMANN N., J.W. PETERSON, D. HAYTOWITZ, P.R. PEHRSSON, V.P. JESUS, S.L. BOOTH. 2004.** "Vitamin K content of fast foods and snack foods in the US diet". J. Food Comp. Anal. 17(3–4): 379–384. doi: 10.1016/J.JFCA.2004.02.005.

[55] **XU Y., L. ZHANG, R. YANG, X. YU, L. YU, F. MA, H. LI, X. WANG, P. LI. 2020.** "Extraction and Determination of Vitamin K1 in Foods by Ultrasound-Assisted Extraction, SPE, and LC-MS/MS". Molecules 25(4): 839. doi: 10.3390/molecules25040839.

[56] **ZHENG X., Y. HOU, H. HE, Y. CHEN, R. ZHOU, X. WANG, T. GONG, W. JIANG. 2021.** "Synthetic vitamin K analogs inhibit inflammation by targeting the NLRP3 inflammasome". Cell. Mol. Immunol. 18: 2422–2430.

[57] **ZHOU S., E. FEENEY. 2022.** "An overview of vitamin K in cheese and the potential role on cardiovascular disease". Proc. Nutr. Soc. 81(OCE4): E112. doi:10.1017/S0029665122001410.

[58] **ZIEMINSKA M., B. SIEKLUCKA, K. PAWLAK. 2021.** "Vitamin K and D Supplementation and Bone Health in Chronic Kidney Disease-Apart or Together?" Nutrients 13: 809.