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Original paper

Assessment of vitamin content in different types of romanian cheese

EVELINA GHERGHINA^{1,3}, FLORENTINA ISRAEL-ROMING¹, DANIELA BĂLAN^{1*},
GABRIELA LUȚĂ¹, AGLAIA POPA¹, MARTA ZACHIA²

¹University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania

²National Research and Development Institute for Food Bioresources-IBA Bucharest, Romania

³Centre for Applied Biochemistry and Biotechnology BIOTEHNOL Bucharest, Romania

Abstract

In recent years there was a preference for healthy natural products hence an increased interest in functional food including fermented dairy products occurred. Dairy products contain nutrients essential for health, of which vitamins could help reduce the risk of certain diseases. Cheese is a highly nutritious food that can play an important role in a well-balanced diet. The aim of this research was to investigate the vitamin composition of various types of cheese and to evaluate the vitamins intake provided by cheese as part of the daily diet. The determinations were performed on traditional Romanian cheese, meaning cow, sheep, goat and buffalo Telemea and some Kashkaval types purchased from local supermarkets. The samples were subjected to vitamins B1, B2, B12, A and E analysis using spectrofluorometric, spectrophotometric, HPLC and microbiological methods. The results showed a higher content of vitamins B2 and E in Telemea cheese while Kashkaval types were distinguished for vitamin B1, B12 and A. Regarding the vitamin content depending on raw material used for obtaining the different types of cheese, those made from goat milk recorded the highest level of the analyzed vitamins, followed by those from sheep milk, while lowest amounts were found in products obtained from cow.

Keywords Cheese, Kashkaval, Telemea, vitamins.

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✉ *Corresponding author: DANIELA BĂLAN, University of Agronomical Sciences and Veterinary Medicine Bucharest, Romania
E-mail: balan.dana@gmail.com

Introduction

Lately the requirement of modern consumers for health-promoting natural products increases the interest in biofunctional foods, containing probiotics, prebiotics, vitamins and minerals such as diverse fermented dairy products (SANDERS, 1998; STANTON, 2005).

Dairy products contain many biochemical compounds that are essential for health, of which vitamins are some of the nutrients that keep the body functioning properly and could help reduce the risk of certain diseases (KWAK, 2012; LINARES, 2017).

Cheese is a product obtained by coagulation of milk that gives a solid phase (*coagulum* or *curd*) and a liquid phase (*whey*). The curd obtained by draining will turn into cheese and may be salted, fermented and/or ripened for certain time in order to develop the flavor and texture characteristic of the cheese variety.

Cheese is a highly nutritious food that can play an important role in a well-balanced diet. The nutritional value of cheese depends largely on its composition which is determined during the production process. So, draining of curd leads to a loss of proteins (with the whey) and lactose; also most of the milk water-soluble vitamins remain into the whey during curd manufacture. Skimming of milk induces a loss of lipids therefore of fat-soluble vitamins, while fermentation and ripening lead to enrichment in group B vitamins.

Consequently, the main vitamins present in the cheese are the fat-soluble vitamins (A, D and E) retained by the curd, which contains 90% of the milk fat. As to the water-soluble vitamin content, it varies significantly from one cheese to another depending on microbial synthesis that occurs in cheese during ripening (FOX, 2017). Some bacterial strains as *Lactobacillus* sp. and *Bifidobacterium* sp. involved in dairy products fermentation can provide an additional source of B vitamins (thiamin, riboflavin, cobalamin, folate and biotin) during dairy fermentation (LEVIT, 2016; VAN WYK, 2011; LAIÑO, 2013). Also the dairy propionibacteria, especially *P. freudenreichii* ssp. *shermanii*, the main component of ripening flora in Swiss-type cheeses, are used for the production of vitamin B12 and there is an increasing interest in their potential use as probiotics (GARDNER, 2005; VOROBEVA, 2008; RABAH, 2017).

Vitamins are involved in intermediary metabolism as cofactors in numerous enzymatic reactions or in non-enzymatic physiological functions such as the visual process (vitamin A), as antioxidants (carotenoids, vitamins E, C and B2), in regulation of calcium metabolism (vitamin D) and in haematopoiesis (vitamin B12, folate and vitamin B6) (ÖSTE, 1997).

Most vitamins are present in a variety of foods, however vitamin deficiencies still exist in many countries as a result of insufficient food intake and because of unbalanced diets. Moreover, B-group vitamins, usually present in many foods, are easily destroyed during cooking and food processing, leading also to insufficient amounts in the daily diet.

For the above reasons, several types of Romanian cheese named *Telemea* and *Kashkaval* were selected for a vitamin content analysis.

Telemea is a traditional Romanian cheese typically made of sheep milk, but nowadays the term is used also for cheese made out of cow milk, and in some cases of goat, or buffalo's milk (BRATU, 2012). Since 2005, the name *Telemea* is a protected designation of origin product of Romania. Similar to the Greek *feta* or Turkish *teleme*, Romanian *Telemea* can have high water content, that gives them a soft, creamy texture and a strong, spicy flavor. Alternatively, it may be left to mature in brine that makes it crisper, more flavored and salty and it can be kept longer in very concentrated brine.

Kashkaval is a semi-hard, yellow cheese made of cow milk, sheep milk or both. It belongs to the family of *pasta filata* cheeses (SANTA, 2014) that is made by giving the curd a hot bath during the production process and is allowed to age for six months when developing a piquant, taste. *Kashkaval* is particularly popular in Eastern Europe, where is used as a generic term for all kinds of yellow cheeses.

The aim of this research was to investigate the vitamin composition of various types of *Telemea* and *Kashkaval* of different provenance and to evaluate the vitamins intake provided by cheese as part of the daily diet. *Regulation (EU) No 1169/2011 of the European Parliament and of the Council* on the provision of food information to consumers contains specification regarding recommended daily allowances for vitamins. In this context, as some dairy products have a significant intake of vitamins, consumers looking for healthy foods should be informed about this, especially as public interest in functional foods has increased.

Materials and Methods

Samples

Several types of traditional Romanian cheese, meaning cow, sheep, goat and buffalo *Telemea* were selected for vitamins content determination. In addition the same analysis were made on some *Kashkaval* types made from cow milk: *Penteleu* (cow 1), *Rucar* (cow 2), *Dalia* (cow 3); sheep milk: sheep 1, *Dobrogea* (sheep 2) and one smoked type of *Kashkaval* (obtained also from cow milk). Dairy products were purchased from local supermarkets.

Analysis of vitamins B1, B2, B12, vitamin A (expressed as β -carotene), vitamin E (expressed as α -tocopherol) was performed in triplicate. The cheese samples were finely crushed and an average sample was weighed for each type. The results were expressed per 100 g of fresh weight (FW).

Analysis methods

Determination of vitamin B1 content

Sample preparation. A weighed amount of sample was digested with sulphuric acid on a boiling water bath and subsequently treated with an enzymatic complex containing phosphatase and protease for releasing the thiamin from the natural ester and protein bonds.

Analysis method. Thiamin content was analysed using a fluorometric method based on the oxidation of thiamin with oxidizing reagent (potassium ferricyanide in alkaline solution) to fluorescent thiochrome which was extracted with isobutyl alcohol. The intensity of fluorescence of the isobutyl alcohol extract was compared with that of the standard solution (100 µg/ml thiamin). Also a blank (control) prepared by adding of sodium metabisulphite in the sample was used. The intensity of fluorescence was measured with a JASCO FP-6300 spectrofluorometer and the fluorescence value of the blank test was subtracted from that of the sample extract. Fluorometric analysis of thiamin involve 370 nm as the excitation wavelength and 460 nm as the fluorescence emission wavelength (YANG, 2015).

Determination of vitamin B2 content

Sample preparation. Fluorometric determination of vitamin B2 was performed after extraction in acidic medium under heating. Acid hydrolysis must be followed by enzymatic hydrolysis which is performed in order to release riboflavin from the FAD and FMN enzymes present in the analyzed product. Therefore digestion with sulphuric acid on a boiling water bath (15 min) and subsequently enzymatic treatment with phosphatase and protease were applied.

Analysis method. Vitamin B2 content was fluorometric measured after extraction in isobutyl alcohol. The intensity of fluorescence of the isobutyl alcohol extract was compared with that of the standard solution (100 µg/ml riboflavin). Also a blank (control) was prepared by adding of sodium metabisulphite in the sample. The intensity of fluorescence was measured with a JASCO FP-6300 spectrofluorometer and fluorescence value of the blank test was subtracted from that of the sample extract. This method is applicable to dairy products for vitamin B2 content ranging from very low (minimum 5 ppm) to very high values. Analysis of riboflavin by fluorometric method was made at 450 nm as the excitation wavelength and 525 nm as the fluorescence emission wavelength (YANG, 2015).

Determination of vitamin B12 content

Total content of vitamin B12 was measured according to the AOAC 960.46 microbiological method using the VitaFast Vitamin B12 test kit (produced by R-Biopharm). Vitamin B12 was extracted from a homogenized sample and the extract is diluted. The diluted extract and the standards were applied to the wells of a microtiter plate coated with *Lactobacillus delbrueckii subsp. lactis (leichmannii)*. The growth of the bacteria is dependent on the vitamin content and induces turbidity proportional to the amount of vitamin present in the culture medium. The measurement of the turbidity was performed with a microplate reader, at 630 nm.

Determination of vitamin A content (expressed as β-carotene)

A spectrophotometric method adapted to the complex matrix of dairy products was used to determine the amount

of vitamin A which was quantified as β-carotene (BALAN, 2014).

Samples preparation. The samples were treated with alcoholic KOH solution in order to perform a grease saponification.

Extraction of β-carotene. The extraction of β-carotene was performed in separatory funnels with portions of acetone till the last extract became colorless. The acetonic extracts were collected in another separatory funnel and hexan were added so two layers are separated. The clear extract of β-carotene (superior layer) was collected and the absorbance was measured at 450 nm. Quantification was realized with a calibration curve prepared with β-carotene solution. The limit of quantification was 0.2 µg/ml β-carotene.

Determination of vitamin E (α-tocopherol) content

The content was analyzed using a reversed phase HPLC with UV detection method (GHERGHINA et al, 2015).

Sample preparation. Saponification of samples with KOH ethanolic solution was performed at 80°C, with continuous agitation, for 15 minutes. Ascorbic acid was used for preventing vitamin E oxidation. After saponification, α-tocopherol was extracted with isoctane. The non-polar solvent was evaporated at 45°C in a centrifugal evaporator and vitamin E was solubilized in methanol.

Analysis method. Separation of tocopherols was carried out by RP-HPLC method, using a Waters Alliance system, with UV detection. The separation was performed with C8 Symmetry column and a mobile phase consisting in methanol + acetonitrile + water solution. The data was achieved and processed with EMPOWER 2.0 specialized software. Calibration curve was obtained using 56.22 µg/ml α-tocopherol standard solution.

Statistical analyses. All measurements were carried out in triplicate, and the results are presented as means ± S.D. The mean values of the quantitative variables were compared by Analysis of Variance (ANOVA) considering the differences as significant for $p \leq 0.05$.

Results

Assessment of vitamin B1 (thiamin) in the analyzed type of cheese

In Telemea type cheeses, vitamin B1 content was found between 6.04 ± 0.21 µg/100 g FW (cow Telemea) and 21.49 ± 0.95 µg/100 g FW (goat Telemea) (Figure 1).

There was a significant difference in the content of vitamin B1 between the two samples of Telemea obtained from goat milk, which may indicate that not only the breed of the animal that provides the raw material (milk) influences the concentration of vitamin B1 in the final product, but also other factors (food, animal age, season, milk processing).

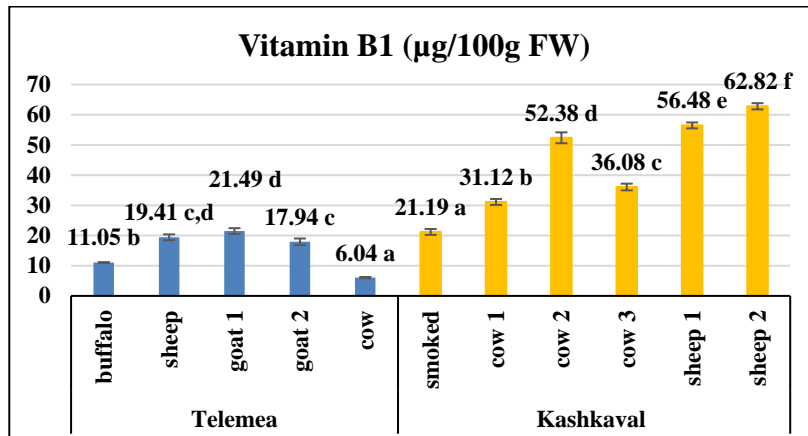


Figure 1. Content in vitamin B1 in the selected types of cheese

Note: For each group averages (\pm SD; n=3) noted with the same letters are not significantly different ($p \leq 0.05$)

For Kashkaval types, the values of vitamin B1 ranged between 21.19 ± 0.97 in smoked Kashkaval and 62.82 ± 1.03 µg/100 g FW (Figure 1) in Kashkaval type Dobrogea (sheep 2), therefore higher than those determined in Telemea cheese.

Assessment of vitamin B2 (riboflavin) in the analyzed type of cheese

Analysis of local cheeses indicated that riboflavin content reached values around 10 times higher than those of vitamin B1 for all analyzed dairy products (Figure 2). For Telemea cheeses, the lowest value of vitamin B2

content was obtained for buffalo Telemea (187.12 ± 9.04 µg/100 g FW), while goat Telemea achieved the highest value (1068.22 ± 24.15 µg/100 g FW). According to the study conducted by Górska-Warsewicz (2019), dairy products are a very important source of riboflavin, the author reporting an average value of 3.79 mg/100 g riboflavin in the studied types of cheeses.

As in the case of vitamin B1, there was a significant difference between the two types of cheese obtained from goat milk in terms of vitamin B2 content, which can be explained by their different origin as a geographical area.

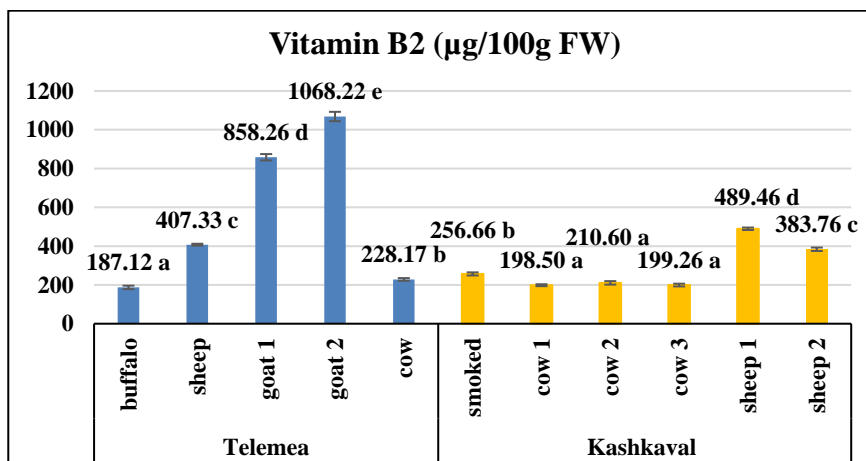


Figure 2. Content in vitamin B2 in the selected types of cheese

Note: For each group averages (\pm SD; n=3) noted with the same letters are not significantly different ($p \leq 0.05$)

For the Kashkaval types, vitamin B2 amounts ranged from 198.50 ± 4.97 to 489.46 ± 6.41 µg/100 g FW (Figure 2). The highest value was found for cheese made from sheep milk, and the lowest for Penteleu cheese (cow 1). No significant differences were observed between the Kashkaval types obtained from cow milk, independent of their brand or their origin (Penteleu, Rucar, Dalia).

Assessment of vitamin B12 (cobalamin) in the analyzed type of cheese

In the case of Telemea type cheeses, the recorded content of vitamin B12 was between 0.10 ± 0.01 µg/100 g FW (in goat Telemea) and 0.49 ± 0.02 µg/100 g FW (in cow Telemea) (Figure 3). There are no significant differences between the two types of goat Telemea.

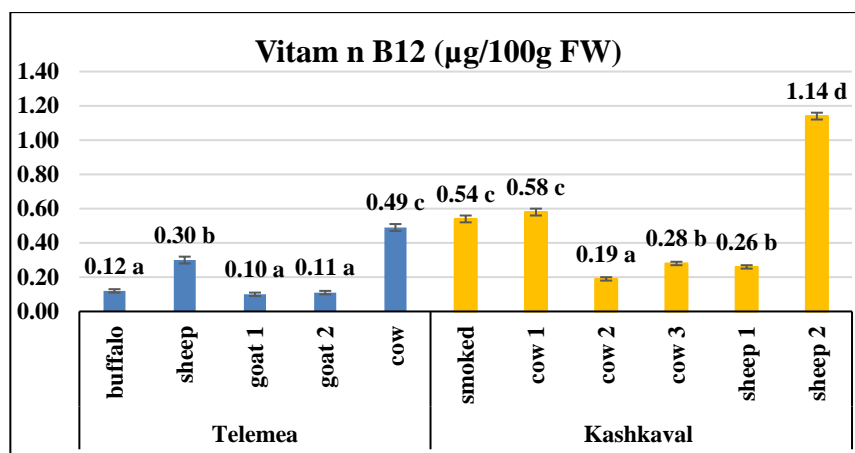


Figure 3. Content in vitamin B12 in the selected types of cheese

Note: For each group averages (\pm SD; n=3) noted with the same letters are not significantly different ($p \leq 0.05$)

The amounts of vitamin B12 were lower than those obtained for Kashkaval type products: between 0.19 ± 0.01 $\mu\text{g}/100$ g FW in Rucar Kashkaval (cow 2), and 1.14 ± 0.02 $\mu\text{g}/100$ g FW in Dobrogea Kashkaval (sheep 2).

The analysis of cobalamin indicate that the results are related with the vitamin B12 in milk, but also with the processing method typical for each variety of Kashkaval.

Mean vitamin B12 concentrations in different cheese products is reported by the national food composition databases of Denmark, Switzerland and Canada between 0.34 - 3.34 $\mu\text{g}/100$ g (GILLE, 2015).

Assessment of vitamin A (expressed as β -carotene) in the analyzed type of cheese

The obtained results showed low values for the β -carotene content (Figure 4) compared to group B vitamins. Vitamin A is partially influenced by both the

original milk composition and the obtaining technology (LUCAS, 2006). A small proportion of the retinol and carotenoids is associated with whey proteins (PUYOL, 1991) and/or concentrated in the milk fat globule membrane (ZAHAR, 1995). As a result, some amount of these components could be lost with the whey during cheese-making or by skimming of milk, while fermentation and ripening of curd induce an enrichment in group B vitamins provided by certain bacterial strains (*Lactobacillus* sp., *Bifidobacterium* sp.) involved in dairy products obtaining.

For Telemea cheeses the β -carotene content varied between 3.68 ± 0.91 $\mu\text{g}/100$ g FW in buffalo Telemea and 29.39 ± 1.47 $\mu\text{g}/100$ g FW in cow Telemea (Figure 4). Also the Telemea obtained from sheep and goat milk recorded low amounts of vitamin A compare to the cow Telemea, which was found almost three times richer in vitamin A.

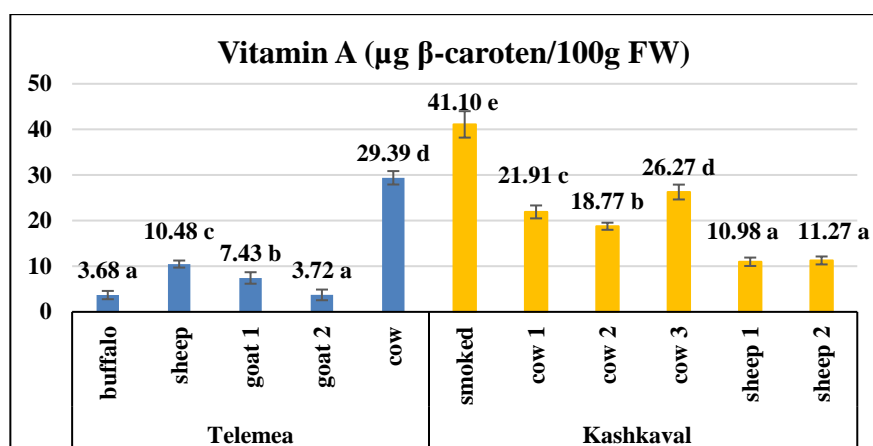


Figure 4. Content in vitamin A in the selected types of cheese

Note: For each group averages (\pm SD; n=3) noted with the same letters are not significantly different ($p \leq 0.05$)

For Kashkaval types, the amounts of β -carotene are generally higher than for Telemea cheese, reaching values between 10.98 ± 0.92 $\mu\text{g}/100$ g FW in sheep Kashkaval and 41.10 ± 2.90 $\mu\text{g}/100$ g FW in smoked Kashkaval.

The highest value was registered in smoked Kashkaval so it seems that the content in β -carotene was not affected by the smoking process but further investigations on this topic are necessary.

Assessment of vitamin E (α -tocopherol) in the analyzed type of cheese

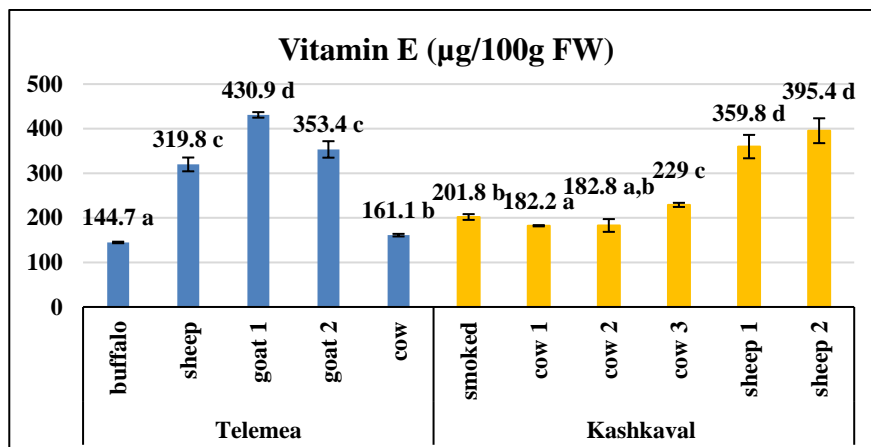


Figure 5. Content in vitamin E in the selected types of cheese

Note: For each group averages (\pm SD; n=3) noted with the same letters are not significantly different ($p \leq 0.05$)

The vitamin E content performed in the Telemea products indicated the lowest value in buffalo Telemea ($144.7 \pm 1.75 \mu\text{g}/100 \text{ g FW}$) and higher values in the two goat Telemea types ($353.4 \pm 18.5 - 430.9 \pm 6.2 \mu\text{g}/100 \text{ g FW}$) and in the sheep type ($319.8 \pm 15.45 \mu\text{g}/100 \text{ g FW}$) (Figure 5). In fact, as can be seen from the Figure 5, products obtained from sheep milk have higher values of α -tocopherol, regardless of whether they are Telemea or Kashkaval: $395.4 \pm 27.85 \mu\text{g}/100 \text{ g FW}$ in Dobrogea type of Kashkaval (sheep 2) and $359.8 \pm 26.25 \mu\text{g}/100 \text{ g FW}$ in the other sample of sheep's Kashkaval (sheep 1).

Discussions

Vitamin content of the analysed types of cheese

As in the case of Telemea cheese, the Kashkaval types obtained from cow milk register lower values of vitamin B1 content (between 31.12 ± 0.99 and $52.38 \pm 1.80 \mu\text{g}/100 \text{ g FW}$), being exceeded by those obtained from sheep milk ($56.48 \pm 0.98 \mu\text{g}/100 \text{ g FW}$ in sheep 1 Kashkaval, as well as $62.82 \pm 1.03 \mu\text{g}/100 \text{ g FW}$ in Dobrogea Kashkaval (sheep 2).

Among the Kashkaval types the lowest vitamin B1 content was found in smoked Kashkaval, being probably influenced by the production technology. Processing conditions as temperature, presence of oxygen, light and duration of heat treatment cause variable losses of vitamins according to the used method and type of food. The most labile vitamins during culinary processes are retinol, vitamin C, folate and thiamin (REDDY, 1999; LESKOVA, 2006). So, thermal processing significantly reduces the content of thiamin but changes of vitamins in dairy products depending on the obtaining technology have not yet been sufficiently investigated.

Regarding the vitamin B2 content, according to other researchers, high amounts were found in the goat Telemea. It seems that the activity of some microorganisms such as *Streptococcus thermophilus* and *Lactococcus*

lactis causes an increased riboflavin content in cheese made from goat milk (PACHECO DA SILVA, 2016).

Analysis performed on the Kashkaval types indicated that the Kashkaval obtained from sheep milk contains higher amounts of vitamin B2 than the one made from cow milk regardless of their brand. However, other authors noted a different riboflavin content in cheese depending on the type of cheese, so the obtaining technology and the origin of milk could influence this parameter. Thus, a study conducted in Italy on four types of cheese (Taleggio, Provolone, Pecorino Romano and Caciotta) revealed values ranging from $133 \mu\text{g}/100 \text{ g}$ to $328 \mu\text{g}/100 \text{ g}$ of vitamin B2 (PANFILI, 1995).

Studying the obtained results it seems that, unlike vitamin B1, the smoking process of cheese did not affect so much the content of vitamin B2, which is comparable or even higher than that of unsmoked Kashkaval types. Studies performed by other authors indicates that although vitamin losses can occur during food processing, particularly in those subjected to heat treatment (OTTAWAY, 2002), riboflavin is regarded as one of the most stable vitamins because both riboflavin and riboflavin-phosphate are stable to heat and atmospheric oxygen, especially in acidic media (GOLBACH, 2014).

Milk and dairy products are food categories which provide a significant dietary cobalamin intake (ZIRONI, 2014; EFSA NDA Panel, 2015).

Although the values recorded for vitamin B12 content are low in the local cheese, they fit within the existing variations in the literature. For example, investigating 120 cheese samples for vitamin B12 values between $0.49 - 0.93 \mu\text{g}/100 \text{ g}$ were found by Aylin and Ayaz (2009). However, Zironi et al. (2014) analysing samples of milk, rennet, whey, ricotta cheese, curd, mozzarella cheese and caciotta cheese showed about 10 times higher vitamin B12 in whey and ricotta cheese than in the milk they are derived from, explainable by the tendency of cobalamin to

concentrate in the proteic fractions along the cheese making process.

In fact, studying the values obtained for the types of cheese analyzed (Figure 3), it seems that cheese making technology influences the content of vitamin B12 because there are significant differences between the samples of cow Kashkaval (Penteleu, Rucar, Dalia) and also between the two samples of sheep Kashkaval.

The lowest values of vitamin A (β -carotene) were obtained for Telemea cheese made from buffalo and goat milk, while the highest for cow Telemea. In fact, some authors mentioned that β -carotene is present in cow milk but absent in buffalo, goat and sheep milk (RAYNAL-LJUTOVAC, 2008; ULLAH, 2017a; ULLAH, 2017b) because these animals metabolize the carotenoids into vitamin A which will be found in milk. Therefore this is the reason why differences in color between bovine and smaller ruminant dairy products are observed (PARK, 2007).

For Kashkaval types, the amounts of β -carotene reached values between 10.98 ± 0.92 and 41.10 ± 2.90 $\mu\text{g}/100$ g FW, which are generally higher than for Telemea cheese. The highest value was registered in smoked Kashkaval so it seems that the content in β -carotene was not affected by the smoking process but further investigations on this topic are necessary.

The differences observed between the β -carotene values determined in the analyzed cheeses can be explained by the different provenance of the milk, considering the fact that several authors have shown that the β -carotene present in the cheese depends almost exclusively on the β -carotene in milk (LUCAS, 2006); also the period of milking is important as the carotenoid content of milk is higher during the summer than in other seasons (HULSHOF, 2006).

The data obtained (Figure 5) reveal a much more limited variation of the vitamin E content in the analyzed samples, a variation that suggests more a correlation with the content of this analyte in the raw material (milk), than with the technological process applied for cheese-making. The Kashkaval types should be expected to have a higher vitamin E content than the Telemea cheese due to their lower water content. But, the maturation of the Kashkaval types occurs over a longer period of time, which suggests a possible loss of vitamin E by oxidative attack.

Contribution of the analyzed cheese to achievement of the vitamins NRVs (*nutrient reference values*, meaning EU guidance levels on the daily amount of vitamin that the average healthy person needs to prevent deficiency).

Cheese cannot be considered valuable source of vitamins, its nutritional importance deriving from other biochemical components. However, the fact that these products contain vitamins, both water-soluble and fat-soluble, in measurable quantities, indicates their contribution to achieving daily doses that ensure the maintenance of human health.

According to European directives (*Regulation EU No 1169/2011*), 1.1 mg is the recommended daily dose (NRV) for vitamin B1. In this context, it can be calculated a maximum intake of 2% of NRV of vitamin B1 achieved

by consuming 100 g of Telemea cheese, while Kashkaval can bring an intake of vitamin B1 of up to 6% of NRV per 100 g of product.

The recommended daily dose of vitamin B2 according to the same European directives is 1.4 mg. Analyzing the data from this point of view it can be noted that the intake of vitamin B2 achieved by consuming 100 g of Telemea cheese can be about 75% of NRV, while in the case of Kashkaval can be obtained an intake of up to 27% of NRV per 100 g of product consumed.

Regarding the vitamin B12, the daily dose for adults is 2.5 μg . The obtained results revealed that Telemea type cheeses do not bring a significant intake of vitamin B12, except for the cow Telemea which could contribute about 20% to achieve the recommended daily dose. Instead, the consumption of 100 g of Kashkaval could provide up to about 50% (Dobrogea sheep Kashkaval) of the daily requirement of vitamin B12.

Conclusions

All previous studies focused on the analysis of vitamin concentrations in milk and there are few references in the literature to the contents of these vitamins in cheeses. The present study reveals that high values of vitamin B2 and E in Telemea cheeses were registered, while Kashkaval types contain vitamins B1, B12 and A in higher amounts.

The highest values of vitamins B1, B2 and E were determined for cheese obtained from goat milk, followed by those from sheep milk, while the lowest values were observed for cheese types obtained from cow milk. Instead cow cheese is distinguished by a higher content of vitamin B12 and A (β -carotene).

Differences in vitamins content have been found between cheese made from milk of the same animal origin, which may indicate that, in addition to the breed of milk supplier, the concentration of vitamins in the final product may be influenced also by other factors related to both the milk as raw material (food, age of the animal, season etc.) and the process of cheese-making.

A large proportion of vitamins B2 and B12 nutrient reference values (NRVs) can be achieved by daily consumption of goat and cow Telemea. At the same time, sheep Kashkaval as part of the daily diet can bring most of the required amount of B2 and B12 vitamins.

In conclusion, investigated dairy products had satisfactory nutritional value with respect to vitamins so consumption of different type of cheese may help humans to achieve the recommended daily intake of vitamins and supply health benefits.

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Conflict of Interest

The authors have no conflict of interest to declare.

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