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Characterization of bioactive compounds and element content in goat milk and cheese products †

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Abstract: Goat milk and cheese are popular dairy products known for their nutritional value and distinct flavors. The presence of bioactive compounds such as carotenoids and volatile compounds in these products contributes to their sensory characteristics and potential health benefits. This study aims to compare the content of bioactive compounds in goat milk and the cheese that it was produced thereof. High Performance Liquid Chromatography (HPLC) equipped with diode array detector (DAD) was used for the quantification of beta carotene and lutein content. Lutein content in milk samples displayed higher values ranged between 0.11 and 0.25 mg/100g per sample, compared to cheese samples. Beta Carotene was not detected in neither of the matrices. For the identification of volatile compounds, Solid Phase Microextraction/Gas Chromatography-Mass Spectrometry (SPME/GCMS) were used. The volatile compounds detected, were classified into terpenes, ketones, aldehydes, acids and esters. Esters constituted the most abundant group of compounds in all samples. The simultaneous analysis of these compounds provides valuable insights into the nutritional composition, flavor profiles, and potential health benefits of goat cheese and milk. At last, the major elements comparison of milk and cheese products, including Calcium (Ca), Phosphorus (P), Potassium (K), Sodium (Na), Magnesium (Mg), Chlorine (Cl) and Sulfur (S), were quantitatively measured across all samples using a wavelength dispersive X-ray Fluorescence (WD-XRF) to establish their elemental profiles. Milk samples exhibited higher concentrations of Potassium. Conversely, cheese products displayed elevated levels on all the other elements.

Keywords: β-carotene; lutein; bioactive compounds; volatile compounds; goat cheese; goat milk; HPLC; SPME/GCMS; elements; WD-XRF

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1. Introduction

Goat cheese and milk are widely consumed dairy products known for their nutritional value and characteristic flavors. Goat's derivatives is a dynamic and growing industry sector. They contain various bioactive compounds, including beta-carotene, lutein, and terpenoids, which contribute to their sensory and health-promoting properties [1]. Therefore, the accurate determination and quantification of these compounds in goat cheese and milk are of great importance.

Beta-carotene and lutein are naturally occurring carotenoids with well-established roles as antioxidants and provitamin A precursors. Their presence in goat cheese and milk

can be indicative of their nutritional quality and potential health benefits, such as the prevention of a variety of diseases, including cardiovascular disease, certain types of cancer and eye diseases [2]. High-Performance Liquid Chromatography (HPLC) has been widely employed as a reliable analytical technique for the separation, identification, and quantification of carotenoids due to its excellent sensitivity and selectivity [3].

Terpenoids are another group of important compounds found in goat cheese and milk. They contribute to the characteristic flavors and aromas associated with these dairy products [4].Headspace Solid Phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as a powerful tool for the analysis of volatile and semi-volatile compounds, including terpenoids, due to its simplicity, sensitivity, and ability to capture the complex flavor profiles of food samples [5].

Dairy products, such as milk and cheese, are essential components of the human diet, providing valuable nutrients and contributing to overall health and well-being. Understanding the elemental composition of these products is crucial for assessing their nutritional value and ensuring quality control in the food industry [6]. The major elements of interest, including calcium (Ca), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), and sulfur (S), were the focus of the analysis. In this context, Wavelength Dispersive X-ray Fluorescence (WD-XRF) spectroscopy has emerged as an efficient analytical technique for elemental analysis in various materials, including food products.

In this study, we aim to analyze beta-carotene, lutein, terpenoids and the major element content in goat cheese and milk samples. This simultaneous analysis will provide valuable insights into the nutritional composition and flavor profiles of these dairy products. Such information can be beneficial for quality control purposes, nutritional assessment, and product development in the dairy industry. Therefore, could contribute to promoting the consumption of these dairy products as part of a balanced diet.

2. Materials & Methods

2.1. Samples pretreatment and analysis using High-Performance Liquid Chromatography (HPLC)

2.1.1. Milk samples collection and cheese preparation

Eight fresh goat milk samples from North-Western Macedonia were strained, pasteurized, cooled to 35-37°C, and treated with a starter culture, 0,02% $CaCl_2$ solution, and commercial rennet. After curdling, the curd was cut into 2 cm pieces, drained for 2 hours at room temperature, and incubated at 18-21°C for 20-24 hours until the pH reached 4.80. The curds were placed in plastic containers, immersed in brine solution and matured at 4°C for 2 months. Prior to analysis the extraction of the samples was done as described on Andrés et al., paper [7].

2.1.2. Chemicals/Reagents and Analysis

Beta-carotene and lutein standards were obtained from HPC Standards GmbH and A2S respectively. High-performance liquid chromatography (HPLC)-grade solvents, including methanol (ChemLab), triethylamine (Fischer Scientific) and acetonitrile (CarloErba,) were used for the preparation of mobile phases and sample dilutions. The HPLC analysis was performed using a separation module Ecom, ECB2000, equipped with pump and degasser (Ecom, ECP2000), oven (Ecom, ECO2000) and diode array detector (Ecom, ECDA). The HPLC system was equipped with a RP-C18 column (Fortis Technologies, 250X4,6mm, 5um) for milk samples analysis and HALO C30 (Advanced material technologies, 150X3,0mm, 2,7um) for cheese sample analysis. DAD detector was set at 450 nm for detection and quantification of beta-carotene and lutein. 3D spectra ranging from 290nm to 600nm was also recoderd. Data acquisition and analysis were performed using the Clarity Chromatography software. The mobile phase consisted of a mixture of methanol and acetonitrile in a specific ratio, typically 90:10 and 1ml TEA to prevent oxidation, for approximately 20 minutes and 10 minutes equilibrium time. A standard calibration curve

for beta-carotene and lutein was prepared by injecting a series of standard solutions with known concentrations into the HPLC system. A sample injection volume of 20uL was used for both the standards and the prepared milk/cheese samples. The HPLC separation was achieved using an isocratic elution, pumped at a flow rate of 0,8 mL/min at 35 °C. The retention times and peak areas of the analytes were recorded against their corresponding response area concentrations and used for quantification.

2.2. Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) for terpenoids detection

Terpenoid standards representing the target compounds such as Hexanal [CAS 66-25-1], limonene [CAS 138-86-3] , nonanal [CAS 124-19-6], and Pentanone-2 [CAS 107-87-9] were supplied by A2S.Acetone [CAS 67-64-1], 2-Butanone [CAS 78-93-3], a-pinene [CAS 80-56-8] and b-pinene [CAS 127-91-3] were obtained from HPC Standards GmbH. 4-methyl-2-pentane [CAS 108-10-1] was supplied by Applichem. Their purity was above 95%. The SPME/GC-MS analysis was performed using an Agilent 8890 Gas Chromatograph coupled with an Agilent 5977B Mass Spectrometer. The GC-MS system was equipped with a HP-5MS column (Agilent Technologies, 250X30m, 0,25um) for separation of terpenoids. For each milk and cheese sample, 5 ml of milk and 3 gr of cheese mixed with 10 uL 4-methyl-2-pentanone (internal standard) was placed in a glass vial[8,9].The vials were sealed and equilibrated at 50 °C temperature for 30 minutes to allow headspace equilibration. After equilibration, the SPME fiber (Sigma Aldrich, USA, 30/50 DVB/CAR/PDMS) was exposed to the headspace of the vial for a predetermined extraction time, typically 30 minutes. After extraction, the fiber was retracted into the needle and immediately inserted into the GC injection port for desorption and analysis. The GC column and GC/ MS conditions was followed as described on Gatzias et al.[8]. The obtained compounds identified according to those registered in the NIST 2020 library, a mass spectrometry database.

2.3. X-ray fluorensence for the determination of major element content in dairy products

The major element content analysis was carried out using WD-XRF (Brucker,TIGER S8) instrument, equipped with Rh x-ray tube, Be 75 um window, set of PET, LIF 200, Ge and LIF 220 crystals. Each element was determined by measuring the characteristic X-ray radiation of the ka line and its background radiation. All measurements were carried out under atmospheric He conditions. The milk samples measured as received whilst the cheese samples measured in dry basis.

3. Results & Discussion

3.1. Analyzing beta-carotene and lutein using High-Performance Liquid Chromatography

The HPLC analysis revealed only the presence of lutein in goat milk and cheese samples. The retention times for beta-carotene and lutein standards were observed at 7 and 12 minutes, respectively with the C18 column used for milk samples and 2 and 2,6 minutes with the column C30. To validate the results, the samples were spiked with standards to determine their appearance on the chromatogram in relation to the sample peak being identified. Quantitative analysis of the samples indicated that the concentration of beta-carotene in goat milk and cheese was either very low and so not detected or not present at all. Lutein concentrations were found to be between 0.11 and 0.25 mg/100g per goat milk sample. In goat cheese samples, lutein content was lower compared to milk reaching on average 0.04 mg/100g. Many factors, notably species, preservation, storage and even carotenoid digestion, affect the actual carotenoid content [10]. Carotenoid digestion is probably linked to dietary lipids for transit and to specific transporters of lipophilic molecules for absorption [10]. Generally, goats do not accumulate a high level of carotenoids, probably due to the high efficiency of vitamin A formation in enterocytes [10]. Therefore, the lower levels of carotenoid content could be linked to that. However, it remains to be investigated how dietary management could affect the carotenoid uptake.

3.2. Headspace Solid Phase Microextraction (HS-SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) for terpenoids detection

The HS-SPME/GC-MS analysis allowed the identification of different classes of compounds in feta cheese samples (Table 1 and 2). The volatile compounds were classified into ketones, aldehydes, acids, esters, and terpenoids. Within the ketones group, 2-Heptanone and 2-Nonanone were identified.). Only Nonanal from the group of aldehydes, was identified. Generally, ketones and aldehydes are either originated from cut forage or produced during drying [10]. However, Nonanal could also be a product of the metabolic activities, as certain lactic bacteria strains can produce straight-chain aldehydes in milk and cheese [11]. Among the volatile acids, three (Hexanoic acid, Octanoic acid, and Butanoic acid) were identified in samples. These compounds, belonging to the second most abundant group, typically occur in fresh forage [10]. Esters constituted the most abundant group of compounds in all samples. Esters can be enzymically or chemically generated by the occurrence of both acids and alcohols [10]. Notable terpene detected in the samples included: α-pinene. Terpenes availability is exclusively dependent on feed intake [8]. These results indicate the presence of diverse volatile compounds in goat cheese and milk, contributing to their characteristic flavors and aromas.

Table 1. Detection of different volatile compounds of different milk samples.

Volatile Components	M1	M2	M3	M4	M5	M6	M7	M8
Total Ketones								
2-Heptanone	+	+	+	+	+	-	-	-
2-Nonanone	+	+	+	+	+	+	-	-
Total Aldeydes								
Nonanal	-	+	+	-	-	-	-	-
Total Acids								
Hexanoic acid	-	+	-	+	+	-	+	+
Octanoic acid	-	+	+	+	+	+	+	+
Butanoic acid	-	-	-	-	+	+	-	+
Total Esters								
Octanoic acid, methyl ester	+	+	-	-	-	-	-	-
Decanoic acid, methyl ester	+	+	-	-	-	-	-	-
Decanoic acid, ethyl ester	-	-	-	-	-	-	-	-
Hexanoic acid, ethyl ester	-	-	-	-	-	-	-	-
Octanoic acid, ethyl ester	-	-	-	-	-	-	-	-
Total Terpenoids								
α-pinene	+	+	+	+	+	+	+	+

Table 2. Detection of different volatile compounds of different cheese samples.

Volatile Components	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8
Total Ketones								
2-Heptanone	+	+	+	-	-	-	-	-
2-Nonanone	+	+	-	-	+	-	-	-
Total Aldeydes								
Nonanal	-	-	-	-	-	-	-	-
Total Acids								
Hexanoic acid	-	+	-	-	-	-	-	-
Octanoic acid	-	+	+	-	-	-	-	-
Butanoic acid	-	-	-	-	+	+	-	-
Total Esters								
Octanoic acid, methyl ester	-	-	-	-	-	-	-	-
Decanoic acid, methyl ester	-	-	-	-	-	-	-	-

Decanoic acid, ethyl ester	-	+	+	+	+	+	+	+
Hexanoic acid, ethyl ester	-	+	-	+	+	-	+	-
Octanoic acid, ethyl ester	-	+	-	-	+	-	+	+
Total Terpenoids								
α-pinene	+	+	+	+	+	+	+	+

3.3. Determination of major element content using X-ray fluorensence

The WD-XRF analysis revealed distinct elemental compositions between milk and cheese products. Milk samples demonstrated higher concentrations of Potassium (K) compared to cheese, with average value of 1872,9 ppm (Table 3). Potassium is vital for promoting bone health, muscle function, and nerve transmission in the human body [12]. On the other hand, cheese samples exhibited elevated levels on the rest of the elements. Sodium values ranged between 1,4 % and 2,5 %, Chlorine values vary from 1,5 % to 3,2 %. Average values concentrations 3645,7 ppm, 5164 ppm and 1773,6 ppm are shown for Phosphorus (P), Calcium (Ca) , and Sulfur (S), respectively (Table 3). Cheese samples shown higher concentrations of Na, Ca, and Cl compared to milk samples, owing to the incorporation of these elements during the cheese-making process. Besides that, Ca comes in greater percentage from dairy products rather than milk, as casein micelles constitute the natural vector of Ca [13]. Magnesium (Mg) levels in cheese were twice as high as in milk. Mg plays an important role in many physiological processes, such as metabolism of proteins and nucleic acids, neuromuscular transmission and bone growth [14]. The elemental composition differences observed between milk and cheese samples highlight the importance of incorporating a variety of dairy products in the diet to obtain a well-rounded nutritional intake. Overall, the WD-XRF analysis provides valuable insights into the major elements present in milk and cheese products, assessing their nutritional value and overall quality. Moreover, the cost-effectiveness of WD-XRF presents an efficient analytical approach for routine quality control and product development in the food industry. These findings contribute to a better understanding of dairy products' elemental content and their potential implications for consumers and manufacturers alike.

Table 3. Analysis of mineral elements in milk (n=8) and cheese (n=8) samples determined through Wavelength Dispersive X-ray Fluorescence (WD-XRF) spectroscopy.

Trait	Mean	Minimum	Maximum
Milk minerals (ppm)			
Calcium(Ca)	2097,3	861,2	2617,7
Clorine (Cl)	1925,2	1500	2615
Potassium(K)	1872,9	1481	2835,0
Phosphorus(P)	1262,9	640,6	2256,7
Sulfur(S)	579,7	345,4	965,1
Sodium(Na)	510,1	350,2	783,0
Magnesium(Mg)	186,0	138,4	371,6
Cheese minerals (ppm)			
Calcium(Ca)	5164,1	3704,8	6548,7
Potassium(K)	1311,4	772,9	1672,0
Phosphorus(P)	3645,7	2895,0	4298,6
Sulfur(S)	1773,7	1586,5	2043,8
Magnesium(Mg)	309,1	256,9	359,0
Cheese minerals (%)			
Clorine (Cl)	2,2	1,5	3,2
Sodium(Na)	1,8	1,4	2,5

4. Conclusions

This study conducted a comprehensive analysis of bioactive compounds and major elements in goat milk and the resulting cheese, providing understanding of their composition and potential health benefits. These findings demonstrated distinct variations between milk and cheese samples. The study sheds light on the transformation of bioactive compounds and elemental composition during the cheese production process, providing valuable insights into the nutritional and compositional changes that occur. Further research is warranted to investigate the impact of processing techniques and storage conditions. Additionally, studying the bioavailability and potential physiological effects of beta-carotene, lutein, and terpenoids in humans provides insights into their health-promoting properties and aid in the development of functional food products. As far as the elemental profiles is concerned, complementary analytical methods may be necessary for a more detailed elemental analysis in future studies as this study focused solely on major elements (Ca, P, K, Na, Mg, Cl and S) and did not explore trace elements, which may also contribute to the overall nutritional quality of dairy products.

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