Properties of Water Soluble Extracts of Feta, Metsovone and Related Cheeses

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Article

Properties of Water Soluble Extracts of Feta, Metsovone and Related Cheeses

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Featured Application: The investigation of antioxidant activity of cheeses has unveiled an array of promising applications in food industry and promotion of consumer health. Cheese, as a widely consumed dairy product, possesses antioxidants that not only contribute to its preservation, but also enrich the quality and health benefits for the consumers.

Abstract: The purpose of the present study was to evaluate the antioxidant activity and peptide levels of Feta cheese and other brined cheeses, and Metsovone cheese, and other smoked cheeses. The antioxidant activity was determined by Folin and FRAP assays, while the peptide content was determined by Bradford and Lowry assays. The assays were applied in the water soluble extract of cheeses. The results showed that Feta cheese and brined cow cheese differ in antioxidant activity. Feta cheese and brined goat cheese also differ in both antioxidant activity and peptide levels. Results also showed that Metsovone cheese and other smoked cheeses exhibit antioxidant activity and significant peptide levels. Moreover, the water soluble extracts of all cheeses show some anti-inflammatory activity, suggesting that may peptides exhibit anti-inflammatory activity.

Keywords: feta cheese; Metsovone cheese; antioxidant activity; peptide content; anti-inflammatory activity

1. Introduction

Cheese is a fermented milk-based product that is produced globally in different varieties. The raw material, milk, used in cheese production is mainly cow, goat and sheep. The characteristics of cheeses are depended on the composition and quality of milk, as well as various other factors [1,2].

Feta cheese is recognized as a Protected Designation of Origin (PDO) product and belongs to brined cheeses [3]. It is made exclusively from sheep milk or a combination of sheep and up to 30% goat milk. Metsovone is a pasta filata type cheese, produced from raw cow milk or a blend of cow, sheep, and goat milk, with goat milk content not exceeding 20% of the mixture [4]. Metsovone cheese is classified as a smoked cheese [5].

The water soluble extract (WSE) of cheese contains mainly small and medium-sized peptides, free amino acids and salts. The water is an efficient solvent for extracting small peptides from cheese, many of which play a crucial role in bioactivities. The level of water-soluble nitrogen is used for cheese ripening index, as it tends to increase during ripening process [6,7].

During ripening process, biochemical and chemical reactions occur, which contribute to the formation of the characteristic texture and flavour of each cheese. The main milk proteins, αs1-casein and β-casein, are degraded into larger and smaller peptides, that influence the texture and flavour of cheeses, by biochemical factors. The larger peptides and oligopeptides enhance cheese flavour, as substrates for enzymatic activities of the microbiota. Peptides are components that are generated and degraded during cheese production, ripening, and preservation [8–11].

Cheese contains antioxidants that inhibit oxidative processes in the cheese and neutralize free radicals, prevent oxidative changes and support body's defense mechanisms. Peptides, which have

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positive effects on metabolic functions and provide health benefits, are defined as bioactive peptides. These peptides have various bioactivities, such as antioxidant, antimicrobial, anti-inflammatory, and other actions [12–14]. Gupta et al. showed increase of antioxidant activity of Cheddar cheese, while after fifth month a decrease was observed, suggesting that antioxidant peptides were not resistant to further proteolysis [15]. Bottesini et al. investigated the antioxidant activity of Parmigiano-Reggiano cheese, which is mostly due to antioxidant-free amino acids and in minor extent to antioxidant peptides [16]. Hernández-Ledesma et al. identified peptides contained at least one proline residue, suggesting that these peptides were likely responsible for the antioxidant activity that was found [17].

Antioxidants are also commonly used to inhibit the action of lipoxygenase and prevent autoxidation of substrates. Lipoxygenases exist in the human body and play a significant role in inflammation. Therefore, the inhibition of lipoxygenases is considered important for disease prevention. Inflammation is associated with diseases, such as cancer, diabetes, asthma, atherosclerosis, and neurodegenerative [18]. Laakso & Lilius reported peptides, derived from milk casein, responsible for inhibition of lipoxygenase activity [19].

In the present study was carried out analysis of water soluble extracts of Feta cheese and other brined cheeses, as well as Metsovone cheese and other smoked cheeses. The purpose was to evaluate the peptide levels and antioxidant activity in different cheeses.

2. Materials and Methods

2.1. Cheese samples

In this study, triplicate cheese samples were sourced from a selection of diverse dairy industries located across Greece. Feta samples were obtained from Dodoni (Epirus 1), Karalis (Epirus 2), Bizios (Elassona, Thessaly), Erymanthos (Achaia, Western Greece). Goat cheeses were collected from Dodoni (Epirus 1), Karalis (Epirus 2), Exarchos (Elassona, Thessaly), Erymanthos (Achaia, Western Greece). Cow cheeses were supplied from Dodoni (Epirus 1), Bizios (Elassona, Thessaly), Vlacha (Central Macedonia). Metsovone samples were from Tositsa Foundation (Epirus) and the other smoked cheeses were supplied from Pappas (Epirus) and Belas Vermion (Central Macedonia).

2.2. Reagents

Folin-Ciocalteu's phenol reagent 2 N was purchased from Sigma-Chemicals (St. Louis, USA). Gallic acid, bovine serum albumin (BSA) 96 %, sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). The reagent 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) was from Fluka (Buchs, Switzerland). Hydrochloric acid ≥ 37 %, lipoxygenase and linoleic acid were from Sigma–Aldrich (Steinheim, Germany). Acetic acid was purchased from Carlo Erba (Val de Reuil, France). Boric acid was from Riedel-de Haën (Seelze, Germany), while Bradford reagent was purchased from Roti(Karlsruhe, Germany). Ethanol ≥ 98 % was purchased by Honeywell (North Carolina, USA). Water HPLC grade and acetonitrile HPLC grade were purchased from Chem-Lab (Zedelgem, Belgium).

2.3. Instruments and Apparatus

The absorbance is measured using a three-decimal precision spectrophotometer, Jenway 6505 UV/VIS (Stone, Staffordshire, U.K.). The pH measurements were obtained using a Consort C831 pH-meter (Turnhout, Belgium), with a precision of two decimal points. The weight of materials was measured using an electronic balance, Kern ABS balance (Lohmar, Germany), with a precision of four decimal points. The Hettich ZentrifugenMikro 22R centrifuge (Tuttlingen, Germany) and Stomacher, Bag Mixer 400 (France), were used for extract preparation. Waters 660E HPLC System with Diode Array Detector Waters 996 (Massachusetts, U.K.) was used.

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2.4. Methods of Analysis

2.4.1. Cheese Extract Preparation

Water soluble extract (WSE) of cheese samples were prepared by the method of Kuchroo & Fox with some modifications [20,21]. 10 g of cheese samples were homogenized with 20 mL of distilled water. The supernatant was filtered through No.42 Whatman filter paper and then, the permeated was filtered using a 0.45 μm membrane filter.

2.4.2. Antioxidant Activity Assays

The antioxidant activity was determined using Folin-Ciocalteu assay [22,23]. In a test tube, 1.9 mL of deionized water, whose pH was adjusted to the pH of the WSE, were mixed with 100 μL of WSE, and followed by the addition of 125 μL of Folin-Ciocalteu reagent and vortexing. After 1 min, 380 μL of 20% sodium carbonate were added, the mixture was vortexed and kept in the dark at room temperature for 2 h. After incubation in the dark at room temperature for 2 h, the absorbance of the mixture was measured at 750 nm against blank. The blank was prepared by adding deionized water with the same pH as WSE's pH instead of the sample. Gallic acid was used as a standard and the results were expressed as mg/L gallic acid equivalents. They were also expressed as mg gallic acid/kg cheese.

The antioxidant activity was determined using FRAP assay [20,24]. The FRAP reagent was freshly prepared by mixing 10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 9 mM 2,4,6-tripyridal-s-triazine (TPTZ) in 0.05 M HCl, and 1 part of 20 mM $FeCl_3$ in 0,05 M (in the ratio of 10:1:1 vol/vol/vol). The FRAP assay was conducted following the methodology described in the study by Perna et al., with specific modifications. The difference was that 1.5 mL of FRAP reagent and 0.25 mL WSE were mixed and the blank reagent was prepared by adding deionized water with the same pH as WSE's pH instead of the sample. Gallic acid was used as a standard and the results were expressed as mg/L gallic acid equivalents. Moreover, were expressed as mg gallic acid/kg cheese.

2.4.3. Peptide assays

The peptide content was calculated using Bradford assay [21,25]. In an Eppendorf tube, 0.75 mL of Bradford reagent (commercial reagent 4-fold diluted with deionized water) was mixed with 0.25 mL WSE. Then, the mixture was incubated at room temperature for 5 min. After 5 min, the absorbance of the mixture was measured at 595 nm against blank (containing 0.25 mL of deionized water with the same pH as WSE's pH instead of WSE). Bovine serum albumin was used as a standard and the results were expressed as mg/L BSA equivalents. They were also expressed as mg BSA/kg cheese.

The peptide content was calculated using Lowry assay [26,27]. Prior to the analysis, the reagent of Lowry was freshly prepared by mixing 50 parts of reagent A: 2% Na_2CO_3 in 0.1 M NaOH, and 1 part of reagent B: 0.5% $CuSO_4{\cdot}5H_2O$ in 1% potassium tartate. In an Eppendorf tube, 200 μL deionized water, whose pH has been adjusted to the pH of WSE, were mixed with 50 μL of WSE. Then, 1.25 mL Lowry reagent wasadded and the mixture was vortexed. After 10 min incubation in the dark at room temperature, 0.25 mL of diluted (1 M) Folin-Ciocalteu reagent was added and the mixture was kept in the dark for 30min. After 30 min, the absorbance of the mixture was measured at 750 nm against blank (containing 50 μL of deionized water with the same pH as WSE's pH instead of WSE). Bovine serum albumin was used as a standard and the results were expressed as mg/L BSA equivalent. Moreover, were expressed as mg BSA/kg cheese.

The peptide profile was evaluated by HPLC analysis [28,29]. Waters 660E HPLC system and the column, MZ Analysentechnik C18 (4.6 mm x 25.0 cm) were used. The elution was gradient, using solvents A (0.1 % v/v trifluoroacetic acid in HPLC-grade water) and B (0.09 % v/v trifluoroacetic acid in a 60:40 v/v mixture of acetonitrile and HPLC-grade water). Solvent A was 100 % for 5 min, followed by change in composition from 0 % to 100 % of solvent B for 20 min, and finally, 100 % solvent B for 13 min. The flow rate was 0.8 mL/min. Before injection, the samples were diluted 10 times with

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deionized water. A volume of 20 μL of diluted WSE was injected into the column and the peptide elution was monitored at 214 nm.

2.4.4. Anti-inflammatory Assay

The anti-inflammatory activity was determined using lipoxygenase inhibition assay [19,30]. In an Eppendorf tube, 900 μL of borate buffer 1M pH=9, 100 μL of WSE and 100 μL of LOX (diluted in borate buffer) were mixed. Then, the mixture was vortexed and kept for 5 min in the dark. After 5 min, 100 μL of 4.18 mM linoleic acid in ethanol were added. The absorbance was measured at 234 nm for 20 min. The control contains deionized water, whose pH was adjusted to the pH of WSE, instead of sample. The control of blank contains ethanol instead of linoleic acid and deionized water with the same pH as WSE's instead of sample. The sample blank contains ethanol instead of linoleic acid. The anti-inflammatory activity was expressed as inhibition of lipoxygenase and was estimated through the formula: % Inhibition= (Acontrol-Asample)/Acontrol.

2.5. Statistical Analysis

To compare the statistical difference of the above measurements among the types of cheese considering the different region, we fitted mixed effect models. Specifically, we assumed the type of cheese as a fixed effect (as we are interested in comparing the measurements for the specific types of cheese), while we assume the region as a random effect (considering the observed regions as a random sample of all possible region where these cheeses are made). For the pairwise comparisons between two different types of cheeses, we used the Kenward-Roger (K-R) Degrees of Freedom Approximation [31,32].

The findings of the fitted models described in section 3., while the descriptive statistics of the variables are in the Supplementary material. The software of RStudio 4.3.1 was used for statistical analysis.

In the Tables below, we provide the estimated pairwise differences along with their level of statistical significance. The Tables in Results provide the pairwise expected differences between the type of cheeses for the variables of interest, the corresponding standard errors, or standard deviations for t-tests (in parenthesis) and p-values (in italics). The reported p-values are adjusted so that each fitted model has a 5% type I error in total (controlling the Familywise Error Rate), so that we can be more confident about the statistical significance of the differences.

The main result demonstrates the statistical difference for most of the under-study variables between feta cheese and the rest (cow and goat).

The colors for the statistical significance of the results are the dark red color corresponds to a strong statistically significant difference (more than 99 %), the dark blue corresponds to a moderate statistically significant difference (between 95 %-99 %), and the yellow corresponds to a mention (between 90 %-95 %).

3. Results and Discussion

3.1. Feta Cheese and Other Ripened Brined Cheeses

In Tables 1–3 antioxidant activity and peptide levels of water soluble extract of Feta cheese, brined goat cheeses, and brined cow cheeses from different regions of Greece are presented.

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Table 1. Antioxidant activity (Folin and FRAP assays) and peptide content (Bradford and Lowry assays) of water soluble extracts of ripened Feta cheese from different regions of Greece.

Region	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
Epirus 1	179±7	3.72±0.02	1627±28	3777±114
Epirus 2	130±3	3.55±0.08	1487±55	2994±74
Thessaly	201±2	3.97±0.06	2468±81	4890±123
Western Greece	162±4	3.49±0.06	1220±31	3204±96

The results are presented as Mean ± SD (standard deviation). The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

Table 2. Antioxidant activity (Folin and FRAP assays) and peptide content (Bradford and Lowry assays) of water soluble extracts of ripened brined goat cheeses from different regions of Greece.

Region	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
Epirus 1	110±3	1.75±0.01	782±28	2200±67
Epirus 2	129±3	2.77±0.05	808±30	2662±75
Thessaly	118±1	2.78±0.08	805±25	3007±72
Western Greece	148±4	2.86±0.07	900±35	2974±51

The results are presented as Mean ± SD. The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

Table 3. Antioxidant activity (Folin and FRAP assays) and peptide content (Bradford and Lowry assays) of water soluble extracts of ripened brined cow cheeses from different regions of Greece.

Region	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
Epirus	100±3	1.98±0.05	1617±34	2277±70
Thessaly	184±6	2.90±0.06	2542±46	4520±65
Central Macedonia	133±3	2.77±0.06	1418±49	3190±81

The results are presented as Mean ± SD. The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

The water soluble extracts of brined cheeses exhibit antioxidant activity. Perna et al. reported antioxidant activity in WSEs of cow cheeses [20].

In Table 4 statistical analysis of the results for antioxidant activity and peptide content of Feta cheese, brined goat cheese, and brined cow cheese, is presented.

Table 4. Expected differences among brined cheeses for antioxidant activity and peptide content.

Cheeses	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
Feta–Cow	30.42 (11.37) 0.01	1.16 (0.14) 0.00	-194 (139.06) 0.17	546.5 (235.81) 0.06
Feta–Goat	42.08 (8.81)	1.14 (0.11)	876.67 (105.46)	1005.25 (178.87)

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	0.00	0.00	0.00	0.03
	11.65	-0.02	1070.66	458.75
	(11.37)	(0.14)	(139.06)	(235.81)
Cow–Goat	0.31	0.89	0.00	0.06

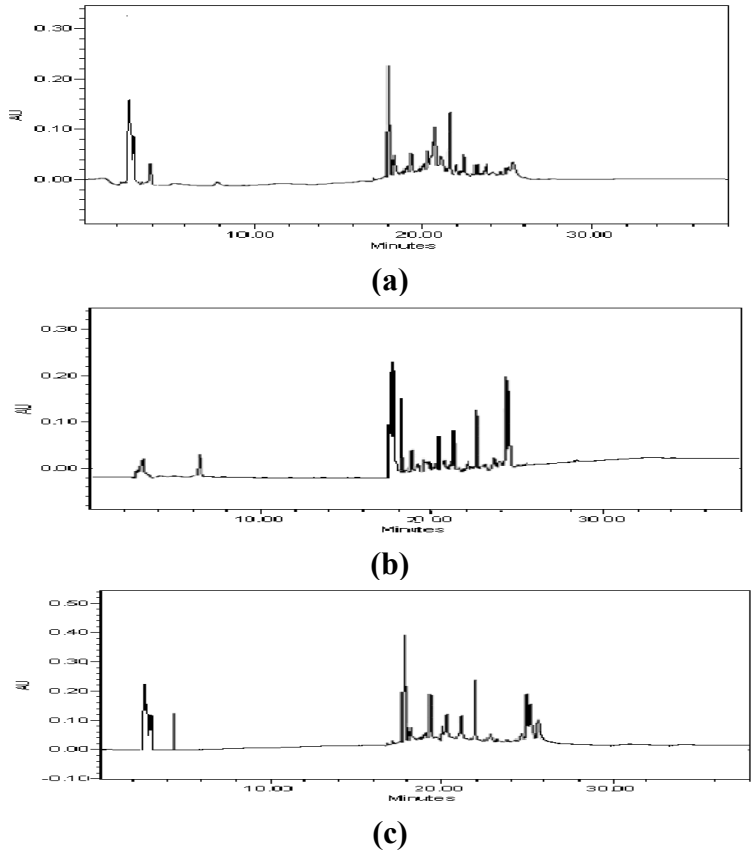
The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

Feta cheese and cow cheese show statistically significant difference in antioxidant activity, determined by both Folin and FRAP assays. The antioxidant activity of Feta cheese is higher than brined cow cheese. Feta cheese and goat cheese differ significantly in antioxidant activity, determined by Folin and FRAP assays, and peptide content, determined by Bradford and Lowry assays. The antioxidant activity and peptide content of Feta cheese are higher than brined goatcheeses.

Stobiecka et al. investigated the antioxidant activity of cow, goat, sheep milk with various assays, including FRAP. The research showed differences in antioxidant activity between different types of milk due to their chemical composition differences. Goat milk had the highest antioxidant activity and cow milk had the lowest antioxidant activity [14]. Perna et al. determined antioxidant activity of cow cheeses by FRAP assay, which was increased during ripening process [20]. Gupta et al. showed the significant peptide levels of WSEs of Cheddar cheese dependent on the ripening process [15].

Comparison of the chromatograms of Feta cheese, brined goat and cow cheeses, shows two similar peak regions. In the first region, two high peaks appear, corresponding to hydrophilic peptides, at similar retention times. In the second region, which corresponds to more hydrophobic peptides, a cluster of different peaks is observed within a similar retention time range. However, there is differentiation among the peaks, and consequently, in their peptide profile. Therefore, there is a general similarity, but individual differences.

In Figure 1, the chromatograms of Feta cheese from different regions of Greece, Epirus 1, Epirus 2, Thessaly and Western Greece, are presented.



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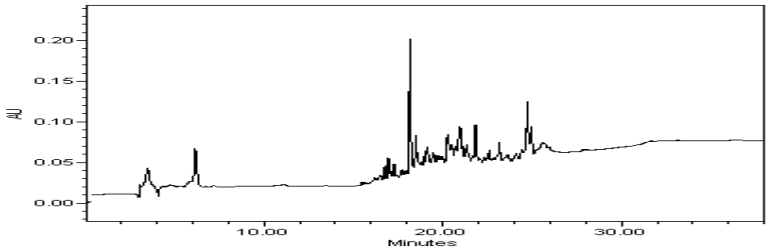
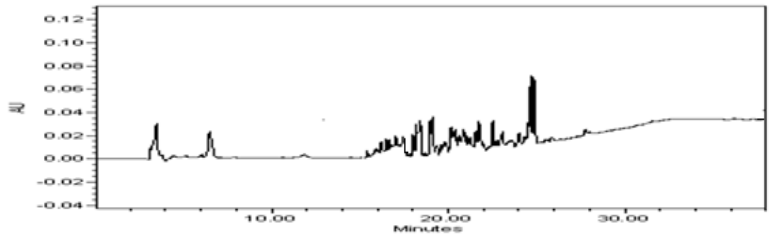


(d)

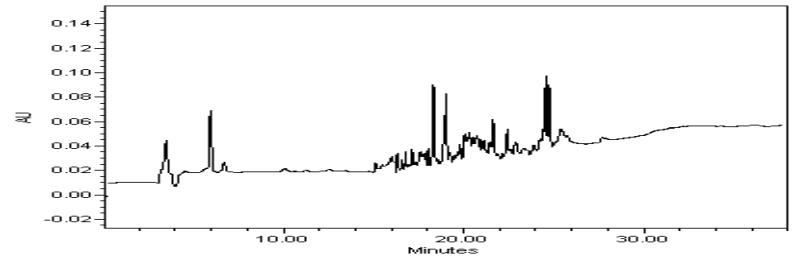
Figure 1. Chromatogram of Feta cheese from (**a**) Epirus 1, (**b**) Epirus 2, (**c**) Thessaly, (**d**) Western Greece.

The chromatograms of Figure 1 show two peak regions. In the first region, corresponding to hydrophilic peptides, the first high peak appears at the same retention time, while the second high peak is shifted in Feta of Epirus 2 and Western Macedonia compared to Feta of Epirus 1 and Thessaly. In the second region, a cluster of various peaks is observed, corresponding to more hydrophobic peptides, within a similar retention time, except for Feta of Epirus 2 where the peaks appear in a more restricted range. Some distinct peaks appear at the same retention times in each chromatogram, while two distinct peaks appear for the cheeses of Epirus 2 and Thessaly. There is differentiation among the peaks, and consequently, in their peptide profile. Therefore, there is a general similarity, but individual differences.

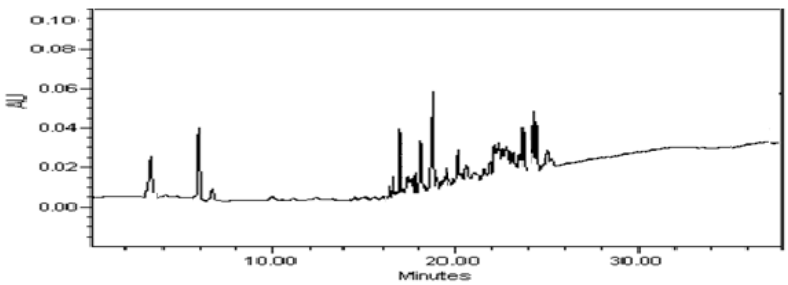
In Figure 2, the chromatograms of ripened brined goat cheese from different regions of Greece, Epirus 1, Epirus 2, Thessaly and Western Greece, are presented.



(a)



(b)



(c)

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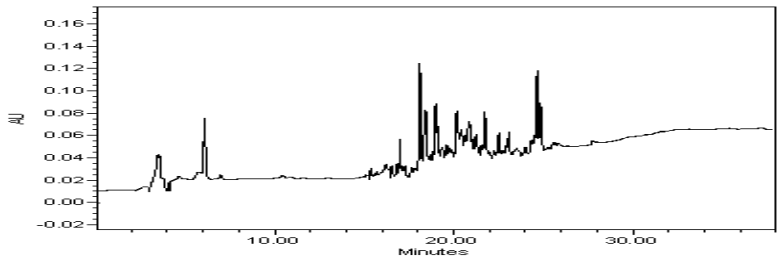
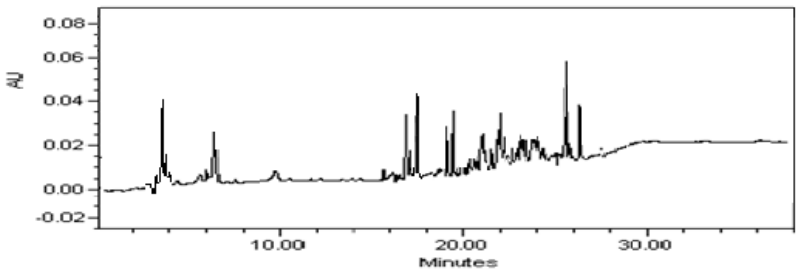


(d)

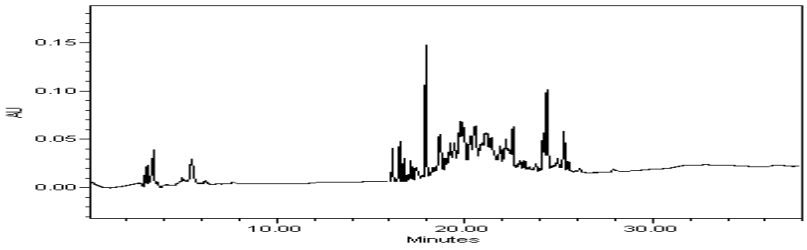
Figure 2. Chromatogram of brined goat cheese from (**a**) Epirus 1, (**b**) Epirus 2, (**c**) Thessaly, (**d**) Western Greece.

The chromatograms of Figure 2 appear two peak regions. In the first region, which corresponds to hydrophilic peptides, the first and second high peaks appear at similar retention times in all goat cheeses, except for Goat of Epirus 1, where the retention times are slightly differentiated. In the second region, a cluster of various peaks of the same range is observed, corresponding to more hydrophobic peptides. In the chromatograms of Thessaly and Western Macedonia a peak appears that is not present in goat of Epirus. The peaks exhibit differentiation, leading to differences in their peptide profile. Therefore, there is a general similarity, but individual differences.

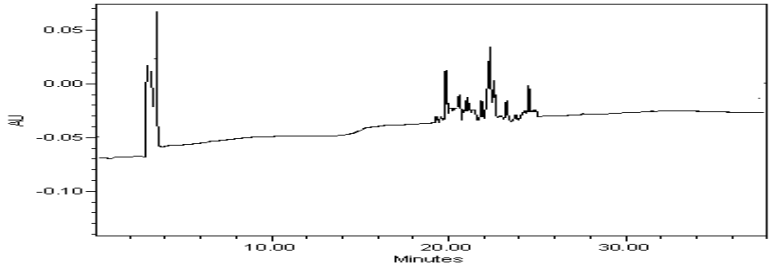
In Figure 3, the chromatograms of ripened brined cow cheese from different regions of Greece, Epirus 1, Thessaly and Central Macedonia, are presented.



(a)



(b)



(c)

Figure 3. Chromatogram of cow cheese from (**a**) Epirus1, (**b**) Thessaly, (**c**) Central Macedonia.

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The chromatograms of Figure 3 show two peak regions. In the first region, corresponding to hydrophilic peptides, the first and second high peaks appear at similar retention times in cow cheeses of Epirus 1 and Thessaly, except for Central Macedonia, where a double peak appears. In the second region, a cluster of various peaks is observed, corresponding to more hydrophobic peptides, and appear within the same elution time range for cow cheeses of Epirus 1 and Thessaly, except for Central Macedonia, where the peaks appear in a more restricted range. In the chromatograms of cow cheeses from Epirus 1 and Thessaly, some of the distinct peaks appear at similar retention time. However, these peaks exhibit differences, leading to variations in their peptide profiles. Therefore, there is a general similarity, but individual differences.

Katsiari et al. carried out HPLC analysis of water-soluble extracts of Feta cheeses made with NaCl or mixtures of NaCl and KCl during ripening and preservation [28]. Moatsou et al. investigated the nitrogenous fractions of traditional Feta cheese during ripening by HPLC analysis [33]. Kocak et al. evaluated peptides profile in white-brined cheeses made from goat milk with adjunct cultures during ripening and preservation, using HPLC analysis [34]. Sahingil et al. evaluated peptide profiles of water soluble extract of white-brined cheeses during ripening and preservation using HPLC analysis [35].

In Table 5 antioxidant activity and peptide level of the water soluble extract of Feta cheese during ripening and storage.

Table 5. Antioxidant activity (Folin and FRAP assays) and peptide content (Bradford and Lowry assays) of water soluble extracts of Feta cheese during ripening and storage.

Age, Days	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
2	120±3	3,27±0,15	2212±43	2204±62
16	142±5	3,56±0,06	1245±72	2577±63
60	179±7	3,72±0,02	1627±28	3777±114
120	217±3	3,41±0,06	1767±64	4247±113

The results are presented as Mean ± SD (standard deviation). The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

The water soluble extracts of Feta cheeses have antioxidant activity. Perna et al. determined antioxidant activity of cow cheeses using FRAP assay and it was found to increase during ripening and storage [20]. Gupta et al. showed that cow cheeses had significant peptide content using Lowry assay, which was increased during ripening and storage [15]. Gandhi et al. determined peptide content using Bradford assay in water soluble extracts of brined cheeses. Reduction in peptide content of 10 days brined cheeses was observed, and then peptide content varied depending on the brine composition [36].

3.2. Metsovone Cheese and Other Ripened Smoked Cheeses

In Table 6 antioxidant activity and peptide content of water soluble extract of Metsovone cheese, aged 3 and 6 months, as well as other smoked cheeses aged 3 months from different regions in Greece.

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Table 6. Antioxidant activity (Folin and FRAP assays) and peptide content (Bradford and Lowry assays) of water soluble extracts of Metsovone cheese, 3 and 6 months, and other ripened smoked cheeses 3 months from different regions of Greece.

Region	Folin (mg gallic acid/L WSE)	FRAP (mg gallic acid/L WSE)	Bradford (mg BSA/L WSE)	Lowry (mg BSA/L WSE)
Metsovone, 3 months	291±12	7.45±0.18	3277±99	5850±153
Metsovone, 6 months	437±5	9,40±0,26	3478±70	7664±103
Epirus, 3 months	230±8	5.11±0.20	2550±114	5110±151
Central Macedonia, 3 months	294±5	9.33±0.08	2872±111	6677±96

The results are presented as Mean ± SD. The results were also expressed as mg gallic acid/kg cheese for antioxidant activity and mg BSA/kg cheese for peptide content.

The water soluble extracts of Metsovone cheese and other smoked cheeses have antioxidant activity, as determined by Folin and FRAP assays. There is also significant peptide level. The antioxidant activity was significant for Metsovone cheese 6 months.

Shaibanl et al. determined significant antioxidant activity in smoked cheeses by Folin assay [37]. Vosgan et al. observed increase of the antioxidant activity during preservation of smoked cheese [38].

In Figure 4, the chromatograms of ripened Metsovone cheese and ripened smoked cheeses from Epirus and Central Macedonia are presented.

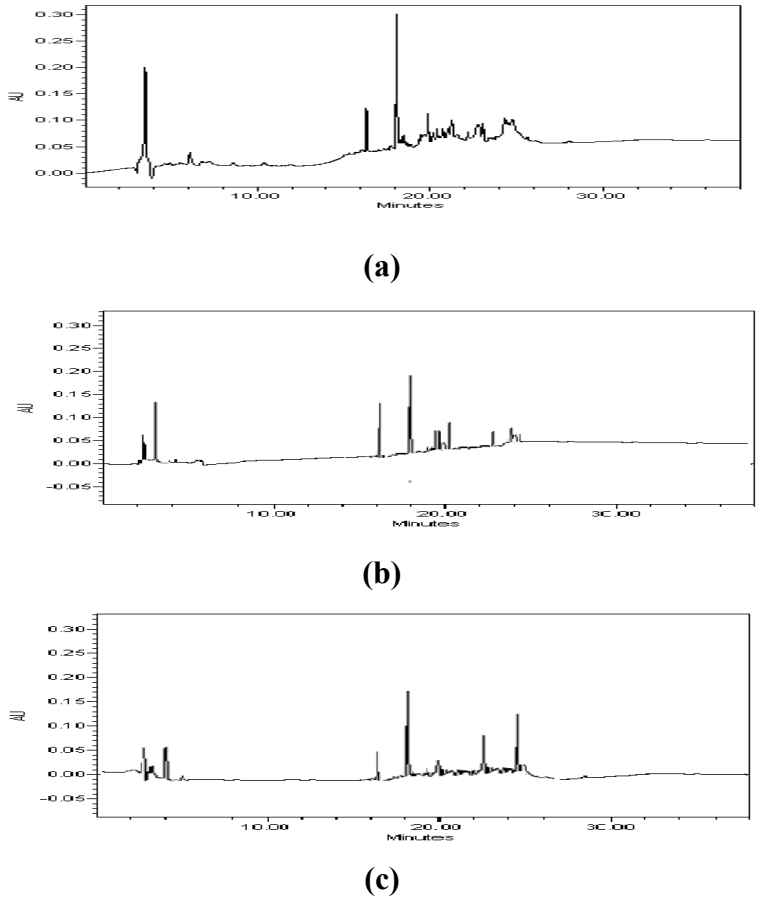

Figure 4. Chromatogram of (**a**) Metsovone, (**b**) smoked cheese from Epirus, (**c**) smoked cheese from Central Macedonia.

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Comparison of the chromatograms of Metsovone and smoked cheeses from Epirus and Central Macedonia shows two peak regions. In the first region of chromatograms of smoked cheeses from Epirus and Central Macedonia, two high peaks appear at similar retention times, while in the chromatogram of Metsovone, a single high peak is observed. In the second region, a cluster of various peaks is observed within the same retention time range and some distinct peaks appear at the same retention time in all chromatograms. Subsequently, in the chromatogram of Metsovone, there is a cluster of peaks, while in the chromatograms of smoked cheeses from Epirus and Central Macedonia, more distinct peaks appear, although they exhibit differences. Therefore, there is a general similarity, but individual differences.

3.3. Anti-inflammatory Activity of Cheeses

In Table S4 is presented the anti-inflammatory activity of Feta cheese and other ripened brined cheeses, expressed as % Inhibition. The anti-inflammatory activity of Feta cheese ranges between 5,0 % and 8,7 %. The brined goat cheese has range of values between 3,3 % and 7,0 %. The values of anti-inflammatory activity of brined cow cheese are ranged between 2,8 % and 6,0 %. During the determination of anti-inflammatory activity, the mixture wasn't clear, so the water soluble extracts of cheeses were diluted 2 times and the results had low values. The above findings suggest that peptides in the water soluble extracts of cheeses may exhibit anti-inflammatory activity.

4. Conclusions

The water soluble extracts of Feta cheese differ from brined goat cheese in both their antioxidant activity and peptide content. Furthermore, the water soluble extracts of Feta cheese differ from brined cow cheese in their antioxidant activity. Feta cheese shows higher antioxidant activity compared to brined cheeses, cow and goat. Additionally, the peptide content of Feta cheese is higher than brined goat cheese. Metsovone cheese and other smoked cheeses show antioxidant activity and contain significant peptide levels. The water soluble extracts of Feta cheese, as well as other brined cheeses, and Metsovone cheese, and other smoked cheeses, show some anti-inflammatory activity.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, I.G.R.; methodology, I.G.R.; software, K.B.; validation, I.G.R. and A.K. and I.L.; formal analysis, A.K. and I.L.; investigation, I.G.R.; resources, I.G.R.; data curation, A.K. and I.L.; writing—original draft preparation, A.K. and I.G.R.; writing—review and editing, I.G.R.; visualization, A.K. and K.B.; supervision, I.G.R.; project administration, I.G.R.; funding acquisition, I.G.R. All authors have read and agreed to the published version of the manuscript.

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Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with "A"—e.g., Figure A1, Figure A2, etc.

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