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The Physicochemical Characterization, Fatty Acid Profile, and Nutritional Indices of the Greek Traditional Cheese "*Tsalafouti*"

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Abstract: *Tsalafouti* cheese, a traditional Greek spreadable cheese, has experienced remarkable popularity over the last decade due to the strong dairy production heritage links that it presents, alongside the special nutritional and sensory attributes that it holds. The aim of this study was to chemically characterize the Greek cheese *Tsalafouti*, with a special focus on its fatty acid profile and the potential nutritional effect it may have. Standard methods were used to assess the physicochemical profile of *Tsalafouti* cheese, while a fatty acid profile was determined using gas chromatography. The analysis revealed average values, including a pH of 3.94, moisture content of 75.05%, protein content of 8.86%, and fat content of 11.3%. The fatty acid profile highlighted the predominance of health-important fatty acids, such as oleic acid, known for its well-established health benefits. Additionally, the health lipid indices of *Tsalafouti* were assessed by calculating the atherogenicity index (IA), the thrombogenicity index (IT), the ratio of hypocholesterolemic to hypercholesterolemic fatty acids (HH), the health-promoting index (HPI), and the PUFA-n-6/PUFA-n-3 ratio. The values of these indices indicate the quality concerning proatherogenicity, prothrombogenicity, and the risk of cardiovascular issues. Notably, all the above health indicators are consistent with known health effects. Importantly, the ratio of ω6/ω3 fatty acids aligns with the nutritional recommendations of the World Health Organization. Based on these results, *Tsalafouti* cheese emerges as a low-fat product and source of protein with potential functional properties, distinguishing itself from other cheeses in the market.

Keywords: *Tsalafouti*; spreadable cheese; low-fat cheese; fatty acids; physicochemical characteristics; nutritional indices

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1. Introduction

Traditional foods play an exceptional role in the culture, history, and lifestyle of a country [1]. The correlation between traditional foods and potential health benefits has become a point of interest for research, especially under the heightened public interest in nutrition and healthy dietary choices. This trend has resulted in an increased consumer demand for traditional foods, with a particular emphasis on dairy-based traditional products [2].

Globally consumed dairy products are abundant in essential nutrients, including protein, fat, vitamins, minerals, and bioactive compounds [3]. The nutritional content of a dairy product, especially when it provides rich protein and low-fat content, can contribute to a healthy and balanced diet for consumers [4,5].

In Greece, sheep milk dominates in cheese production, often supplemented by goat milk [6]. There is a lot of research that presents the nutritional and biological significance of sheep milk, citing advantages in its fatty acid profile, such as a high percentage of short- and medium-length saturated fatty acids (SFAs), high conjugated linoleic acid (CLA)

content, and low omega-6/omega-3 ratio, additionally coupled with high protein content and diverse bio-functionalities [7–10].

There are a lot of acid-soft spreadable cheeses that are produced in many regions of Greece in great variety and mainly from sheep and goat milk [11].

The most popular Greek varieties of acid-soft spreadable cheese for which there are published data are the following PDO cheeses: Galotyri, Katiki Domokou, Pichtogalo Chanion, Anevato, Kopanisti, and Xygalos of Sitia [11–13]. Galotyri is one of the oldest and most popular Greek acid spreadable cheeses traditionally produced with pasteurized milk at 90 °C, which is acidified naturally without starters in the regions of Epirus and Thessaly [13]. The traditional production of all these varieties of cheese has been industrialized and standardized for the production of commercial-quality products. Such industrial products are made from pasteurized milk with the addition of commercial starters distributed by food retailers and supermarkets [14]. Nevertheless, there are other varieties of acid-soft spreadable cheeses that are still produced today with traditional practices in small dairies in the mainland areas or islands of Greece and are distributed in these areas for local consumption. All these types of local traditional products promote the heritage and tradition of these regions and, moreover, enhance the financial sustainability of local societies [15].

A Greek traditional cheese product with a spreadable texture that has similar characteristics to cheeses belonging to this category is "*Tsalafouti*" [16,17].

Traditionally, the production of *Tsalafouti* starts in early July until mid-August, using the last milk of the lactation period, which has a more viscous composition. The process involves boiling and salting the milk during the boiling phase. Subsequently, it is covered and allowed to cool, usually in flowing water (such as a river) or in a cave and stirred once or twice a day. After approximately 20 days, it achieves a thickened consistency and is ready for consumption [17].

The industrial production of *Tsalafouti* cheese exhibits some differentiations compared to its traditional production. The process begins with the reception and filtration of raw sheep milk, or a combination of goat and sheep milk, followed by pasteurization and cooling. Natural coagulation occurs, with optional additions of rennet and/or culture. Subsequently, salt is added, initiating a stirring phase lasting for 5–6 days at 10–18 °C. Upon the completion of stirring, the cheese is packaged in small plastic containers and stored below 4 °C as the final production step [15,17].

The aim of the present study was to evaluate the physicochemical characteristics of the Greek traditional cheese '*Tsalafouti*' and also to determine its profile of fatty acids and lipid quality index in an attempt to characterize it and highlight possible functional properties.

2. Materials and Methods

2.1. Sampling

Twelve samples of Tsalafouti were collected from cheese producers on the mainland of Greece, of which six came from the Karditsa prefecture, five from the Etoloakarnania prefecture, and one from the mountainous prefecture of Evritania. *Tsalafouti* producers were using milk collected between May and October, which corresponds to the mid-to-late milking season of small ruminants. The samples from Etoloakarnania are coded with the letter A, those from Karditsa with the letter K, and those from Evritania with the letter E preceding the corresponding numbers. Each sample, divided into sterile 250 mL collectors, was stored in a freezer at −20 °C until analysis. Prior to analysis, the samples were thawed at temperatures of <6 °C and mixed gently to ensure homogeneity. All analyses were performed in triplicate.

2.2. Physicochemical Characteristics

The physicochemical characteristics were evaluated by the following analysis: pH was determined by a pH meter (Hach, HQ 11d), fat content by the Van Gulik method [18], salt content by the modified Mohr method as described in Fernandes et al. [19], and the protein

content according to the Kjeldahl method for dairy products [20]. Moisture was assessed by drying to a constant weight at 105 ± 10 °C with a moisture analyzer (KERN MLB50-3, KERN & Sohn GmbH, Balingen, Germany), and the acidity percentage of *Tsalafouti* cheese (in % lactic acid) was assessed by the titrimetric method according to AOAC 947.05. All materials and reagents used for the analyses in this study were of analytical grade, and all analyses were performed in triplicate.

2.3. Analysis of Free Fatty Acids

2.3.1. Fat Extraction

The lipid extraction of *Tsalafouti* samples was performed following the protocol chloroform–methanol–water described by Folch et al. [21], with minor modifications as described by Danezis et al. [22]. The fat extract was stored in amber vials at −18 °C pending fatty acid (FA) analysis.

2.3.2. Preparation of Fatty Acid Methyl Esters

The fat extracted from *Tsalafouti* samples was subjected to basic transmethylations according to ISO 12966-2 [23] with some modifications. About 100 mg of extracted fat was dissolved in 2 mL of n-hexane, added to 1 mL of KOH 5% in methanol, and mixed vigorously at room temperature for 5 min. After phase separation, the upper layer, which contains fatty acid methyl esters (FAMEs), was transferred into vials. A sample of 1 μL of the vial was analyzed by GC [24].

2.3.3. Gas Chromatographic Analysis of Fatty Acid Methyl Esters

The protocol of Pizzillo et al. [25] was used, with some modifications. A gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan), equipped with a flame ionization detector (FID), automatic injector, and a split injection port, was used. The GC conditions were as follows: injector temperature was set at 260 °C; injection volume was 1.0 μL; split ratio was 1:50; carrier gas, helium, had a flow rate of 0.7 mL/min; detector temperature at 260 °C. FAMEs were separated using a capillary column of 60 m, internal diameter of 0.25 mm, and film thickness of 0.25 μm (model: GE Forte (60 m × 0.25 mm × 0.25 um-BPX70)). The column temperature was initially held at 60 °C for 15 min and then increased at a rate of 3 °C/min to 150 °C and kept for 2 min; finally, the temperature was raised to 220 °C at a rate of 3 °C/min and kept for 50 min. The individual peaks were identified by comparison to reference standards of Supelco 37 Component FAME Mix (Sigma-Aldrich, Steinheim, Germany). The percentage composition of fatty acids was computed from the GC peak areas, using the normalization method (without corrective factors) of the Shimadzu GC solution data system program. The *Tsalafouti* cheese fatty acids percentages were calculated as mean values from duplicate GC-FID analysis [25].

2.3.4. Lipid Quality Indices

Lipid health quality indices of *Tsalafouti* cheese were evaluated by calculating the atherogenicity index (IA), index of thrombogenicity (IT), polyunsaturated fatty acid/saturated fatty acid ratio (PUFA/SFA), hypocholesterolemic/hypercholesterolemic ratio (HH), and health-promoting index (HPI). The lipid quality indices, known as atherogenicity and thrombogenicity indices (AI and TI), were determined according to specifications from Ulbricht and Southgate [26], following the calculation formula. The ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) is an index commonly used to assess the impact of diet on cardiovascular health (CVH). A higher PUFA/SFA ratio is generally considered more favorable for cardiovascular health [27]. The health-promoting index (HPI) was proposed by Chen et al. in 2004 to assess the nutritional value of dietary fat, specifically in the context of ruminant products such as dairy products. It is designed to evaluate the balance between different types of fatty acids in a diet and their potential impact on human health [28].

(IA): Index of atherogenicity.

Calculation formula:

$$(C12:0 + (4 \times C14:0) + C16:0)]/(PUFA + MUFA)$$

(IT): Index of thrombogenicity.
Calculation formula:

$$(C14:0 + C16:0 + C18:0)/[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n\text{-}6) + (3 \times \Sigma n\text{-}3) + (\Sigma n\text{-}3/\Sigma n\text{-}6)]$$

(PUFA/SFA): Polyunsaturated fatty acid/saturated fatty acid ratio.
Calculation formula:

$$\Sigma PUFA/\Sigma SFA$$

(HH): Hypocholesterolemic/hypercholesterolemic ratio.
Calculation formula:

$$(cis\text{-}C18:1 + \Sigma PUFA)/(C12:0 + C14:0 + C16:0)$$

(HPI): Health-promoting index.
Calculation formula:

$$\Sigma UFA/[C12:0 + (4 \times C14:0) + C16:0]$$

2.4. Statistical Analysis

The data are expressed as means ± standard deviation. Variations among values of each parameter of *Tsalafouti* cheeses were examined using a one-way analysis of variance (ANOVA). The effects of the production area and production period, considering fatty acid profiles and physicochemical characteristics, were evaluated using simple linear regression for all the tests, and a confidence level of 95% ($p < 0.05$) was set. The Stata software, version 17 (Stata Corp LLC, 4905 Lakeway Drive, College Station, TX 77845-4512, USA), was used for the analysis.

3. Results and Discussion

3.1. Physicochemical Characteristics of Tsalafouti Cheese

The means of *Tsalafouti* cheese physicochemical characteristics from different Greek producers, during a production period from May to October, are reported in Table 1.

Table 1. Physicochemical characteristics of *Tsalafouti* cheese.

Sample	Humidity %	Fat %	FDM%	Proteins %	pH	Acidity %	NaCl %
A1	75.51 ± 0.05	11	45	8.72 ± 0.14	3.67 ± 0.02	1.52 ± 0.01	2.09 ± 0.10
K2	76.78 ± 0.85	10	43	7.40 ± 0.05	3.82 ± 0.36	1.38 ± 0.14	1.99 ± 0.32
K3	76.82 ± 0.55	10	43.1	7.63 ± 0.32	3.49 ± 0.03	1.45 ± 0.07	1.88 ± 0.15
K4	83.56 ± 0.45	7	42.6	5.34 ± 0.11	3.88 ± 0.01	1.49 ± 0.03	2.04 ± 0.23
K5	80.44 ± 2.84	9.5	48.6	7.28 ± 0.21	4.09 ± 0.06	1.41 ± 0.17	1.85 ± 0.08
K6	73.80 ± 3.49	8.5	32.4	7.36 ± 0.25	4.03 ± 0.06	1.27 ± 0.29	1.98 ± 0.24
E7	75.52 ± 0.01	11.5	47	8.04 ± 0.09	3.83 ± 0.32	1.45 ± 0.01	2.19 ± 0.08
A8	72.84 ± 3.97	17	62.6	13.85 ± 0.06	4.17 ± 0.09	1.90 ± 0.10	1.28 ± 0.17
A9	64.78 ± 2.00	15.5	44	12.50 ± 0.13	4.25 ± 0.06	2.23 ± 0.28	1.48 ± 0.33
A10	68.03 ± 0.3	15	46.8	12.00 ± 0.10	4.31 ± 0.02	1.57 ± 0.04	1.25 ± 0.16
A11	72.21 ± 0.34	13	46.8	9.17 ± 0.14	3.98 ± 0.30	1.37 ± 0.13	1.76 ± 0.13
K12	80.27 ± 0.31	8	40.5	7.06 ± 0.10	3.73 ± 0.02	1.54 ± 0.05	1.71 ± 0.18
Average	75.05 ± 5.26	11.3 ± 3.172	45.2 ± 6.92	8.86 ± 2.573	3.94 ± 0.246	1.55 ± 0.264	1.79 ± 0.31
p-Value	0.172	0.02		0.017	0.084	0.092	0.011
PDO specification	73–66	17–10	50–37	>7	3.8–4.5	N/A *	1.3–2

* Not applicable. Values are means ± standard deviation ($n = 3$).

The percentage of fat, protein, and salinity showed a statistically significant difference ($p < 0.05$) between the samples, in contrast to the moisture content, acidity, and pH values, which did not show a statistically significant difference ($p > 0.05$). The noted differences in the physicochemical parameters could be attributed to the various factors affecting the final composition of milk and dairy products (including cheese), namely not only the breed and individuality of the animal, stage of the lactation period [29], health, season [30], animal nutrition [31], and farm management practices (organic versus conventional), but also the production process. Nevertheless, the results of the physicochemical analyses of *Tsalafouti* cheese are similar to the results of Pappa et al. [32].

Samples A8, A9, and A10, which came from the same producer, showed higher protein levels and fat contents than all other samples, possibly attributable to their protein content and fat content, which vary widely depending on the species, race, state of the lactation period, environmental conditions, diet, etc. [8,33]. According to Ali et al. [34], qualitatively and quantitatively, the protein in cheese depends mainly and to a large extent on the quality and quantity of the protein in raw milk. Milk that has a high protein content will yield cheese with a high percentage of protein. According to Regulation (EC) No 1924/2006 on nutrition and health claims for foods, *Tsalafouti* cheese can be considered a source of protein since 12% of energy is provided by proteins [35]. There were also statistically significant differences between the samples in terms of salt contents ($p < 0.05$). The mean salt content was lower in sample A8 (1.28%) and sample A10 (1.25%), and it was higher in sample E7 (2.19%). These differences among the samples of *Tsalafouti* cheese are probably due to the differences that exist in the amount of salt applied by the producers [36].

The moisture content of the *Tsalafouti* samples did not show a statistically significant difference ($p \geq 0.05$) and ranged from 64.78% for sample A9 to 83.56% for sample K4. Similarly, moisture content was also presented in soft creamy cheeses in the literature such as *Tsalafouti* (80.3%) in the Koutsoukis et al. study [16], Galotyri cheese (76.9%) in the Samelis and Kakouri study [37], and Tvorog cheese (72.18%) in the Bonczar et al. study [38]. According to research about spreadable cheeses by Kasapian et al. [39], Danezis et al. [22], and Lapidakis and Fragkiadakis [12], it was observed that the values of humidity are at high levels in all samples, which may be due to the high-heat treatment of the milk (87–90 °C) [40]. According to Giroux et al. [41], the heat treatment of milk increases the recovery of protein in cheese due to the denaturation of whey proteins. The formation of whey protein/κ-casein complexes on the surface of casein micelles interferes with curd contraction, slows curd shrinkage, and increases moisture retention [41]. The high water-binding capacity of the denatured whey protein also contributes to moisture retention [40–42]. Another reason for the increased moisture content of the *Tsalafouti* samples is the absence of the ripening stage or the short ripening time [43,44].

Subsequently, the fat contents ranged between 7.00% in sample K4 and 17.00% in sample A8 and showed a statistically significant difference between samples ($p \geq 0.05$). The comparison with well-known spreadable cheeses showed the following: The fat contents of the samples were at levels close to the fat of Sitia Xygalos (8.83%) and Katiki Domokos (11.3%) in the study of Danezis et al. [22] and Queso Blanco cheeses (15%) and sour cheeses from pasteurized goat milk (17.3%) [45]. There is no statistically significant difference between *Tsalafouti* cheeses ($p \geq 0.05$) in terms of pH values, which were between 3.49 in sample K3 and 4.31 in sample A10. Food safety regulations state that pH values that are below 4.5, as is the case here, support product safety in all samples [44]. In addition, the pH values of the *Tsalafouti* samples were compared with those of the corresponding types of cheeses, such as cream cheese, Neufchatel, and cottage cheese, where their values were at higher levels (4.6–4.8), and it was observed that in most cases of creamy textured cheeses in the literature, the pH values were higher [44]. However, levels like the pH values of the *Tsalafouti* samples were the pH values that Galotyri had (3.83) in the study of Danezis et al. [22] and Katiki Domokos (4.32) [39]. According to Britten and Giroux [43], the reduced pH values in cheese are a result of the higher moisture content, which increases the amount of residual lactose in cheese and promotes the production of lactic acid by

starters. The higher moisture content also reduces the concentration of casein and colloidal metals, resulting in lower regulatory capacity. These two factors lead to the production of acid and therefore the appearance of a lower pH in the cheese [43,46]. Koutsoukis et al. determined selected physicochemical characteristics of *Tsalafouti* cheese, such as protein content, moisture, and FDM, and reported values close to this study [16]. According to the PDO accreditation, the submitted specification is shown also in Table 1. As presented in Table 1, most samples (~70%) are within the proposed standard for PDO *Tsalafouti* regarding fat content, protein, pH, and salinity.

3.2. Fatty Acid Profile and Nutritional Indices of Tsalafouli Cheese

Twenty-seven fatty acids were identified in the *Tsalafouti* samples, with the majority being myristic (14:0), palmitic (16:0), stearic (18:0), and oleic (18:1n-9) acids. (Table 2). The fatty acid profile was not affected by the different producers and season variability as there were no statistically significant differences among the samples ($p \geq 0.05$). On the contrary, variations were found in the fatty acid profiles of cheeses from distinct production regions, with statistically significant differences observed in the percentages of C8:0, C10:0, C11:0, C12:0, C12:1, iso-C13:0, C14:0, C20:0, and C24:0 fatty acids ($p < 0.05$). The fatty acid profile of the cheese mainly reflects the composition of the fatty acids of the milk from which it comes and is not affected by the production process of cheese [45]. The results show that the *Tsalafouti* samples from the prefecture of Etoloakarnania probably come from animals that grazed in lowland areas, while the samples from Karditsa and Evrytania come from mountainous areas. Cooler temperatures and higher biodiversity in mountain pastures affect the milk's fatty acid profile, while botanical composition also plays a role [31,47–50]. The findings are consistent with other studies linking animal diet to milk characteristics [51,52]. The main fatty acids in all samples of *Tsalafouti* cheese are also the predominant fatty acids in ruminant milk [16]. High percentages of these fatty acids have also been found in the studies of Danezis et al. [22] in cheeses such as Domokos Katiki and Anevato, Galotyri, and Xygalo Sitias:

- It is difficult to compare the results of this study with other studies of soft-acid spreadable cheeses due to the limited number of such studies focused on the profile of fatty acids in soft-acid spreadable cheeses.

Nevertheless, Table 3 attempts a comparison of PDO spreadable Greek cheeses with *Tsalafouti* cheese.

In the present study, the sums of fatty acids had the following series of SFA > MUFA > PUFA, which are consistent with the findings of other studies, including Mileriene et al. [53]. Saturated fatty acids ranged from 60.47% (sample K12) to 72.66% (sample A1), monounsaturated fatty acids from 24.18% (sample A1) to 35.58% (sample K12), and polyunsaturated from 3.16% (sample A1) to 4.83% (sample K4). The percentage of saturated fatty acids was at levels close to that of the saturated fatty acids of the creamy spreadable cheeses Katiki Domokos (70.6%), Galotyri (70%), and Anevato (71.2%) (Table 3) [22]. Of the group of saturated fatty acids, palmitic acid was the main saturated fatty acid that accounted for more than 24% of the total fatty acids in all samples; the palmitic acid of Anevato (27.8%) was found at similar levels. However, in Kopanisti cheese, palmitic acid was observed at lower levels, constituting only (20.1%) of the total fatty acids [22]. Regarding the effects of monounsaturated fatty acids, the contents of this study were higher than the contents of Galotyri cheeses (24.6%) and Anevato (24.5%) [43], with the exception of the percentage of monounsaturated fatty acid content of sample A1 (24.18%). Oleic acid predominated among monounsaturated fatty acids, with contents ranging between 20.26% (sample A1) and 30.92% (sample K12); similar values were found in Anevato (20.8%) and spreadable cow's cheese (25.9%) [52]. The production of oleic acid occurs in the mammary gland by the action of the enzyme stearoyl-CoA desaturase on fatty acid 18:0 and in the large abdomen from the biohydrogenation of fatty acids C18:2 and C18:3. Oleic acid is known to have properties that can help fight cancer and reduce the risk of atherosclerosis [48]. Also, oleic acid can enhance HDL cholesterol and lower LDL cholesterol, which can help in preventing

cardiovascular diseases [54]. Finally, the results of the contents of the polyunsaturated fatty acids of the samples K4 (4.83%), K5 (4.46%), K6 (4.77%), and E7 (4.30%) were similar to that of the Pichtogalo of Chania (4.61%) and Anevato (4.22%), while the contents of the polyunsaturated fatty acids of all samples were at lower levels than the contents of the polyunsaturated fatty acids of Galotyri cheeses (5.40%) and Sitias Xygalo (5.53%) [22].

In the category of polyunsaturated fatty acids, linoleic acid was predominant, with values ranging from 2.48% (sample A1) to 3.92% (sample K4), which were higher than the correspondent value of Anevato (2.28%) and at the same value of Katiki Domokos (2.47%) [22]. The large amount of n-6 PUFA, such as linoleic acid, promotes the pathogenesis of many diseases, including cardiovascular diseases, cancer, and inflammatory and autoimmune diseases, while increased levels of n-3 PUFA exert sedative effects [55]. Linolenic (n-6) and alpha-linolenic (n-3) fatty acids, although at low percentages, were also found in the *Tsalafouti* samples assessed.

The values for the ratio between omega-6 and omega-3 fatty acids (n-6/n-3) (Table 4) were between 2.47 and 4.31.

According to Simopoulos et al. [56], the recommended value for this ratio would be 2:1 or 3:1; therefore, samples A11, K2, K4, and K5 would not be suitable for consumption. However, the World Health Organization accepts higher levels of the ratio, ranging from 5:1 to 10:1,as still being within acceptable limits for maintaining health. Nevertheless, the ratio of omega-6 to omega-3 is an important indicator of the impact of essential fatty acids on human health, and *Tsalafouti* cheese has one of the lowest ratios among PDO spreadable Greek cheeses [57].

Regarding the atherogenicity and thrombogenicity indices, despite the lack of an established parameter for those values, the lower the result, the lower the risk of platelet aggregation and coronary diseases due to a higher concentration of anti-atherogenic fatty acids (monounsaturated and omega-3 and -6). For *Tsalafouti* cheese, results varied between 1.65 and 2.99 for the atherogenicity factor and between 1.99 and 3.06 for thrombogenicity; see Table 4.

The consumption of foods with a lower AI can reduce the levels of total cholesterol and LDL-C in human blood plasma [27].

The H/H ratio plays a crucial role in the functional activity of fatty acids in the metabolism of lipoproteins for plasma cholesterol transport and in cardiovascular health. According to Chem J and Lui H, the H/H ratio and the HPI index for dairy products in the literature are 0.32–1.29 and 0.16–0.68, respectively. Dairy products with a high HPI value are assumed to be more beneficial to human health [58]. In *Tsalafouti* cheese, results varied between 0.513 and 0.923 regarding the H/H ratio and between 0.332 and 0.604 regarding the HPI index, which shows a corroboration of other research, and in comparison, to other PDO Greek cheeses, *Tsalafouti* cheese has the second-highest ratio among cheeses.

Table 2. Fatty acids % *Tsalafouti* samples.

Fatty Acids	A1	K2	K3	K4	K5	K6	E7	A8	A9	A10	A11	K12	Mean	StDev	*p*-Value Sample	*p*-Value Season	*p*-Value Area
C4:0	2.31 ± 0.01	2.39 ± 0.02	2.25 ± 0.04	2.17 ± 0.06	2.50 ± 0.04	1.92 ± 0.15	2.46 ± 0.04	2.36 ± 0.03	2.62 ± 0.16	2.15 ± 0.07	2.47 ± 0.05	2.13 ± 0.04	2.34	0.21	0.620	0.067	0.636
C6:0	1.99 ± 0.08	1.89 ± 0.03	1.95 ± 0.04	1.74 ± 0.03	2.07 ± 0.05	1.70 ± 0.04	1.97 ± 0.02	2.34 ± 0.01	2.20 ± 0.03	1.79 ± 0.01	2.42 ± 0.02	1.43 ± 0.06	1.95	0.26	0.919	0.064	0.123
C8:0	2.55 ± 0.04	2.09 ± 0.08	1.99 ± 0.06	1.94 ± 0.02	2.06 ± 0.08	1.89 ± 0.07	2.09 ± 0.08	2.46 ± 0.02	2.50 ± 0.07	2.01 ± 0.1	2.77 ± 0.07	1.34 ± 0.13	2.15	0.35	0.783	0.077	0.039
C10:0	9.66 ± 0.15	6.83 ± 0.17	6.57 ± 0.10	6.87 ± 0.06	6.66 ± 0.18	6.38 ± 0.07	6.31 ± 0.08	7.59 ± 0.09	8.10 ± 0.15	6.42 ± 0.07	9.04 ± 0.10	3.96 ± 0.19	7.10	1.40	0.699	0.108	0.036
C10:01	0.36 ± 0.03	0.22 ± 0.05	0.21 ± 0.04	0.23 ± 0.03	0.27 ± 0.02	0.25 ± 0.03	0.17 ± 0.02	0.26 ± 0.02	0.28 ± 0.02	0.20 ± 0.01	0.22 ± 0.02	0.18 ± 0.02	0.25	0.05	0.430	0.818	0.061
C11:0	0.10 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.06	0.01	0.955	0.185	0.012
C12:0	5.45 ± 0.08	3.88 ± 0.07	3.75 ± 0.06	3.88 ± 0.03	3.78 ± 0.05	3.87 ± 0.04	3.17 ± 0.08	4.23 ± 0.02	4.42 ± 0.06	3.61 ± 0.04	4.48 ± 0.05	2.61 ± 0.10	3.97	0.66	0.992	0.248	0.020
C12:01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.09	0.02	0.051	0.242	0.004
Iso-C13:0	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08	0.01	0.585	0.062	0.023
C13:00	0.13 ± 0.03	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	0.14 ± 0.01	0.13	0.01	0.513	0.610	0.722
C14:0	12.27 ± 0.08	10.42 ± 0.12	10.73 ± 0.12	10.24 ± 0.36	10.82 ± 0.19	11.02 ± 0.27	9.68 ± 0.22	10.9 ± 0.65	11.19 ± 0.29	10.69 ± 0.15	10.83 ± 0.07	9.21 ± 0.4	10.82	0.72	0.498	0.775	0.022
C14:1 (ω5)	1.06 ± 0.04	1.03 ± 0.11	1.11 ± 0.05	0.98 ± 0.07	1.03 ± 0.01	1.03 ± 0.01	1.03 ± 0.03	1.06 ± 0.04	1.07 ± 0.04	1.06 ± 0.01	0.9 ± 0.08	1.17 ± 0.04	1.09	0.07	0.384	0.225	0.660
C15:0	1.03 ± 0.04	1.06 ± 0.05	1.09 ± 0.11	1.06 ± 0.04	0.98 ± 0.09	0.98 ± 0.01	1.11 ± 0.01	1.09 ± 0.01	1.11 ± 0.04	1.14 ± 0.01	1.02 ± 0.07	1.23 ± 0.02	1.12	0.08	0.903	0.636	0.903
C15:1	0.27 ± 0.03	0.27 ± 0.02	0.29 ± 0.02	0.23 ± 0.01	0.24 ± 0.03	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.01	0.26 ± 0.04	0.29 ± 0.02	0.24 ± 0.01	0.28 ± 0.02	0.26	0.03	0.718	0.632	0.768
C16:0	27.89 ± 0.14	26.06 ± 0.46	26.39 ± 0.24	27.17 ± 0.23	29.29 ± 0.24	27.07 ± 0.32	24.41 ± 0.33	25.07 ± 0.26	24.7 ± 0.10	27.42 ± 0.12	25.86 ± 0.11	25.97 ± 0.14	26.6	1.41	0.631	0.149	0.839
C16:1	1.91 ± 0.11	1.99 ± 0.09	2.04 ± 0.06	2.15 ± 0.10	2.29 ± 0.04	2.23 ± 0.11	2.07 ± 0.05	1.99 ± 0.07	1.98 ± 0.14	2.03 ± 0.05	1.59 ± 0.15	2.39 ± 0.07	2.11	0.19	0.350	0.039	0.070
Aiso-C17:0	0.63 ± 0.04	0.55 ± 0.04	0.57 ± 0.02	0.54 ± 0.03	0.55 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.56 ± 0.01	0.56 ± 0.01	0.56 ± 0.01	0.53 ± 0.03	0.62 ± 0.04	0.58	0.04	0.508	0.701	0.818
C17:0	0.61 ± 0.03	0.69 ± 0.03	0.68 ± 0.02	0.68 ± 0.03	0.57 ± 0.02	0.56 ± 0.01	0.78 ± 0.01	0.63 ± 0.01	0.63 ± 0.03	0.78 ± 0.02	0.67 ± 0.03	0.89 ± 0.02	0.69	0.09	0.469	0.702	0.362
C17:1	0.29 ± 0.02	0.25 ± 0.01	0.27 ± 0.01	0.28 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.23 ± 0.01	0.27 ± 0.02	0.29 ± 0.02	0.20 ± 0.02	0.42 ± 0.01	0.28	0.05	0.792	0.229	0.443
C18:0	8.28 ± 0.24	10.28 ± 0.25	10.47 ± 0.22	8.82 ± 0.13	8.08 ± 0.21	8.86 ± 0.11	12.29 ± 0.24	10.15 ± 0.21	9.69 ± 0.12	10.27 ± 0.15	10.62 ± 0.15	10.18 ± 0.26	9.94	1.19	0.371	0.352	0.308
cis-9 C18:1	20.26 ± 0.17	25.18 ± 0.37	24.96 ± 0.23	25.31 ± 0.35	23.28 ± 0.16	25.55 ± 0.22	25.87 ± 0.11	24.00 ± 0.52	23.58 ± 0.15	24.54 ± 0.06	21.59 ± 0.20	30.92 ± 0.67	24.65	2.46	0.927	0.291	0.064
C18:2n-6	2.57 ± 0.09	3.22 ± 0.12	2.95 ± 0.09	3.92 ± 0.10	3.58 ± 0.02	3.75 ± 0.03	3.06 ± 0.04	2.84 ± 0.01	2.85 ± 0.01	2.70 ± 0.03	2.96 ± 0.01	2.92 ± 0.01	3.14	0.43	0.722	0.530	0.080
C18:3n-3	0.68 ± 0.01	0.76 ± 0.01	0.77 ± 0.01	0.91 ± 0.01	0.88 ± 0.03	1.02 ± 0.02	1.24 ± 0.01	1.00 ± 0.02	0.99 ± 0.01	0.95 ± 0.02	0.73 ± 0.01	1.03 ± 0.01	0.92	0.16	0.480	0.223	0.118
C20:0	0.34 ± 0.04	0.36 ± 0.01	0.37 ± 0.02	0.32 ± 0.01	0.31 ± 0.01	0.31 ± 0.02	0.51 ± 0.03	0.29 ± 0.01	0.28 ± 0.01	0.39 ± 0.03	0.29 ± 0.01	0.41 ± 0.01	0.35	0.07	0.301	0.820	0.010
cis-11 C20:1	0.03 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.13	0.22	0.954	0.988	0.539
C22:0	0.13 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.24 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.21 ± 0.01	0.13 ± 0.01	0.23 ± 0.01	0.17	0.04	0.694	0.573	0.062
C24:0	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0	0	0	0	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.0	0	0	0.01	0.01	0.23	0.874	0.018

Results refer to the mean ± standard deviation of *Tsalafouti* samples analyzed in triplicate.

Table 3. Comparison of PDO spreadable Greek cheeses with Tsalafouti cheese.

Fatty Acids	Tsalafouti %	Anevato %	Galotyri%	Katiki Domokou%	Kopanisti%	Pichtogalo Chanion%	Xynogalo Sitias%
C4:0	2.34	0.12	0.09	0.027	0.070	0.106	0.107
C6:0	1.95	1.31	0.978	0.537	0.394	1.23	0.943
C8:0	2.15	2.43	2.01	1.36	0.668	2.14	1.58
C10:0	7.1	9.53	8.48	7.47	2.17	8.74	6.55
C11:0	0.06	0.087	0.12	0.107	0.034	0.11	0.067
C12:0	3.97	4.7	5.27	5.07	3.32	5.16	3.71
C14:00	10.82	11.30	12.00	13.00	5.20	12.6	11.00
C14:1 (ω5)	1.09	0.353	0.583	0.527	0.021	0.392	0.433
C15:00	1.12	0.823	1.18	1.24	0.496	1.03	0.993
C15:01	0.26	0.25	0.295	0.31	0.096	0.16	0.237
C16:00	26.6	27.8	28.7	30.0	20.1	30.4	29.7
C16:01	2.11	1.16	1.4	1.46	0.664	1.3	1.3
C17:00	0.69	0.603	0.73	0.787	0.248	0.588	0.723
C17:01	0.28	0.18	0.233	0.227	0.066	0.242	0.227
C18:00	9.94	12.3	9.97	10.2	7.91	9.06	10.9
cis-9 C18:1	24.65	20.8	18.1	18.8	21.7	18.5	21.6
C18:2n-6	3.14	2.28	2.42	2.47	28.8	2.26	2.45
C18:3n-3	0.92	0.54	0.683	0.753	2.19	0.12	0.773
C20:00	0.35	0.127	0.125	0.163	0.098	0.118	0.197
C22:00	0.171	n.d.	0.13	0.14	0.022	0.018	0.167
C24:00	0.009	n.d.	0.06	0.077	0.076	0.004	0.017
ΣSFA [1]	67.36	71.2	70.0	70.6	40.9	71.4	67.0
ΣMUFA [2]	28.63	24.5	24.6	24.3	25.8	24.0	27.5
ΣPUFA [3]	4.12	4.22	5.4	5.13	33.3	4.61	5.53
Σn-6	3.10	2.28	2.42	2.47	28.8	2.26	2.45
Σn-3	0.913	0.54	0.683	0.753	2.19	0.512	0.773
n-6/n-3	3.4	4.22	3.54	3.28	13.15	4.41	3.17
IA*	2.24	2.70	2.75	3.02	0.98	3.07	2.43
IT **	2.13	2.67	2.65	2.81	0.71	2.73	2.52
PUFA/SFA ***	0.061	0.059	0.077	0.073	0.814	0.064	0.082
HH ****	0.695	0.571	0.522	0.498	1.92	0.48	0.611
HPI *****	0.443	0.37	0.366	0.338	1.34	0.333	0.427

[1] Total Saturated fatty acids (SFA):C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. [2] Total Monounsaturated fatty acids (MUFA):C14:1 + C15:1 + C16:1 + C17:1 + cis-9 C18:1 + trans-11 C18:1 + trans C18:1 + C24:1. [3] Total Polyunsaturated fatty acids (PUFA):cis-9, trans-11 C18:2 (CLA1) + cis-10, trans-12 C18:2 (CLA2) + C18:2n-6c + C18:2n-6 + t C18:3n-3 + C18:3n-6 + C20:2n-6 + C20:3n-3 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:2n-6 + C22:6n-3. * Index of atherogenicity. Calculation Formula: [C12:0 + (4 × C14:0) + C16:0]/ΣUFA. ** Index of thrombogenicity. Calculation formula: (C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA) + (0.5 × Σn-6 PUFA) + (3 × Σn-3 PUFA) + (n-3/n-6)]. *** Polyunsaturated fatty acid/saturated fatty acid ratio. Calculation Formula:ΣPUFA/ΣSFA. **** Hypocholesterolemic/hypercholesterolemic ratio. Calculation Formula: (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0). ***** Health-promoting index. Calculation Formula:ΣUFA/[C12:0 + (4 × C14:0) + C16:0]. n.d.: not detected.

Table 4. Sum of Fatty acids and Nutritional indices of *Tsalafouti* Cheese samples.

Fatty Acids	A1	K2	K3	K4	K5	K6	E7	A8	A9	A10	A11	K12
ΣSFA [1]	72.66	66.94	67.28	65.86	68.04	65.48	65.85	68.14	68.49	67.74	71.40	60.47
ΣUFA [2]	27.34	33.06	32.72	34.14	31.96	34.52	34.15	31.86	31.51	32.26	28.60	39.53
ΣMUFA [3]	24.18	29.08	29.00	29.31	27.49	29.75	29.85	28.02	27.67	28.61	24.96	35.58
ΣPUFA [4]	3.16	3.98	3.72	4.83	4.46	4.77	4.30	3.84	3.84	3.65	3.69	3.95
Total ω-3 FA	0.68	0.76	0.77	0.91	0.88	1.02	1.24	1.00	0.99	0.95	0.73	1.03
Total ω-6 FA	2.48	3.22	2.95	3.92	3.58	3.75	3.06	2.84	2.85	2.70	2.96	2.92
SFA/UFA	2.66	2.02	2.06	1.93	2.13	2.05	1.93	2.14	2.17	2.10	2.49	1.53
n-6/n-3	3.65	4.24	3.83	4.31	4.07	3.68	2.47	2.84	2.88	2.84	4.05	2.83
IA *	2.99	2.17	2.23	2.11	2.40	2.17	1.94	2.29	2.34	2.29	2.57	1.65
IT **	3.06	2.50	2.57	2.36	2.62	2.34	2.25	2.46	2.45	2.57	2.88	1.99
PUFA/SFA ***	0.043	0.059	0.055	0.073	0.065	0.073	0.065	0.056	0.056	0.054	0.052	0.065

Table 4. *Cont.*

Fatty Acids	A1	K2	K3	K4	K5	K6	E7	A8	A9	A10	A11	K12
HH ****	0.513	0.722	0.702	0.729	0.632	0.722	0.809	0.692	0.680	0.675	0.614	0.923
HPI *****	0.332	0.461	0.447	0.474	0.418	0.460	0.515	0.437	0.426	0.437	0.388	0.604

[1] Total Saturated fatty acids (SFA):C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. [2] Total Unsaturated fatty acids (UFA):MUFA + PUFA. [3] Total Monounsaturated fatty acids (MUFA):C14:1 + C15:1 + C16:1 + C17:1 + cis-9 C18:1 + trans-11 C18:1 + trans C18:1 + C24:1. [4] Total Polyunsaturated fatty acids (PUFA):cis-9, trans-11 C18:2 (CLA1) + cis-10, trans-12 C18:2 (CLA2) + C18:2n-6c + C18:2n-6 + t C18:3n-3 + C18:3n-6 + C20:2n-6 + C20:3n-3 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:2n-6 + C22:6n-3. * Index of atherogenicity. Calculation Formula: [C12:0 + (4 × C14:0) + C16:0]/ΣUFA. ** Index of thrombogenicity. Calculation formula:(C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA) + (0.5 × Σn-6 PUFA) + (3 × Σn-3 PUFA) + (n-3/n-6)]. *** Polyunsaturated fatty acid/saturated fatty acid ratio. Calculation Formula:ΣPUFA/ΣSFA. **** Hypocholesterolemic/hypercholesterolemic ratio. Calculation Formula: (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0). ***** Health-promoting index. Calculation Formula:ΣUFA/[C12:0 + (4 × C14:0) + C16:0].

4. Conclusions

This study of the physicochemical characteristics, fatty acid profile, and lipid quality of the traditional Greek cheese "*Tsalafouti*" is the first attempt at in-depth research of this traditional cheese that highlights its quality and potential nutritional value. The fatty acid profile assessment revealed that the ratio of ω6/ω3 fatty acids is within the recommendations of the World Health Organization, and the fat quality indices are also at healthy levels. *Tsalafouti* cheese has a low percentage of fat compared to other cheeses and at the same time a good source of protein, so it could be accepted as a low-fat cheese, possibly with functional properties. These characteristics, combined with its positive fatty acid profile, underline its suitability for health-conscious consumers. Moreover, local traditional products promote the heritage and tradition of their production regions, and they enhance the financial sustainability of local societies. *Tsalafouti* is currently under evaluation to become a PDO product; thus, a special focus on highlighting its authenticity is required in order to utilize its potential as a nutritional product.

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