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Effect of The Composition of Grazing Matter on The Quality Characteristics of The Traditional Greek Dairy Product "Tsalafouti".

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Abstract: *In the subalpine grassland of "Kostilata", which is located in northwestern Greece, on Mount Tzoumerka, 98 plant species thrive, the group of grasses is dominant and from the milk of the animals grazing there, the traditional Greek dairy product "tsalafouti" is produced. The present study was aimed at correlating the composition of grazing matter with the production of "tsalafouti". The grassland area was divided into three zones based on their altitude above the sea level: zone A (1100 m - 1400 m), zone B (1401 m - 1800 m) and zone C (1801 m - 2393 m). In zone A, the crude protein, NDF, ADF and total fat contents of the grazing matter were 5.8%, 59.3%, 39.4% and 1.6% respectively, in zone B were 7.7%, 54.9%, 36.1% and 1.7% respectively and in zone C were 10.7%, 48.2%, 32.3% and 1.9% respectively, with statistically significant differences (P<0.05). The moisture content of "tsalafouti" produced from the milk of the animals grazing in zones A, B and C was 79,4%, 79,2% and 79.5% respectively, the total fat on dry weight was 45.1%, 43.3% and 41.3% respectively, with a statistically significant difference (P<0.05), the protein content was 6,5%, 7,1% and 7,6%, respectively with a statistically significant difference (P<0.05) and the lactose content was 3,8%, 3,9% and 4.0% respectively.*

Keywords*: grazing matter, "Kostilata" subalpine grassland, traditional dairy product, "tsalafouti".*

I. Introduction

Altitudinal zone is an important factor that determines the production and nutritional value of grasslands [1 - 3]. The subalpine grasslands have a rich flora and are utilized primarily by nomadic livestock farming. Animal nutrition is the main factor that determines the quality of animal products [4, 5]. It is well known that ruminant animals cover a large part of their dietary needs by grazing, which ranges from 25% to 75% [6], while animal feed represents 50% to 90% of the production costs of each animal product [7]. The amounts of crude protein, NDF and ADF in grassland plants are the most important factors that define the quality of the grazing matter produced [8 - 10]. The same plant species at the same stage of growth show significant differences in their chemical composition when they grow in different environments, even if they are not very close to each other [8, 11]. On the one hand, livestock grazing in grasslands is an important economic food source; on the other hand the products obtained from these breeding systems are rich in nutrients and contribute to the enhancement of human health [7, 12]. Milk produced from grazing animals is rich in nutrients, like vitamins and fats [13, 14]. Products produced from the milk of grazing animals are of high nutritional value [4, 15, 16]. Furthermore, dairy products produced from the milk of animals grazing in alpine grasslands are richer in beneficial fatty acids than those produced from the milk of silage fed animals in lowland areas [15]. This rich heritage is crucial to the viability of livestock farming in mountain areas, as its animal products are characterized by high quality, authenticity and originality [17, 18]. The breeds of farm animals from which the milk is produced, the way in which the animals are handled and managed, the soil and climatic conditions of each area along with the method of preparing and ripening the dairy products, are the factors that make a diary product stand out as part of the tradition of a region [19]. Traditional technology without starter cultures may result in the production of cheeses with better organoleptic characteristics compared to those using starter cultures [20]. Lactic bacteria that contaminate milk from the environment of the stall play a key role in formulating the flavor characteristics of raw milk cheeses. These microbes are quantitatively and qualitatively richer in raw milk cheeses [19, 21]. In Greece, there is a wide variety of traditional dairy products, some of which are not known to the general public. They are made from cow's, sheep's or goat's milk or blends of them and are characterized by their unique, special flavor and rich aroma. Their classification is based on their fat on dry weight and moisture content (Table 1). Some Greek cheeses with spreadable texture have been characterized as "Protected Designation of Origin" (PDO) products (Table 2).

Table 1. Maximum moisture and minimum fat content on dry weight in fresh cheese with spreadable texture (Greek legislation, Code of Food and Beverage, article 83).

Quality	Maximum moisture (%)	Minimum fat on dry weight (%)
Excellent quality	58	70
First quality	62	60
Second quality	75	60
Cheese from partially skimmed milk	75	50-60

Table 2. Chemical composition of Greek traditional spreadable cheeses with "Protected Designation of Origin" (PDO) (Code of Food and Beverage).

Cheese	Type of milk	Maximum moisture (%)	Minimum fat on dry weight (%)
Anevato	S-G or blend	60	45
Galotyri	S-G or blend	75	40
Katiki Domokou	S-G or blend	75	40
Kopanisti	C-S-G or blend	56	43
Pehtogalo of Chania	S-G or blend	65	50
*C = Cow's milk, S = Sheep's milk, G = Goat's milk			

The longer the ripening time of cheese, the greater the loss of moisture, while salting may eventually lead to additional loss of moisture. The proteolytic activity of microbial enzymes is an important factor which, in addition to the protein content of the final product, significantly affects its organoleptic properties, due to volatile substances produced [22]. Furthermore, the coagulation of milk with rennet affects the content of proteins in the dairy product [23]. The fat content of milk is affected by animal feed, while the protein content of milk is affected much less or not at all [24 - 29]. The chemical composition of cheeses with spreadable texture differs from one another. Some Greek and European cheeses with spreadable texture are presented in Table 3.

Table 3. Chemical composition of Greek and European cheeses [22].

Cheese	Moisture (%)	Protein (%)	Fat on dry weight (%)
Galotyri	71.0	10.0	48.3
Cottage cheese	79.0	14.0	20.0
Ricotta	72.0	11.2	45.4

"Tsalafouti" is produced in many areas of Greece, but mainly in the mountainous regions of Agrafa and Tzoumerka. The way it is produced varies from region to region, both in the manufacturing process and in the type of milk used. The objectives of the present work was to study the effect of the chemical composition of grazing matter on the quality characteristics of the traditional Greek diary product "tsalafouti", to record its manufacturing process, to determine its chemical composition, to classify it in the corresponding category of dairy products according to the Greek legislation and to compare it with other similar dairy products.

II. Materials And Methods

2.1 Area of grazing

The research was conducted during the years 2015 and 2016, in "Kostilata" subalpine grassland which is located 80 km northeast of Arta, at Theodoriana, Greece, where about 5000 sheeps are grazing during the summer months. In this region, the traditional product "tsalafouti" is produced, exclusively by the breeders themselves, during the second half of July and the first half of August. This area extends at an altitude of 1100 to 2393 m above the sea level and depending on the altitude, different physicochemical and climatic conditions prevail within the grassland and different plant species also thrive. For the needs of the research, grazing matter was collected from three different altitudinal zones, and specifically from zone A (1100 m - 1400 m), zone B (1401 m - 1800 m) and zone C (1801 m - 2393 m) and "tsalafouti" produced from the milk of the animals grazing in each of these zones was collected.

2.2 Sampling and determination of the chemical composition of grazing matter

To determine the chemical composition of the grazing matter in each zone, 20 samples of forage were collected in the second half of July and 20 samples in the first half of August. Then, the samples were placed in an oven for drying at 65°C for 48 hours [30]. The determination of the crude protein of forage was carried out according to the Kjeldahl method [31], Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined by the method of Van Soest et al and Mertens [32, 33] and the determination of total fat was performed using the Soxhlet method [34].

2.3 "Tsalafouti" and its production process

"Tsalafouti" is produced exclusively from milk of sheeps which are in the last stage of their lactation period and which are milked once a day or once every two days. It has high moisture content, creamy texture

and sweet-tasting flavor. It is consumed mainly as a side dish and is known in the region for its special flavor and texture. It is available for sale from the producers themselves and the entire amount produced is channeled to the Prefecture of Arta and the neighboring villages of Trikala.

The production process of "tsalafouti" is as follows:

1. After milking, the milk is drained for the retention of foreign bodies resulting from the milking process, e.g. stones, animal hair etc.
2. Milk is heated by fire (Picture 1), is stirred at the same time and when it begins to inflate (approximately at 85 to 90^{o}C), it is withdrawn from the fire.

Picture 1. Milk heating

3. A small amount of salt is added during heating or at the end of it.
4. After the milk has cooled down it is transferred in plastic containers or wooden barrels, which are placed in cool and shady places at low temperature, such as caves (Pictures 2a and 2b), near streams or in running water or even into the ground, in such a way as the rim of the container protrudes.
5. The product is stirred daily (Picture 2b)

Pictures 2a and 2b. Ripening area of "tsalafouti".

6. After the completion of the above procedure, the milk of the next milking is also added to the collection containers.
7. The ripening of "tsalafouti" lasts about five to six days.

After completion of the production process, the product is placed in smaller containers and is available for consumption.

2.4 Sampling and determination of the chemical composition of "tsalafouti"

In order to determine the chemical composition of "tsalafouti", a total of thirty samples were collected, in particular from five livestock producers, which bred their animals in each zone in the years 2015 and 2016, from 10 to 15 of August, when "tsalafouti" was ready for consumption. The determination of moisture was made by drying the samples until constant weight [35]. The determination of total fat was carried out using the Gerber method [36], protein content was determined by the Kjeldahl method [37] and the determination of lactose was performed with the LACTOSTAR automatic milk analyzer.

2.5 Statistical analysis

The results were compared for significant differences by one-way ANOVA test, while mean differences were checked using Tuckey's test ($P < 0.05$). Statistical analyses were performed with OriginPro 9.0 software.

III. Results And Discussion

The chemical composition of the grazing matter of "Kostilata" grassland per altitudinal zone is presented in Table 4. The highest crude protein and total fat content of the grazing matter was found in the samples of zone C with 10.7 ± 1.8% and 1.9 ± 0.2% respectively, followed by the samples of zone B with 7.7 ± 1.3% and 1.7 ± 0.1% respectively and the lowest content was found in the samples of zone A with 5.8 ± 0.9% and 1.6 ± 0.1% respectively, with statistically significant differences ($P<0.05$). The highest content of grazing matter in NDF and ADF was found in the samples of zone A with 59.3 ± 2.9% and 39.4 ± 2.0% respectively, followed by the samples of zone B with 54.9 ± 1.6% and 36.1 ± 1.6% respectively and the lowest content was found in the samples of zone C with 48.2 ± 2.3% and 32.2 ± 1.4% respectively, with statistically significant differences ($P<0.05$).

Table 4. Chemical composition (Dry Matter %) of grazing matter of "Kostilata" grassland per altitudinal zone

	Year	Crude protein (%)	NDF (%)	ADF (%)	Total Fat (%)
Zone A	2015	5.7 ± 0.9a	57.9 ±2.0a	39.7 ± 2.5a	1.6± 0,1α
	2016	5.9 ±0.9a	60.8 ± 2.9b	39.1 ± 1.4a	1.6 ± 0,1α
	Mean	5.8 ± 0.9^{1}	59.3 ± 2.9^{1}	39.4 ± 2.0^{1}	1.6 ± 0.1^{1}
Zone B	2015	7.9 ± 1.4a	55.0 ± 1.2a	36.8 ± 1.5a	1.8 ± 0.1α
	2016	7.5 ± 1.3a	54.8± 2.0a	35.4± 1.6a	1.7 ± 0.1α
	Mean	7.7 ± 1.3^{2}	$54.9 \pm 1,6^{2}$	36.1 ± 1.6^{2}	1.7 ± 0.1^{1}
Zone C	2015	10.8 ± 1.8a	48.2 ± 1.8a	33.1 ± 2.7a	1.9± 0.3α
	2016	10.6 ± 1.7a	48.3 ± 2.8a	31.5 ± 1.9a	1.9 ± 0.2α
	Mean	10.7 ± 1.8^{3}	48.2 ± 2.3^{3}	32.3 ± 1.4^{3}	1.9 ± 0.2^{2}

*Average rates followed by different letter (a, b), in the same column differ significantly ($P < 0.05$).
**Mean rates followed by different exponent ($^{1, 2, 3}$), in the same column differ significantly ($P < 0.05$).

Among the altitudinal zones of the subalpine grassland of "Kostalata" different temperatures were recorded. Higher temperatures were specifically recorded in the altitudinal zone A, followed by the temperatures of the altitudinal zone B and the lower ones were recorded in the altitudinal zone C. In each of the above zones, different plant species thrive [38]. The different chemical composition observed in the samples of grazing matter between the altitudinal zones is attributed to the higher temperature observed in the lower altitudinal zones. Low temperature contributes to the increase in protein content of plants and reduces their NDF and ADF content [39, 40]. Higher crude protein and total fat content and lower NDF and ADF content, at the same period of time, in higher altitude grasslands compared to lower altitude grasslands was also mentioned by other researchers [41, 42]. The chemical composition of "tsalafouti" is presented in Table 5. "Tsalafouti" contains protein in an amount of 7.1%, fat on dry weight in an amount of 43.9%, lactose in an amount of 3.9% and its moisture is 79.4%. Although its characteristics are similar to those of soft cheeses (cheese from partially skimmed milk), it cannot be classified as cheese, according to the Greek Code of Food and Beverage (Table 1).

Table 5. Chemical composition of "tsalafouti" diary product

Type of milk	Moisture (%)	Fat on dry weight (%)	Proteins (%)	Lactose (%)
Sheep's milk	79.4 ± 2.0	43.9 ± 2.4	7.1 ± 0.6	3.9 ± 0.3

The increased moisture of "tsalafouti" is due to the fact that there is no drainage of the product in combination with the short ripening time. The yield of milk in cheeses with spreadable texture depends, among other factors, on drainage time [43]. The longer the ripening times of cheese, the greater the loss of moisture [22]. Fat content of "tsalafouti" is comparable to that of most Greek soft spreadable cheeses and the corresponding European ones. Milk boiling destroys natural lipases, but the microorganisms involved in coagulation produce extracellular and intracellular lipases. The former can achieve rapid lipolysis while the latter are released after lysis of the bacterial cells [22]. However, the short ripening time does not allow any lipolysis process which would lead to a further reduction in fat content. The chemical composition of some of the aforementioned Greek PDO cheeses is somewhat different from that of "tsalafouti", with the exception of "galotyri" and "katiki Domokou" which have marginally lower moisture content (Table 2). The wrong classification of "tsalafouti" as cheese is due to its organoleptic characteristics alone, which indeed resemble those of other soft cheeses with spreadable texture. The higher protein content of cheeses with spreadable

texture (Table 3) is due to the addition of rennet, which binds milk proteins during coagulation, while in the milk used for the production of "tsalafouti" no rennet is added and therefore the loss of gel proteins is greater. A similar result was found in "galotyri" samples, which had been curded either with rennet or with natural acidification. The samples that had been coagulated with rennet were richer in protein [23].
The chemical composition of "tsalafouti" samples per altitudinal zone is given in Tale 6.

Table 6. Chemical composition of "tsalafouti" per altitudinal zone.

	Year	Moisture (%)	Fat on dry weight (%)	Proteins (%)	Lactose (%)
Zone A	2015	79.2 ± 1.8a	45.3 ± 1.8a	6.4 ± 0.4a	3.8 ± 0.2a
	2016	79.6 ±1.9a	45.0 ± 1.4a	6.6 ± 0.5a	3.9 ± 0.4a
	Mean	79.4 ± 1.7[1]	45.1 ± 1.6[1]	6.5 ± 0.5[1]	3.8 ± 0.2[1]
Zone B	2015	80.3 ± 2.7a	43.4 ± 1.5ab	7.0 ± 0.2b	4.0 ± 0.2a
	2016	78.3 ± 2.3a	43.2 ± 1.0a	7.1 ± 0.2ab	3.8 ± 0.2a
	Mean	79.2 ± 2.6[1]	43.3 ± 1.2[2]	7.1 ± 0.2[2]	3.9 ± 0.2[1]
Zone C	2015	80.3 ± 1.5a	41.5 ± 1.8b	7.6 ± 0.2c	3.9 ± 0.2a
	2016	78.7 ± 1.8a	41.1 ± 1.0b	7.6. ± 0.4bc	4.2 ± 0.3a
	Mean	79.5 ± 1.9[1]	41.3 ± 1.4[3]	7.6 ± 0.3[3]	4.0 ± 0.3[1]

*Average rates followed by different letter (a, b, c), in the same column differ significantly ($P < 0.05$).
**Mean rates followed by different exponent ([1, 2, 3]), in the same column differ significantly ($P < 0.05$).

"Tsalafouti" samples showed statistically significant differences ($p < 0.05$) in protein and fat content in relation to the different altitudinal zones. The results show that as the altitude increases, the fat content on dry weight decreases but the protein content increases. The above differences are due to the fact that animals grazing in different altitudes receive forage of different nutritional value (Table 4). The existing variations in milk quality are predominantly attributed to animal feed, which is one of the most important factors influencing the composition and profile of fatty acids in milk [29, 44]. Moreover, the aforementioned differences may also be due to the different microflora of each altitudinal zone.

IV. Conclusion

The altitude zone affects the chemical composition of the grazing matter, which in turn affects the chemical composition of "tsalafouti". The coagulation of "tsalafouti" is due to the effect of the natural microflora of each region. This product does not meet the requirements of Greek Legislation to be classified as "cheese", although its composition is similar to that of other Greek traditional spreadable cheeses with Protected Designation of Origin. A comprehensive study of the physicochemical, microbiological, biochemical and organoleptic characteristics of "tsalafouti" is required, to develop such specifications, so that the product can be certified as cheese of protected geographical indication or as mountain cheese or even be included in the category of organic products to be upgraded qualitatively and commercially and established in the Greek market.

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