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Quality indicators of traditional Bulgarian artisanal sheep's cheese

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Abstract. The aim of the present study is focused on the evaluation of the quality parameters of artisan sheep cheese produced by old Bulgarian technology. The study was carried out in three stages - at the end of the ripening process (45th day), during and at the end of storage (180th and 360th day). An increase of 9.0 % in dry matter and a decrease in water content and titratable acidity values were observed over the entire period studied. There was a decrease in oleic fatty acid (C18:1n9c) from 28.16% at day 45 to 26.09% at day 360 of ripening. In the case of palmitic acid (C16:0) the values recorded were similar from 30.24% (45th day) to 30.74% (360th day). Changes in the composition of organic acids were observed, with lactic acid levels decreasing from 27.66% at day 45 to 8.62% at day 360. After microbiological analysis, it was found that the main microflora present in the samples were representatives of lactic acid microorganisms as compared to the non-starter microflora.

1 Introduction

The growing interest in artisan dairy products from milk has been rising in recent years. The higher consumers' interest, in turn, necessitates the development of legal frameworks for the possibilities of small domestic productions, which would permit trade in these products [1, 2]. Numerous studies have been evaluated the nutritional and microbiological status of traditional artisan sheep and goat milk products, and not a tiny part of them end with recommendations for lowering the unwanted microbial presence [3]. Small family farms that process harvested sheep's milk are expanding their production program, paying serious attention to traditional products for the market. In this way, they successfully target their diggers by adding concepts of "traditional," "local," "domestic," or "homemade" product to the brand.

The Balkans, including Bulgaria, fall into a geographical region distinguished by centuries-old traditions in raising sheep, whose milk is processed according to traditional technologies. The resulting dairy products are distinguished by specific taste, aroma, and textural characteristics [2]. These distinctive indicators in the composition of craft products are related to the environment's microbiological, technological, and seasonal conditions [4]. For this reason, artisanal products are often listed as "risky" for mass marketing, and the terms of supply are limited.

The specific indicators related to traditional artisanal products' physical, chemical, microbiological, and sensory indicators vary depending on the type of raw material used, the microbial purity, and the technological conditions for their production. The differences in phytoreceptors in the environment's different seasonal and geographical conditions also determine the differences in the contained fatty acids, proteins, and other components [1, 3, 4].

In ripening conditions, dairy products form typical substances that determine the product's unique nutritional and taste potential. The ripening of dairy products obtained by traditional technology is a complex and dynamic biochemical process [5]. It is primarily associated with protein breakdown, lipolysis of lipid components, and, last but not least, the metabolic breakdown of lactose and lactate. Preparing traditional artisan cheeses and the conditions of formation of the coagulant without the use of cultivated starter cultures is carried out by enzyme systems (proteinases and peptidases), helping to form short peptides and aromatic compounds. Fermentation processes carried out by the representatives of autochthonous microorganisms determine the product's specific organoleptic indicators in the different ripening stages [6, 7].

Traditional artisan cheeses acquire a specific milky-sour taste during ripening, which cannot yet be related to the activity of certain strain cultures. Isolation of some representatives of the species *L. plantarum* and *L.*

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delbrueckii, as well as other species of lactic acid bacteria, is quantitatively and qualitatively inconsistent, related to different environmental conditions [8, 9, 10].

Dynamic market conditions and modern understandings of healthy and local eating are factors in choosing foods from local (regional) farms and producers. Due to the constantly growing interest in traditional lactic acid products in nutrition, the present study aimed to focus on determining the quality characteristics of artisanal sheep's cheese prepared using conventional technology.

2 Materials and Methods

2.1. Materials

Sheep's milk (month of May 2021), obtained by manual milking of autochthonous Karakachan sheep (50 animals) from the Sliven region (Topolchane village 42.65°N 26.433333°E), was used for producing artisanal sheep's cheese. The animals are entirely pasture-fed, with no additional feeding. The raw milk is obtained from the morning hand milking of the sheep. Freshly milked milk is clarified by straining through cheesecloth and then curdled using rennet enzyme without adding starter cultures. The resulting coagulum is uncut and processed, after which the cheese cubes are drained and shaped into fresh cheese in a rectangular shape of up to 2 kg. The resulting cheese is placed in packages and dry salted. Ripening takes 45 d.

2.2. Technology of cheese production

The artisanal sheep`s cheese technology and pictures of the end products are shown in Fig. 1 and 2.

The artisanal cheese was obtained in the University of Food Technologies - Plovdiv laboratory conditions at the department "Technology of milk and dairy products." The production conditions are adapted according to classic craft technology. After ripening, the cheese (see 2.1.) is crushed and placed on the bottom of a clay pot. After that, the clay vessel and the cheese are covered with condensed and salted sheep's milk. The container is closed with a lid and paraffined. The cheese is ripened and stored at a temperature of 6 - 8°C for 360 d (Fig.1).

2.3 Methodology

To obtain an accurate measurement of the moisture content of a cheese using evaporation methods according ISO 5534:2004 [11]. Then calculated %Total solids = (100 - %Moisture). The amount of sodium chloride was obtained according to ISO 5943:2006 [12]. The amount of fat - according to ISO 3433:2008 [13]. Acidity was determined according to BNS 1111-80 [14] and potentiometrically by using a pH meter.

The total protein content, nitrogen, and its fractional amounts were determined according to [15], with the adaptation of product characteristics [16].

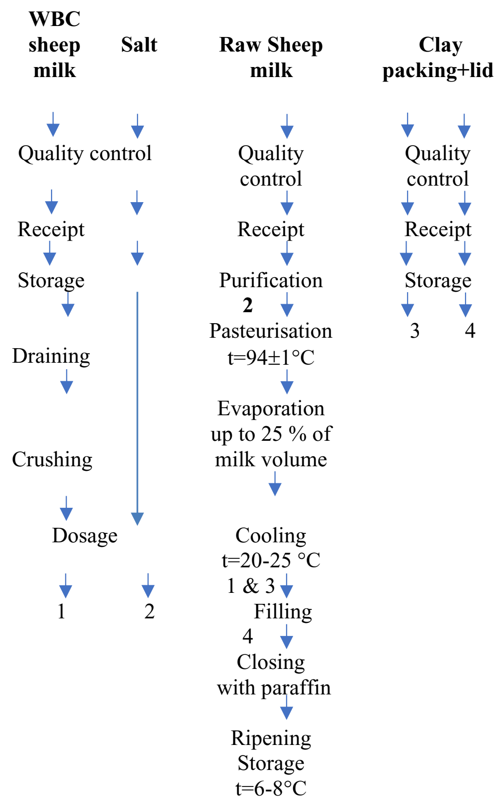

Fig.1. Technological scheme for obtaining artisan cheese from sheep's milk

Fig. 2. Picture of the end products of artisanal sheep cheese

The microbiological analysis of artisanal sheep`s cheese, the count of lactococci was enumerated on M17 agar incubated for 3 d under aerobic condition at 30°C and 37°C. Total lactobacilli during ripening and storage were enumerated on MRS agar under anaerobic incubation at 37°C using an anaerobic jar for 72 h. Selective enumeration of lactic acid bacteria count was made according to [17]. The psychrophilic microorganisms (ISO 17410:2019) [18] were determined.

Lipid extraction. Methanol (500µl) was added to 100.0 mg of lyophilized and powdered sample. The following internal standards were used: 50µl ribitol and 50µl nonadecanoic acid (each in mg/ml concentration); then, vortex for 10s and incubate (30min/70°C/300rpm); cooling to room temperature and adding 300µl distilled water + 500µl chloroform; then, vortex for 10s, centrifuge (10 min/22°C/13000 rpm) were added.

The resulting lower phase (apolar phase - 300µl) was evaporated to dryness under vacuum. To the dry residue 1.0ml (1M) solution of sulfuric acid in methanol (transmethylation) was added, followed by incubation at 96°C for 90min. The cooled solution was extracted with hexane (3 × 500ml). The combined organic layers were (silating reagent) and 50µl pyridine were added followed by incubation at 70°C for 30 min.

Chromatographic conditions for determination of fatty acids. For the determination of the fatty acids, 1.0 µl of the sample was used, which was injected into a system consisting of an Agilent GC 7890A gas chromatograph and an Agilent MD 5975C mass spectral detector. An HP-5MS column with parameters: length of 30m, diameter 0.32mm, and film coating thickness of 0.25µm was used under the following conditions for the determination of fatty acids: the initial temperature was 70°C, then hold 1min, and increase to 300°C at 5°C/min, hold 10min; injector and detector temperatures were 250°C; carrier gas was helium with a flow rate of $1 ml.min^{-1}$; mass detector scan range was m/z=50-550; injected sample volume was 1µl in 20:1 flow split mode [29].

The experiment was carried out in triplicate. The statistical analysis was performed with Microsoft Excel 2010, and the results were presented as mean value ± SD (n = 3).

3 Results and duscussion

The physicochemical analysis of artisanal sheep's cheese showed dynamic changes in its ripening and storage period ($45^{th}/180^{th}/360^{th}$ d). The results of the physicochemical evaluation (Table 1) show an increase in dry matter and protein content.

Table 1. Physicochemical parameters of artisanal sheep's cheese in the ripening process

Indicators	**Days**		
	45	**180**	**360**
Dry matter, %	47.02± 0.01	49.55± 1.77	52.50± 1.11
Total protein, %	16.44± 1.91	19.33± 0.55	24.55± 1.04
Total nitrogen, %	25.97± 1.66	28.71± 1.06	34.15± 2.83
Water content, %	53.07± 0.13	50.54± 1.22	48.12± 2.03
Sodium chloride, %	3.72± 0.04	4.11± 0.44	4.87± 0.55
Total fat content, %	30.05± 1.07	32.07± 1.14	33.15± 1.29
Titratable acidity, ˚T	242.00± 0.22	183.00± 1.66	124.7± 2.58
pH	4.44± 0.55	4.83± 0.13	4.94± 0.44

** Values are expressed as mean ± Standard deviation (n=3)*

The obtained results differ from those reported previously by other authors. Still, the tendency to increase the dry matter by about 5.0 to 10.0% in the ripening period of sheep's cheese is also reported by other researchers [19, 20]. The total protein concentration of 16.44% on the 45th d of ripening increased by about 8.0% respectively for the same ripening stage; the total nitrogen increased by about 9.3%. The increase in nitrogen fractions during cheese storage has also been reported by other researchers [10, 20, 21], and their studies stated that those storage methods significantly affected nitrogen concentrations. The decrease in water content during ripening is related to the created biochemical processes and binding of free water in protein matrices. In the storage conditions for artisanal sheep's cheese, the semi-permeability of the vessels and the achievement of equilibrium moisture were observed. The ripening conditions at an ambient temperature of 12 - 15°C created additional requirements for developing lactic acid microflora and diffusion distribution of the water phase from the interior to the periphery of the product. The creation of conditions for the redistribution of the liquid phase also led to higher levels of dry matter on the 360th d (52.50%) compared to the initial levels of 47.02%.

The volume of sodium chloride in the samples increases slightly as a consequence of the increase in their dry matter. At the end of storage, the titratable acidity decreased, reaching values of 124°T. The active acidity of the cheese was maintained at relative values throughout the ripening period, which is a prerequisite for suppressing the development of some unwanted representatives of the microflora.

In the composition of artisanal sheep's cheese, ten organic acids have been identified (Table 2), which were also involved in the formation of the aroma-flavor potential of the product.

Table 2. Organic acid content of artisanal sheep's cheese in the ripening process

Organic acids	**Days, mg/g dry weight**		
	45	**180**	**360**
Acetic acid	0.92±0.00	0.09±0.00	0.09±0.00
Propionic acid	0.11±0.00	0.08±0.00	0.08±0.00
Butyric acid	0.17±0.00	0.11±0.00	0.10±0.01
Pyrovic acid	0.38±0.01	0.22±0.01	0.13±0.00
Lactic acid	27.66±0.03	18.62±0.02	8.62±0.03
Oxalic acid	0.09±0.00	0.09±0.00	1.18±0.01
Succinic acid	0.16±0.00	0.19±0.01	0.27±0.00
Fumaric acid	0.07±0.00	0.08±0.00	0.21±0.01
Malic acid	0.05±0.00	0.11±0.00	0.28±0.01
Citric acid	0.06±0.00	0.11±0.00	0.13±0.01

** Values are expressed as mean ± Standard deviation (n=3)*

Changes were reported in the levels of lactic acid, which in the initial ripening period had the highest levels (27.66 mg/g dw), while at the end of the period, they decreased about three times (8.62 mg/g dw). The analysis reported an increase in the levels of citric acid (from 0.06 to 0.13 mg/g dw), fumaric acid (from 0.07 to 0.21 mg/g dw), and malic acid (from 0.05 to 0.28 mg/g dw) during the storage period. The organic acids in the initial stages of ripening and primarily preserved throughout the ripening process maintain an acidic reaction in the product, unsuitable for the development of psychrotrophic microorganisms [5] (Table 3).

Table 3. Microbiological profile of artisanal sheep's cheese during ripening

Number of viable microorganisms	**Days, cfu/g**		
	45	**180**	**360**
Total number of lactobacilli	1.8*10^5	2.1*10^4	1.1*10^3
Total number of lactococci	1.0*10^9	1.4*10^7	1.2*10^5
Psychrotrophic	1.1*10^3	1.2*10^2	1.2*10^2

In traditional artisanal cheeses, the increased acidity is a factor for active proteolytic processes, which are caused by microflora encountered during milking or processing of the cheese at the beginning of ripening. The results also confirmed the presence of a more significant number of lactococci in the initial period of growth (1.0*10^9), and at the end of the storage, they decreased to 1.2*10^5. The same tendency was observed in the representatives of lactobacilli, which also reduced at the end of ripening (1.1*10^3). Other researchers [10, 21] who studied the microbial stability of similar artisanal cheeses (with a different technological production model) reported the presence of lactic acid bacteria (about 53%) belonging to the genus *Enterococcus* sp., and about 26% to *Lactobacillus* ssp., emphasizing their species variability [4]. The results from the point of view of the microflora in artisan sheep's milk cheeses were dynamic. Some of them have identified representatives of *Enterococcus, Lactobacillus, Streptococcus, Lactococcus*, and *Pediococcus*. The present study found no pathogenic microorganisms, which confirmed the fondings reported by some authors [2, 4, 5, 21, 22]. Other authors [5] report on artisanal cheeses in which molds and yeasts have a higher availability in the ripening process due to the penetrating air in the vessel (Tulum).

Proteolytic changes due to the contained microflora and diffusion processes as a result of storage in clay vessels affected the fatty acid profile of the cheese during the all studied period [3, 21]. The changes in the quantitative and qualitative composition of the contained fatty acids also determined the specific aroma-taste profile of the cheese [6 - 9]. Factors such as phytoreception, breed, age of the animals, stage of lactation, presence of diseases, stress, temperature conditions of the environment, etc., played an essential role in the fatty acid composition.

The short-chain fatty acids (C4:0 - C14:0) and those with C16:0 were synthesized due to fermentation processes in the rumen of animals [22]. At the same time, long-chain fatty acids (C18:0 - C22:0) were mainly synthesized from lipids, which were absorbed into the bloodstream through the small intestine. The study showed that fatty acids' profile remains relatively close during ripening, with some of them having lower values at the end of ripening (Table 4), and they are not statistically distinguishable.

The fatty acid profile of the artisanal cheese determined 21 fatty acids. The test showed that saturated fatty acids made up 69.32% of the fatty acids by the 45th day of storage, of which short-chain fatty acids made up 11.76%. The share of unsaturated fatty acids during the same ripening period was mainly represented by palmitoleic and oleic fatty acids, which consisted of about 30.55% of unsaturated fatty acids. Representatives of long-chain fatty acids formed about 1.66% of the fatty acid profile of the sample [21, 22].

Table 4. Fatty acid composition of sheep's cheese in the process of ripening

Compound	**Days, g/100g dry weight**		
	45	**180**	**360**
C4:0	2.51±0.01	2.26±0.00	1.99±0.00
C6:0	1.74±0.00	1.28±0.01	1.32±0.00
C8:0	1.42±0.00	1.15±0.00	1.07±0.00
C9:0	0.11±0.00	0.13±0.00	0.10±0.01
C10:0	3.15±0.00	3.08±0.01	3.04±0.01
C11:0	0.13±0.00	0.11±0.00	0.10±0.00
C12:0	2.70±0.00	2.35±0.00	2.04±0.01
C13:0	0.07±0.00	0.05±0.00	0.05±0.00
C14:1	0.36±0.00	0.33±0.00	0.36±0.01
C14:0	12.18±0.01	14.44±0.04	15.46±0.02
C15:0	0.85±0.00	0.77±0.00	0.64±0.00
C16:1	0.73±0.00	0.62±0.00	0.60±0.00
C16:0	30.74±0.02	31.65±0.02	31.60±0.03
C17:0	0.80±0.00	0.77±0.00	0.75±0.00
C18:2n6c	1.54±0.00	1.43±0.00	1.17±0.00
C18:1n9c	28.16±0.01	27.98±0.02	29.09±0.02
C18:3	0.12±0.00	0.10±0.00	0.11±0.00
C18:0	12.29±0.01	11.15±0.01	10.29±0.01
C20:0	0.10±0.00	0.08±0.00	0.08±0.00
C22:0	0.08±0.00	0.07±0.00	0.08±0.00
C24:0	0.09±0.00	0.09±0.00	0.07±0.00
Cholesterol	3.19±0.00	3.55±0.00	4.01±0.01
Cholic acid	1.55±00	1.62±0.00	1.98±0.00

** Values are expressed as mean ± Standard deviation (n=3)*

In the second stage of study (180th d), the amount of saturated fatty acids remained at relative values (69.76%), of which short-chain fatty acids were 10.36%. Unsaturated fatty acids were 30.13%, represented by palmitoleic and oleic fatty acids, constituting about 1.53%.

In the third stage of the study (360 d), saturated fatty acids made up 69.04%, of which short-chain fatty acids were 9.66%. The profile of unsaturated fatty acids was dominated by palmitoleic and oleic fatty acids, which comprised 30.89%, and long-chain fatty acids were 1.28%.

Milk fat in spherical colloidal groups constituted about 98% of lipids. Small amounts of mono- and diglycerides, cholesterol, cholesterol esters, and phospholipids were found in sheep's milk. Cholesterol levels from the ripening to the end of the storage increased by over 1.0%.

In small ruminants, the transfer of fatty acids from dietary lipids was quicker, which determined the higher levels of unsaturated fatty acids in the composition of dairy products from small ruminants [21, 23]. On average, the amount of saturated fatty acids in sheep's milk and products obtained from it was about 70%.

The levels of carbohydrates that participated in the ripening processes and formed the aroma-flavor characteristics of the cheese are determined (Table 5).

Table 5. Carbohydrate composition of sheep's cheese in the process of ripening

Carbohydrates	Days, mg/g dry weight		
	45	180	360
Galactose isomer 1	0.11±0.00	0.12±0.00	0.15±0.01
Glucose isomer 1	0.20±0.01	0.23±0.02	0.29±0.00
Galactose isomer 2	0.17±0.02	0.14±0.00	0.13±0.00
Glucose isomer 2	0.09±0.00	0.15±0.00	0.21±0.01
Lactose isomer 1	0.26±0.01	0.94±0.00	1.66±0.00
Lactose isomer 2	0.15±0.00	0.27±0.00	0.65±0.01

* Values are expressed as mean ± Standard deviation (n=3)

The data showed that the carbohydrate values varied in close range during the different stages of the test. The amounts of glucose and lactose showed the most pronounced variability compared to galactose.

The relatively low levels of lactose in the product suggested its potential for use in the nutrition of individuals with lactose intolerance, which needs further investigation [23, 24]. Our findings revealed that during the studied period there was a tendency the lactose isomer 1 to be incresead from 0.26 mg/d dw to 1.66 mg/g dw. The galactose isomer 2 tended to decrease, reducing its levels by 4% at the end.

The amino acid composition of the cheese samples was also determined in order to assess the quality of artisanal cheese (Table 6).

Table 6. Amino acid composition of ripening sheep's cheese in the process of ripening

Amino acids	Days, g/100g dry weight		
	45	180	360
Alanine	0.89±0.01	1.22±0.00	2.41±0.00
Glycine	0.39±0.00	0.37±0.00	0.36±0.01
Valine	1.61±0.01	1.22±0.01	1.70±0.00
Leucine	4.73±0.02	5.22±0.01	7.50±0.01
Isoleucine	0.84±0.00	0.93±0.00	2.59±0.00
Proline	0.79±0.00	1.98±0.00	3.56±0.01
Serine	0.52±0.01	0.77±0.00	2.65±0.00
Threonine	0.60±0.00	1.11±0.00	2.22±0.01
Aspartic acid	0.87±0.00	1.33±0.00	1.90±0.00
Methionine	0.73±0.00	1.22±0.01	2.86±0.01
Pyroglutamic acid	nd	4.66±0.01	4.97±0.00
Glutamic acid	4.11±0.00	4.22±0.00	4.64±0.01
Phenylalanine	3.05±0.00	3.01±0.00	3.08±0.01
Lysine	2.04±0.00	2.01±0.00	2.03±0.00
Tyrosine	0.47±0.00	0.58±0.01	0.92±0.00
Tryptophane	nd	0.45±0.00	0.64±0.00

* *Values are expressed as mean ± Standard deviation (n=3).*
**nd – not detected.*

The amino acid content was comparable to sheep's milk cheeses. On the 45th d of ripening, it was noticed that the values of leucine were the highest (4.73 g/100g dw), and the value of glycine was the lowest (0.39 g/100g dw).

The lower salt and water ratios allow lactococci to develop aminopeptidase activity during ripening. Some amino acid values increased as a result of these processes. The amount of alanine increased threefold, reaching 2.41 g/100g dw. A similar trend was reported for leucine, which increased up to 30.5%. Isoleucine, threonine, and proline increased by an average of 30 - 35% during test period.

Essential amino acids consisted of an average of 13.99 g/100 g dw of the total amount, which determined an average of 64% of the identified amounts of amino acids on the 45th d of ripening. In the second stage (180th day), the essential amino acids were 15.54 g/100g dw, constituting an average of about 51.28% of the total amount. In the third (last) stage (360th day), the amounts of essential amino acids constituted 22.98 g/100g dw, which included about 52.19% of the total amount of identified amino acids.

Our study's amino acid composition profiles were similar to those reported previously by other authors, causing quantitative and qualitative differences [9, 23, 24].

The amino acid proline is essential for nutrition, which has increased its levels by more than 50% at the end of cheese storage. It plays a vital role in arginine and polyamines synthesis, directly affecting cell signaling in collagen synthesis. Proline is one of the leading amino acids that need to be included in infancy to ensure proper growth, hemoglobin synthesis, the humoral immune response, an intoxicating effect on nerve cells, and the formation of amyloid precursor protein [25, 26].

The other metabolic compounds' content was also evaluated in order to assess the quality of the artisanal sheep's cheese (Table 7).

Table 7. Other components in the composition of sheep's cheese in the process of ripening

Compound	Ripening and storage period (days), mg/g dw		
	45	180	360
Urea	0.11±0.00	0.67±0.01	1.10±0.00
Threitol	1.10±0.01	1.15±0.01	1.52±0.01
Sorbitol	1.44±0.00	1.29±0.00	2.81±0.00
Myo-inositol	0.22±0.00	0.20±0.00	0.18±0.00
Phosphate	3.09±0.01	3.15±0.00	3.37±0.00

* *Values are expressed as mean ± Standard deviation (n=3).*

The urea levels in artisanal cheeses were directly related to the amounts of proteins and nitrogen fractions in the cheese composition. Urea levels increased by less than 1% from the 45th to the 360th d. The increase in the urea values could be related to the lower degree of hydrolysis of β-casein, which is resistant to proteolysis [27]. As a result of the proteolytic processes and the availability of polyols and urea, specific amounts of triethyl, sorbitol, and myo-inositol are also identified [28]. Their participation in artisanal cheese could be related to the carbohydrate metabolism of some microorganisms and the absence of some enzyme complexes. The amount of phosphates in artisan sheep's milk cheese under ripening and storage conditions did not change significantly, increasing by an average of 0.2%. Phosphorous compounds in the composition of dairy products were represented by their inclusion primarily in the composition of phospholipids. Other forms found in dairy products are colloidal and casein phosphate.

4 Conclusion

The present study evaluated quality characteristics of a traditional artisan sheep's milk cheese. The analysis presented the possibilities of applying the traditional artisanal technologies to produce dairy products, resulting in the final products possessing valuable components for human nutrition. The cheese's microbial stability, fatty acid, and amino acid profile revealed the product's quality indicators. Future research will be focused on a more comprehensive microbiological analysis of the artisanal cheeses during the ripening process.

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