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Microbiological, Physical, Chemical and Sensory Characteristics of Artisanal Georgian Tenili Cheese

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Abstract.

Georgian artisanal tenili cheese belongs to pasta filata-type cheeses made by hand in the Samtskhe-Javakheti region of Southern Georgia. Microbiological, physical-chemical characteristics and acceptance of tenili cheese by the consumers in Georgia. Tenili cheese samples made with different technologies (traditional, non-traditional), ripened in different utensils (clay pot, glass jar) at different ripening periods (1/2/3/5 month) were analyzed on microbiological, chemical, texture, color and sensory characteristics. According to the results techniques applied during processing and utensils for ripening directly influence its microbiological, chemical, color, texture and sensory characteristics. Higher number of lactic acid- and propionic acid bacteria withal the chemical composition

is a precondition for using tenili cheese as a functional food. Non-traditionally made tenili cheese received higher approval of Georgian population due to its characteristics (higher fat content, stronger fibrous structure and intense color) compared to traditionally made cheese.

Keywords: artisanal cheese; consumer acceptance; non-traditional technology; traditional technology.

Introduction

The technology of dairy products making varies in all parts of Georgia. In the region of Samtskhe-Javakheti milk and its products have historically played an important role and were characterized by great diversity. Nevertheless, many traditional products are no longer produced in Samtskhe-Javakheti with the same intensity as before, traditional dishes still play an essential role in the daily life of the local population [1]. One of the traditional products is artisanal cheese tenili, which is called the pride of Meskhetian cuisine and is referred as "magic in a clay pot" [2].

Historically the geographical area of tenili cheese production is the Samtskhe-Javakheti region, but currently it is mainly produced only in two villages – Chobareti (Aspindza municipality) and Andriatsminda (Akhaltsikhe municipality). Tenili cheese is the only cheese in Georgia that has both the status of geographical indication since January 24, 2012 (registration number N5) [3] and its outstanding technology has the status of intangible cultural heritage at the national level, from November 15, 2013 [4].

After the signing of the Association Agreement (deep and comprehensive free trade area) between Georgia and the European Union, trade and economic relations have reached a qualitatively new level, emerged new opportunities for Georgian products to enter the EU market. Food production in the EU market is built entirely on quality and safety policies. The system of geographical indications is designed to ensure the quality of agricultural products, protect the interests of consumers, and provide conditions of fair competition for producers. Tenili cheese has been protected by geographical indication in the EU Member States under an agreement [5].

Preliminary research made in the Samtskhe-Javakheti region for description of tenili cheese preparation revealed that there are two technological methods: traditional and non-traditional. The non-traditional method is the easier and cheaper compared to the traditional method.

Despite the recognition of tenili cheese nationally (GI, intangible cultural heritage) and internationally (agreement between Georgia and EU), no studies have been done in Georgia regarding the microbiological, chemical, texture, colour and sensory characteristics of traditionally produced tenili cheese. Only one work has been published in Georgia with the aim of developing the tenili cheese production by a machinery method [6].

The goal of the research is to determine the microbiological, chemical, texture, colour and sensory characteristics of tenili cheese made with different technologies and ripening utensils at different ripening stages.

Main Part

1. Materials and methods

1.1 Cheese making

Tenili cheese samples were prepared with traditional and non-traditional technologies in the village Chobareti of the Samtskhe-Javakheti region, from where tenili cheese originates. Cow's row milk was purchased from the local farmer and analysed before cheese making by the milk analyzer (Ultrasonic milk analyzer, Model number 32824, Milkotester Ltd., Belovo, Bulgaria) and pH-meter (Foodcare pH-meter, Model number 99161, Hanna Instruments Inc., Woonsocket, USA) (Table 1). Technological differences between traditional and non-traditional methods of tenili cheese making are explained in Table 2.

Table 1.

Parameters of row milk used for tenili cheese making with traditional and non-traditional methods

Parameters	Skimmed milk for traditional production	Milk for non-traditional production
Fat (%)	0.2	4.6
Solids-Non-Fat (SNF)(%)	9.9	9.3
pH	5.3	6.7
Density	36.5	31.1
Protein (%)	3.6	3.4
Lactose (%)	5.4	5.1
Added water (%)	0	0
Salt (%)	0.8	0.8

Table 2.

Differences between traditional and non-traditional technologies

Traditional technology	Non-traditional technology
Skimmed milk	Milk without skimming
No rennet	Rennet (home-made by the farmer) 1.0 % (v/v)
Naturally acidified milk is used for coagulation (37-40°C) which continues directly with hand processing	Head-cheese method (after coagulation at 37-40°C cheese heads are prepared and, after the cheese obtains light acidic taste, it is cut into hot water for further processing of the cheese mass by hand)
Hot whey for hand processing	Hot water for hand processing

The hand-processing step is common for both technologies. At this stage, the cheese mass is slowly stretched during the processing by pressing and moving into the hot whey/water direction. After making one cycle of rope, cheese is folded, and the cheesemaker continues this process until strands become thin like hair, which should not be broken while hand processing. Once stretching is finished, the cheese mass is immersed in the cold water for 2 minutes to avoid sticking of cheese strands to each other and then it is transferred to the brine (15-18%) for 4 minutes to strengthen the cheese strands. Then cheese is hung to dry and three days later, cheese is immersed in cream, and pressed into the ripening utensil (traditionally – in clay pots, but today for this purpose glass jars are used, too). The utensils full of cheese are left for two days, then it is turned upside down and stored in a cool place (12-16 °C) lying on the ash.

Totally 16 tenili cheese samples were prepared according to the above-described technologies: traditional and non-traditional; For each technologically made tenili cheese samples clay pots and glass jars were used as ripening utensils.

1.2 Sampling

Microbiological analyses of tenili cheese samples were carried out at different ripening stages during 5 months (totally four times); the rest cheese samples were stored at -18°C after each microbiological analysis, and later after defrosting, chemical, colour, texture and sensory analyses were conducted. In addition, microbiological analyses of tenili cheese samples were performed without ripening directly after cheese making.

A full design of three factors experiment was used to characterize the samples: the first factor with two levels was the technology (traditional, non-traditional), the second factor with two levels was the utensil (clay pot, glass jar) and the third factor with four levels was the ripening month (1, 2, 3 or 5 months). The combination of all the levels of each factor leads to 16 samples (table 3).

Table 3.

Design of experiment

Number of samples	Technology	Ripening utensil	Ripening month (duration of ripening months)
1	Traditional	Clay pot	September (1)
2	Traditional	Clay pot	October (2)
3	Traditional	Clay pot	November (3)
4	Traditional	Clay pot	January (5)
5	Traditional	Glass jar	September (1)
6	Traditional	Glass jar	October (2)
7	Traditional	Glass jar	November (3)
8	Traditional	Glass jar	January (5)
9	Non-traditional	Clay pot	September (1)
10	Non-traditional	Clay pot	October (2)
11	Non-traditional	Clay pot	November (3)
12	Non-traditional	Clay pot	January (5)
13	Non-traditional	Glass jar	September (1)
14	Non-traditional	Glass jar	October (2)
15	Non-traditional	Glass jar	November (3)
16	Non-traditional	Glass jar	January (5)

2. Analyses

2.1. Microbiological analysis

The isolation of microorganisms was conducted by the method of serial dilutions [7]. Quantitative and qualitative analysis of different microorganism's groups were performed on selective/differential media according to the manufacturer's instructions: total viable bacteria were enumerated on Plate Count Agar (Liofilchem, Italy) after incubation for 3 days at 30°C; lactic acid bacteria – on MRS (De Man, Rogosa and Sharpe) agar (Biolife, Italy) at 37°C after anaerobically incubation for 3 days; propionic acid bacteria – on Propionibacter Isolation Agar Base (HiMedia, India) at 30°C after 10 days of incubation anaerobically; *Bacillus cereus* were enumerated on Polymyxin pyruvate Egg yolk Mannitol Bromothymol blue Agar (PEMBA) (HiMedia, India) at 30°C, incubation period – 3 days; *E. Coli* were counted on Violet Red Bile Lactose (VRBL) Agar (Biolife, Italy) at 37°C after 18-24 h; coagulase positive staphylococci – on Baird-Parker Medium (Liofilchem, Italy) at 37°C for 24 h; *Salmonella* colonies were identified on Bismuth Sulphite Agar (Biolife, Italy) at 37°C for 18-20 h after pre-enrichment in Buffered Peptone Water (Biolife, Italy) at 37°C for 18-20 h and selective enrichment in Rappaport Vassiliadis (RV) broth at 42°C for 24 h; yeasts were enumerated on Oxytetracycline Glucose Yeast Extract Agar (OGYE Agar) (HiMedia, India) at 25 °C after 2-5 days of incubation; mycelial fungi – on Sabouraud Glucose Agar (Biolife, Italy) after 3 days at 30°C; *Listeria monocytogenes* colonies were identified on Listeria Palcam Agar (Liofilchem, Italy) at 37 °C for 24-48 h of incubation. For each analysis, three replicates were carried out.

2.2. Chemical analysis

Total protein content was calculated according to the GOST 23327-98 [8] with the Kjeldahl method; 6.38 was used as the conversation factor. For fat separation solvent extractor (Semi-automatic solvent extractor, velp scientifica, Usmate, Italy, SER 148/6) was used. pH was determined by insertion of the probe in cheese samples (Seven2Go™ pH Meter, Metler toledor, Schwerzenbach, Switzerland, S2). Dry matter and moisture content were determined by GOST 3626-73

[9]. For each sample and each analysis, three replicates were performed.

2.3. Texture analysis

Hardness and adhesiveness were measured by texture analyser (TA-XT plus texture analyser, Stable Micro-systems Ltd, Godalming, UK; Serial No. 12949) with 3 mm diameter, 3 mm high, 60 angle probes. The test mode was compression, test speed – 2 mm/sec and distance – 20 mm. For texture analyses 10 g tenili cheese samples were placed in the cap with 5.5 mm diameter and 3.5 mm height. The force value (KG) was used to compare cheese samples according to the positive peak and negative peak. A positive peak is used to determine hardness and a negative peak – adhesiveness (relaxation phase). For each sample three replicates were carried out.

2.4. Color analysis

At different points on the surface of cheese samples, three measurements were carried out by reflectance colorimeter spectrophotometer (spectrophotometer CM-5, Konica Minolta, Osaka, Japan, 1103809) with reflection mode geometry d/8 illuminant D65/10°, analysis diameter: LAV 30 mm. During the analyses of CIELAB color space, lightness (L*) changing dark to light (0-100%), red to green (a*) and yellow to blue (b*) were measured. Collected results were interpreted with the data software Colibri, version 3.8.12. build number 20099.

2.5. Sensory analysis

For sensory analyses of 16 cheese samples hedonic test was used. Presentation order of samples for each assessor was conducted according to the Latin square design. The assessors were asked to rate each cheese sample according to their preference from 0 (dislike extremely) to 10 (like extremely). The experiment was conducted in the sensory laboratory in a separate booth. Water was provided for rinsing of the mouth. For each sample (3 g) a plastic cup coded with a 3-digit number was used. No time restriction was imposed to the assessors. The experiment was carried out with 62 consumers from Tbilisi, Georgia (29 males, 33 females, mean age: 31 years).

2.6. Statistical analyses

For all instrumental analyses (microbiological, chemical, texture and color analyses), the results were shown as means and standard deviations (±SD). Instrumental data was analyzed by ANOVA considering the technology, ripening utensil, and ripening month as fixed factors and microbiological, chemical, texture and color parameters as dependent variables. Using the Tukey test (considered significantly different at $p<0.05$) was performed post-hoc pairwise test.

The liking data were analyzed by ANOVA considering technology, ripening utensil, ripening month and the participants as factors and liking scores as the dependent variable. A post-hoc Tukey test ($p<0.05$) was performed, where a significant effect of cheese was found.

To link liking data with instrumental data, a principal component analysis (PCA) was applied to the mean values of three replicates to identify correlations among parameters and to group the cheeses accordingly. The liking score was added as a supplementary variable.

All the statistical analyses were performed with XLSTAT Base (Microsoft, Paris, France).

3. Results and discussion

3.1 Instrumental analysis

Microbiology. According to the results of microbiological analyses the presence of *Salmonella*, *Bacillus cereus* and *Listeria monocytogenes* were not confirmed in any of the samples. Coagulase-positive staphylococci were confirmed only in tenili cheese samples without ripening. In ripened samples (1/2/3/5-month) coagulase-positive staphylococci did not confirm. Existence of *E. coli* was confirmed in tenili cheese samples at early stage of ripening (Table 4, Table 5).

The results of microbiological analysis for traditionally and non-traditionally made tenili cheese samples were statistically significant (P <0.001). Lactic acid bacteria and propionic acid bacteria were clearly the dominant microorganisms for cheeses made by both technologies (traditional, non-traditional), which are in line with that reported for pasta filata types of cheeses made by row milk [10].

Table 4.

Microbial composition of tenili cheese made by traditionally and non-traditionally (lg cfu/g) without ripening*

Group of microorganisms	Technology	
	Non-traditional	Traditional
Total plate count	7.06 ± 2.05	10.09 ± 0.82
Lactic acid bacteria	7.08 ± 2.62	10.08 ± 2.45
Propionic acid bacteria	6.90 ± 2.19	6.62 ± 1.4
Yeasts	5.71 ± 0.82	5.73 ± 8.18
Moulds	4.56 ± 1.63	5.37 ± 3.3
E. coli	0	2.7 ± 0,15
Coagulase positive staphylococci	3.2 ± 1.63	4.1 ± 0,71

** Presented values are means of three replicates (n=3), ± standard deviation*

Traditionally made cheese was characterized by a higher number of lactic acid bacteria and total viable bacteria compared to the non-traditionally made cheese. The existence of *E. coli* is confirmed only in traditionally made tenili cheese, which may be explained by the stage of technology of traditionally made tenili cheese, when the cheese is processed by hand in its own whey, while in the non-traditional technology only water is used. Whey is favourable environment for growing spoilage/pathogenic and lactic acid bacteria [11]. According to the results, non-traditionally made cheese confirmed a higher number of propionic acid bacteria, mycelial fungi and yeast content compared to traditionally made cheese.

Table 5.

Microbial composition of tenili cheese samples*

Variant		Total plate	Lactic acid bacteria	Propionic acid bacteria	Yeast	Moulds	*E.coli*
Technology	Non-traditional	7.02±1.16 a	6.72±1.88 a	7.38±0.84 b	5.91±0.86 b	4.42±1.18 b	0 a
	traditional	8.17±2.24 b	6.82±2.09 a	6.87±0.95 a	5.77±0.9 a	3.97±1.1 a	0.7±3.83 b
	***P*-value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Utensil	clay pot	7.48±1.06 a	6.7±1.07 a	7.56±0.38 b	5.91±0.37 b	4.24±0.67 a	0.38±3.78 b
	glass jar	7.71±0.8 b	6.84±0.91 a	6.69±0.44 a	5.77±0.5 a	4.15±0.48 a	0.32±2.42 a
	***P*-value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.025**	**0.005**
Ripening duration	1-month	9.99±0.75 d	9.8±0.3 c	8.05±0.18 d	7±0.06 d	5.65±0.15 d	1.4±2.96 b
	2-month	7.93±0.6 c	6.97±0.24 b	7.56±0.29 c	6.04±0.08 c	4.59±0.44 c	0 a
	3-month	6.59±0.26 b	5.25±0.18 a	6.68±0.34 b	5.6±0.09 b	3.64±0.17 b	0 a
	5-month	5.87±0.17 a	5.05±0.14 a	6.2±0.33 a	4.71±0.22 a	2.9±0.13 a	0 a
	***P*-value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

** Presented values in lg cfu/g, are means of three replicates (n=3), ± standard deviation; different superscript letters mean that within a row, values significantly differ at p<0.05 (Tukey)*

The results of the microbiological analysis in terms of ripening utensils of tenili cheese samples were statistically significant. According to the research results, propionic acid bacteria (P <0.001), yeasts (P <0.001), mycelial fungi (P <0.025) and *E. coli* (P <0.025) were higher in clay pot, while total viable bacteria (P<0.001) and lactic acid bacteria (P <0,001) were higher in glass jars. Propionic acid bacteria are aerotolerant [12], *E. coli* is able to grow in aerobic environment where oxygen is required for efficient metabolism [13] and fungi are essentially aerobic microorganisms [14]. The obtained result is justified by the capillary and high number of porous (macro, micro and sub-micro) structure of the clay which, could significantly affected on microbiological composition of the product ripened in a clay pot [15]. Results of microbiological analyses of tenili cheese samples after production, without ripening are given in the Table 4, and Table 5 show the results of microbiological analysis for cheese samples at different ripening continuance; all obtained results are statistically significant (P<0.001). Based on the research, it is obvious that the number of microorganisms we studied (except *E. coli*) increased significantly at the beginning, and gradually decreased later during ripening. It is known from the literature that *Lb. bulgaricus* is characterized by rapid growth at the initial stage, followed by a significant decrease. This is due to its autolytic characteristic [16]. This result is also in compliance with findings from other author where in other type of pasta filata cheese caciocavallo amount of lactic acid bacteria are higher at the early stage of ripening and decreases gradually after the first month [17]. The number of lactic acid and propionic acid bacteria increases during the first month of ripening and as a result of their metabolism, the pH decreases [18], *Salmonella* spp., *E. coli*, staphylococci, are neutrophils [19] and low pH creates unfavorable conditions for their development; therefore, pathogenic microorganisms are decreasing in cheese samples at early stage of ripening. The absence of *Listeria monocytogenes* in cheeses is justified by the different native microbiota of row milk [10]. Tenili cheese ripened for 5 months is characterized by the minimal number of microbes. The gradual decrease could be explained by competition for lack substrate, producing inhibitory metabolites and the secretion of bacteriocins [16].

Chemical analyses. Table 6 illustrates statistically significant results of chemical analyses, for traditionally and non-traditionally made tenili cheese samples.

According to the results, the samples of tenili cheese produced by non-traditional technology compared to the traditional one is characterized by high-fat content (59.97%) (P <0.001), due to the technological process: milk without skimming is used for the production of non-traditional tenili cheese, while for the traditional technology, skimmed milk is used. Traditional tenili cheese is characterized by high protein (P<0.005), moisture (P<0.001) content and pH (P<0.008) value. It could be assumed that higher protein content in traditionally made cheese is caused by hand-processing step in whey (Table 2), which is an important source of protein, 20 % of milk protein is in whey which is soluble more than casein and has a higher quality rating [20, 21]. In non-traditional technology water is used for the same procedure.

Since cheese is made with skimmed milk in the traditional technology, the hand-processing step is much more difficult compared to non-traditional technology, cheese threads are difficult to stretch and they break easily due to the lack of fat. As a result, traditionally made threads are structurally different and much thicker than non-traditionally made cheese threads. After the next technological step, when cheese made with both technologies is hung for drying, moisture contents are different. It is higher in cheese made by the traditional method (45.5 %) compared to the non-traditionally made cheese (38.07 %). The pH is slightly higher in cheese made by traditional technology (P<0.008). Moisture content is directly proportional to pH [22]. That explains the high pH while being high moisture content.

Obtained results of chemical analyses, for tenili cheese samples ripened in a glass jar and clay pot were statistically significant (P<0.001) except for protein (P<0.577) and fat (P <0.156). Moisture content (42.1%) and pH (4.76) are higher in the cheese samples ripened in the glass jar, accordingly, dry matter (58.4) is higher in the cheese ripened in the clay pot. The difference in

moisture content may be caused by the porous structure of the clay [15]. pH and moisture are related, high moisture affects the pH and causes its increase, which may explain the higher pH of cheese ripened in glass jars [22]. Protein and fat amounts do not change statistically significantly in different ripening utensils.

Table 6.

Chemical composition of tenili cheese samples*

Variant		Fat, %	Protein, %	Moisture, %	Dry matter, %	pH
Technology	Non-traditional	59.97 ± 4.06 b	22.34 ± 1.22 a	38.07 ± 2.74 a	61.94 ± 2.75 b	4.66 ± 0.2 a
	Traditional	49.44 ± 4.98 a	23.31 ± 0.85 b	45.5 ± 2.98 b	54.5 ± 2.98 a	4.68 ± 0.24 b
	***P*-value**	**<0.0001**	0.005	**<0.0001**	**<0.0001**	**0.008**
Utensil	Clay pot	54.11 ± 3.87 a	22.92 ± 0.73 a	41.46 ± 3.03 a	58.54 ± 3.04 b	4.58 ± 0.11 a
	Glass jar	55.3 ± 3.11 a	22.74 ± 0.38 a	42.1 ± 1.43 b	57.9 ± 1.43 a	4.76 ± 0.09 b
	***P*-value**	0.156	0.577	0.014	0.014	**<0.0001**
Ripening duration	1-month	50.52 ± 3.23 a	22.73 ± 0.7 a	44.78 ± 2.63 d	55.22 ± 2.64 a	4.92 ± 0.08 d
	2-month	54.23 ± 4.1 b	22.99 ± 0.36 a	42.21 ± 1.86 c	57.79 ± 1.86 b	4.72 ± 0.05 c
	3-month	55.36 ± 2.94 b	22.67 ± 0.55 a	40.58 ± 2.14 b	59.42 ± 2.14 c	4.6 ± 0.07 b
	5-month	58.71 ± 2.57 c	22.93 ± 0.69 a	39.55 ± 2.1 a	60.45 ± 2.1 d	4.44 ± 0.08 a
	***P*-value**	**<0.0001**	0.879	**<0.0001**	**<0.0001**	**<0.0001**

***Presented values are means of three replicates (n=3), ± standard deviation; different superscript letters mean that within a row, values significantly differ at P<0.05 (Tukey)**

Results of chemical analyses, for tenili cheese samples ripened for different month were statistically significant (P<0.001) except for protein (P<0.879).

Fat content is increasing with ripening, which is caused by the technological process. After the cream is smeared on the cheese and pressed in the ripening utensils and stored upside down, over time the cream is moving to the bottom. Traditionally, the middle part of cheese in ripening utensils is preferable for consumption, which is neither too dry like the top part nor too creamy like the bottom part. Over time the amount of fat increases in the middle part of ripening utensil. As the cheese ripens, its moisture content decreases, because of the release of water from the ripening utensils and level of pH reduces [22]. According to the results, protein does not change statistically significantly with ripening.

Texture and colour. Obtained data of texture and colour analyses, for traditionally and non-traditionally made tenili cheese samples were statistically significant (P <0.001) (Table 7).

Tenili cheese made by non-traditional technology is harder than cheese made by traditional method. In traditional technology during the stretching process cheese mass breaks easily and the final product is softer compared to the cheese made with non-traditional technology.

Traditionally made cheese is characterized by high adhesiveness (12.19), due to its broken fibrous structure. Non-traditional cheeses are characterized by brighter L*, reddish a* and yellowish b* colours compared to the traditionally made cheeses, due to the high-fat content.

Results of texture and colour analyses of tenili cheese samples ripened in a glass jar and clay pot were statistically significant except for hardness and adhesiveness.

Cheese ripened in a clay pot is characterized by a brighter L*, reddish a* and yellowish b* colour compared to the cheese ripened in a glass jar. As already discussed above in terms of microbiology clay pot contains higher number of mycelial fungi (lg cfu/g - 5.91) compared to a glass jar and they have an impact on the colour of cheese samples.

The texture and colour of tenili cheese samples ripened for different periods were statistically significant (P<0.001).

Table 7.

Texture and color analyses of tenili cheese samples*

Variant		Hardness ($kg\ s^{-1}$)	Adhesiveness ($kg\ s^{-1}$)	Color L*	Color a*	Color b*
Technology	Non-traditional	285.67 ± 76.45 b	8.41 ± 2.78 a	87.86 ± 0.97 b	1.81 ± 0.36 b	21.2 ± 1.07 b
	Traditional	155.58 ± 50.65 a	12.19 ± 5.65 b	83.15 ± 1.97 a	0.45 ± 0.22 a	19.79 ± 1.42 a
	***P*-value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Utensil	Clay pot	217.91 ± 48.1 a	10.66 ± 2.27 a	85.88 ± 1.46 b	1.26 ± 0.4 b	20.94 ± 0.73 b
	Glass jar	223.35 ± 44.63 a	9.94 ± 2.56 a	85.13 ± 1.37 a	0.99 ± 0.35 a	20.05 ± 0.65 a
	***P*-value**	0.140	0.276	**0.002**	**<0.0001**	**<0.0001**
Ripening duration	1-month	295.9 ± 42.55 d	14.96 ± 2.53 d	84.35 ± 1.49 a	0.92 ± 0.35 a	21.93 ± 0.32 d
	2-month	251.67 ± 34.8 c	11.21 ± 1.56 c	84.82 ± 1.32 a	1 ± 0.36 a	21 ± 0.55 c
	3-month	197.77 ± 40.72 b	9.31 ± 1.65 b	85.83 ± 1.33 b	1.19 ± 0.39 b	19.72 ± 0.68 b
	5-month	137.18 ± 21.96 a	5.73 ± 0.98 a	87.03 ± 1.31 c	1.4 ± 0.39 c	19.34 ± 0.42 a
	***P*-value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

***Presented values are means of three replicates (n=3), ± standard deviation; different superscript letters mean that within a row, values significantly differ at P<0.05 (Tukey)**

The hardness of the cheese decreases with ripening, which is caused by the softening of the fiber structure. The adhesiveness also decreases, which is caused by losing moisture content along with the ripening of the cheese. During ripening, the colour of tenili cheese becomes brighter L*, reddish a* and the yellow b* colour fades gradually.

3.2 Consumer test

In consumer test analyses (Figure 1), results show a significant effect of technology (p<0.001) and ripening utensil (p <0.043): assessors liking scores are higher for tenili cheese made with non-traditional method than with traditional method and for tenili cheese ripened in a glass jar than in clay pot. In terms of ripening month there is no significant difference (p <0.459) between the liking scores among 1, 2, 3, 5 months.

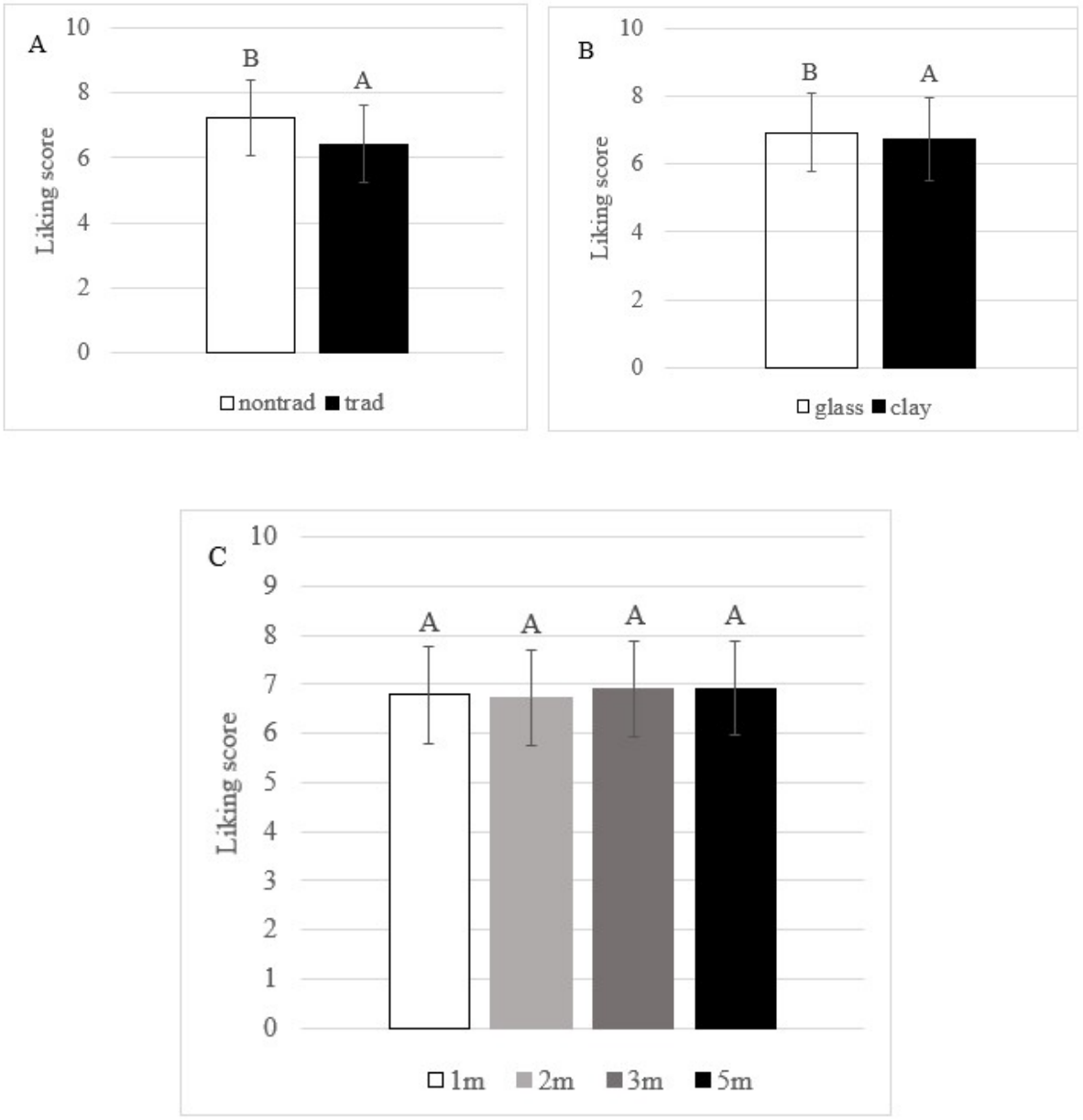

Figure 1. Consumer test results. Mean liking scores of a) non-traditional and traditional technology, b) glass and clay utensils, c) ripening duration. Bars indicate the standard deviation. Means with the different letters are significantly different based on Tukey's post-hoc test ($\alpha < 0.05$).

3.3 Instrumental analysis & consumer test: Principal component analyses (PCA)

The two first dimensions of the PCA explained 83.34% of the variance. Fig. 2 illustrates the clustering essentially based on the technological process, ripening duration. Two separate clusters emerge from the PCA: cluster #1 contains all the 8 non-traditionally made tenili cheese which are characterized by a higher amount of fat, dry matter, colour L* and a*, and cluster #2 groups of all traditionally made tenili cheeses which contains higher percentage of protein and moisture. PCA also reveals a gradient according to the time of ripening going

to the top right to the bottom left of the plot: at their first month of ripening, cheeses were characterized by the higher number of microorganisms and higher values of pH, colour b*, adhesiveness and hardness. These values decrease gradually with the ripening. However, no clear differences between ripening utensils (clay pot, glass jar) were observed.

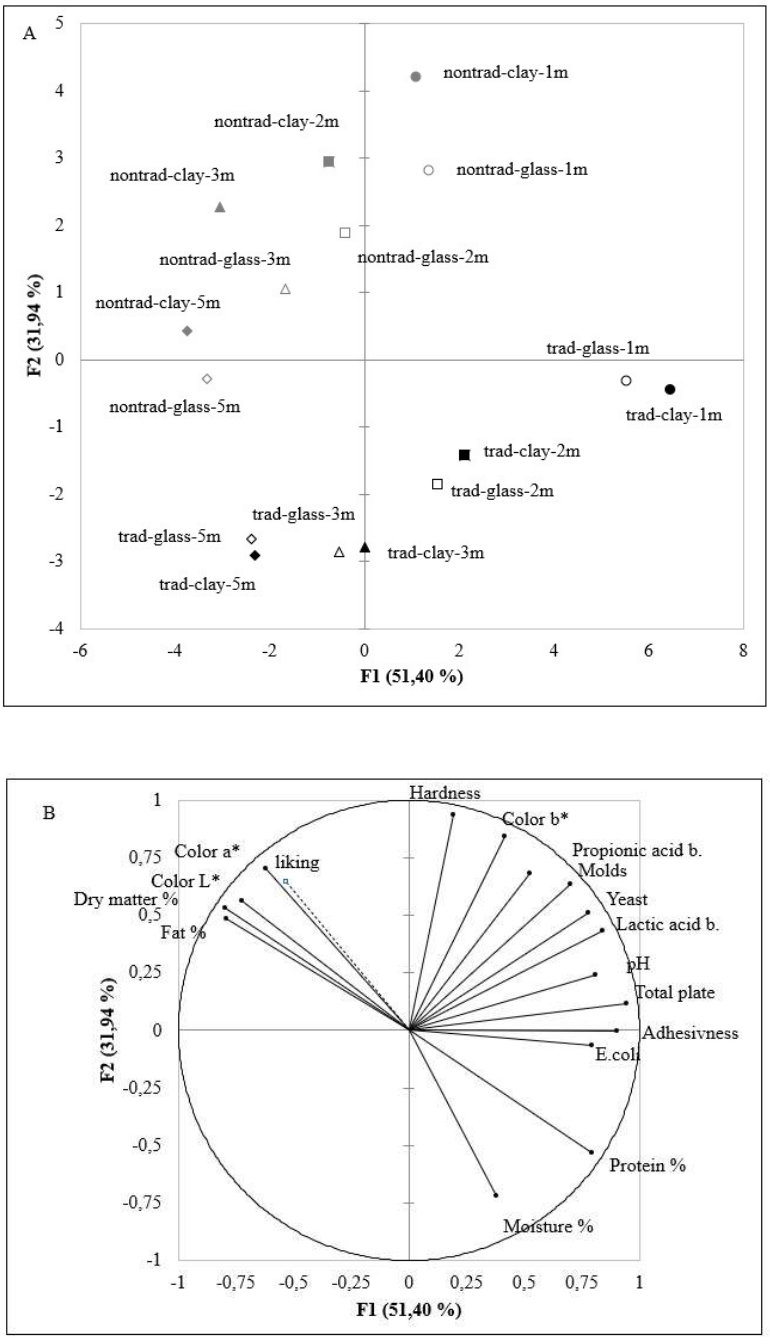

Figure 2. Principal component analyses (PCA) for dimension 1 and 2: a) 16 tenili cheese samples (grey signs-non-traditional cheeses, black signs-traditional cheese, full signs-clay pot, empty signs-glass jar, ● – 1-month, ■ – 2-month, ▲ – 3-month, ♦ – 5-month of ripening); b) microbiological, chemical, texture, colour variables. Liking scores are considered as supplementary variables.

Finally, as already showed with ANOVA, Georgian consumers preferred non-traditionally made tenili cheese compared to traditional tenili cheese.

Conclusion

Based on the results we can conclude:

- The quality of the artisanal Georgian cheese tenili is determined by the chemical composition of used milk as well as the techniques applied during processing and utensils for ripening since these directly influence its microbiological, chemical, colour, texture and sensory characteristics;
- In our study tenili cheese is safe for human consumption during 5 months of ripening. Coagulase-positive staphylococci were confirmed only in fresh cheese and was not detected during ripening, the contamination of tenili cheese by *E. coli* was confirmed only during the first ripening period. The presence of *Salmonella* and *Listeria monocytogenes* were not confirmed in any of the samples. The received results are in full compliance with the regulation of Georgian government №581 „Regarding the approval of the technical regulation on microbiological criteria of foodstuffs" which is created according to the EU Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs;
- The microbiota of tenili cheese made by traditional and non-traditional technology is growing at the initial stage of ripening. Lactic acid and propionic acid bacteria are the dominant microorganisms for cheeses made both as traditional as non-traditional technologies; utensils for ripening impact on the number of this bacteria: traditionally made cheese was characterized with higher number of lactic acid bacteria while in non-traditionally made cheese confirmed higher number of propionic acid bacteria, due to the conditions caused by the structure of clay and glass. High number of Lactic acid and propionic acid bacteria in tenili cheese withal the chemical composition is a precondition for using tenili cheese as a functional food;
- Differences in processing of traditional and non-traditional technologies of tenili cheese making (skimmed milk, processing of cheese mass in whey/water) affect their chemical, texture and colour characteristics: non-traditionally made tenili cheese is characterized by high-fat content, strong fibrous structure and intense colour, while traditionally produced cheese – by relatively low-fat content, broken fiber structure, more adhesiveness and pale colour;
- According to the results of consumer acceptance in Georgia: cheese made with non-traditional technology received higher approval compared to traditionally made cheese. Cheese aged in a glass jar is superior to cheese ripened in a clay pot. For a taste in terms of liking, there is no difference between cheeses ripened for different periods.
- Our work contributes to a better understanding of the microbiological and technological aspects in the practice of making tenili cheese; Future appropriate structured studies with a wider selection of samples and with the joint operation of the Government of Georgia, relevant non-governmental organizations, scientists and the local population of Samtskhe-Javakheti, allow obtaining successive scientific data and sustainable development of this sector to determine standards for production and control of tenili cheese.

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