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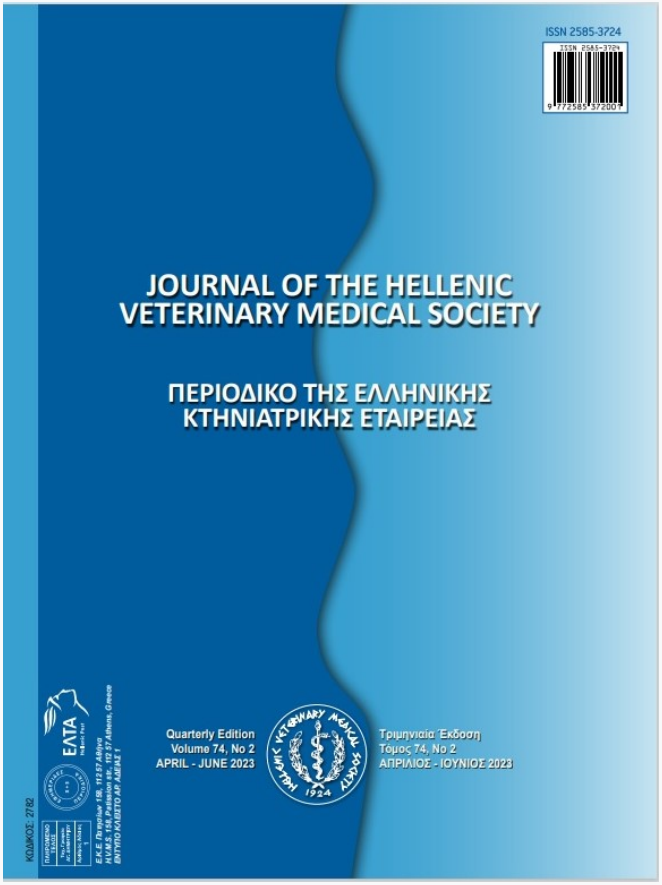

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Biochemical and microbiological characteristics of industrial Tsalafouti cheese

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ABSTRACT: Tsalafouti is a white cheese with mild sour, acidic taste. Its spreadable texture has no gas holdings.In the present work, Tsalafouti cheese was industrially manufactured and its biochemical, organoleptic and microbiological characteristics were investigated. At 30 days of storage,the mean pH was 4.05 and moisture content was 68.06%, fat 14.9%, fat-in-dry matter 46.52%, salt 1.55%, ash 2.4%, and proteins 10.92%. Level of proteolysis remained stable during storage. The volatile compounds 3-methyl butanol, acetoin, ethanol, acetone, were found in high levels on day 30. During the organoleptic evaluation Tsalafouti was very much appreciated until day45.Total viable counts enumerated on skim-milk containing media at 37°C remained constant within 7.5-8.0 log CFU/g from day 15to day 90of storage of the industrially ripened cheese. However, typical mesophilic lactic acid bacteria (LAB) increased to 8.1-8.5 log CFU/g and predominated the technological biota, whereas typical thermophilic LAB, inclusive of enterococci, remained subdominant by 1-3 log units throughout storage. Yeasts were beneficial for the sensory quality of industrial Tsalafouti from day 30 to 45 when their counts were below 6-7 log CFU/g; however, yeasts turned to be the primary spoilage agents after their counts increased to 7.5 log CFU/g with prolonged storage (day 60 to 90). All industrial cheese samples were microbiologically safe as due to their low pH (3.85 to 4.13). These data could be useful for the better recognition of traditional Greek Tsalafouti cheese.

Keywords: Tsalafouti;cheese; industrial manufacture

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INTRODUCTION

Artisanalcheeses are frequently linked to a certain local region and are generallymanufacturedaccording to traditional methods in dairy farms, using simple equipment,with family members as a staff and withoutcertification or label. Their production is usually based on practical skills and empirical experience and they are mainly sold on local markets (Koutsoukis*et al.,* 2017). Theypreserve traditional knowledge as a cultural heritage and have economic impacts in maintaining local employment. With increased globalization and tourism, the various types of traditional products have begun to spread outside their regions of origin. In order to meet consumer demands, they should be produced in a largerscale. The shift from small-scale, local production to the large-scale production in a mechanized, often automated way characterizes their industrial manufacture. A major development in the production of industrial cheeseswas the application of scientific principles to their manufacture, such as the use of pasteurized milk, defined times, temperatures, starter cultures to control fermentation, specific ripening conditions and packing, which greatly improved their quality and safety. When blending traditional and industrial practices to control product quality, the result is standardized, flavor products that can be enjoyed by consumers yearround, everywhere (Johnson, 2017; Donelly, 2016; De RoestandMenghi, 2000).

Tsalafouti is a traditional Greek cheese resembling to fresh acid- and acid/rennet curd cheeses with spreadable texture and no gas openings. It can be consumed plain, in sweet, in savoury dishes or it can be used in various preparations such as cheesecakes, desserts and sauces. Its soft texture makes Tsalafouti, also, very suitable for snacking, a form of eating which is becoming more widespread and its ease of handling makes it attractive and convenientto consumers. The relative low fat content due to its high moisture levels can provide a healthy alternative to diet-consious consumers (Schulz-Collins and Senge, 2004; Lucey, 2003; Guinee *et al.,* 1993). The final cheese has pleasant organoleptic characteristics which are much appreciated by the consumers, resulting in an increase of Tsalafouti demand in recent years. Artisanal Tsalafouti is traditionally made from 'boiled' (90^{o}C) sheep's milk, in the mountains, at the end of the lactation period; after boiling, the milk is left to curdle(acidify) and ripen naturally by a diverse indigenous lactic acid bacteria (LAB) biota growing slowly at approximately 10^{o}C for 15-20 days and consisting mainly of mesophilic LAB(Pappa *et al.*, 2022a).In an attempt to standardize its artisanal manufacturing technology, Pappa*et al.* (2022b)successfully produced Tsalafouti in a semi-industrial way in the pilot-plant laboratory of the Dairy Research Department (Ioannina, Greece)using a mesophilic commercial lactic starter culture consisting of mixed *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*strains. After inoculation with the starter, the production method of semi-industrial Tsalafouti included a short equilibration step at 30^{o}C for 1 h, followed by a slow fermentation and ripening process at 10^{o}C for 10 to 30 days and then cold aerobic storage of the mature product at 2-4^{o}C for up to 90 days (Pappa *et al.,* 2022b). Although the artisanal Tsalafouti has been studied before (Koutoukis*et al.,* 2017; Pappa *et al.,* 2022a), to our knowledge there are no records regarding the characteristics of the industrialized product. Additionally, nowadays, Tsalafoutihas been submitted for registration as a PDO cheese (Ministry of Rural Development and Food, 2022). Therefore, in the present work, the biochemical, organoleptic and microbiological characteristics of commercial Tsalafouti cheesemanufactured in an industrial way were studied.

MATERIALS AND METHODS

Industrial production

Industrial Tsalafouti was producedfrom sheep milk in two dairy companies (that of Koutroumanos and that of Tsiamakis) located in Evrytania, Central Greece,at 2017. During manufacturea small quantity of rennet and salt were added andin each dairy company three replicates took place. Tsalafouti was left to ripen for 15 days at the dairy companies. At 15th day of ripening and storage, samples were sent from the companies to the Dairy Research Department for the analyses, in an ice box. Samples were checked to confirm temperature upon receipt (<10^{o}C) and for any damage due to shipment. Then, the cheese samples were transferred to cold rooms (2-4^{o}C) for storage up to 90 days.

Biochemical analyses

Moisture, fat, fat in dry matter (FDM), salt,ash and protein contents were analyzed as described by Bontinis *et al.* (2008). Acidity was performed using the method of Ling (1963). pH was measured with direct readings of the micro-pH 2001 meter (Crison, Barcelona, Spain).The fat-in-dry-matter (FDM) content was calculated by the formula: FDM% = F x 100/100 - M, where F: % fat of cheese and M: % moisture of cheese and the salt-in-moisture (SM) content by the

formula (Lawrence and Gilles, 1980): SM% = S% x 100/M, where S: % NaCl of cheese and M: % moisture of cheese.

Viscosity was measured with a viscometer (Brookfield Engineering Laboratories Inc, Massachusetts, USA; model RVT), at 4˚C, using No 7 spindle at a speed of 2.5 rpm. Viscosity values were derived from the maximum deflection of the needle on the scale after 1 min of shearing.

Proteolysis was assessed by measuring the nitrogen fractions.Water soluble nitrogen (WSN), nitrogen soluble in 5% phosphotungstic acid (PTA-N) and nitrogen soluble in 12% trichloroacetic acid (TCA-N) were determined as described by Mallatou*et al.* (2004). Total nitrogen (TN) content was determined using the Kjeldahl method (IDF, 1986).

For the study of volatile compounds, the Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometer (SPME-GC-MS) analysis (model GCMSQP2010, Shimadzu, Tokyo, Japan) as described by Kondyli*et al.* (2016) was made. Identification was done by comparing ion spectra and retention times with authentic standards when available and spectra from the mass spectral library NIST05 (Gaithersburg, MD, USA). Calculation was performed by integrating the peak areas (arbitrary units) of total ion chromatograms (TIC) using the Shimadzu GCMS Solution software (Shimadzu, Tokyo, Japan).

All materials used were of analytical grade and all analyses were carried out in duplicate.

Organoleptic evaluation

Samples were assessed organoleptically by a team of five members of the permanent staff of the Dairy Research Department who were familiar with dairy products. Panelists evaluated the appearance, body-texture and flavour of Tsalafouti on a 1 (poor) to 10 (excellent) point scale and were asked to notice any defects such as the existence of rancid flavour or gas holes etc. Importance was given predominantly to the attributes of flavour and body-texture over the appearance (IDF, 1987). Thus, the scores obtained for these two attributes were multiplied by 5 and 4, respectively. The total score was obtained by adding the scores of the three attributes and it described the overall acceptability.

Microbiological analyses

On each sampling day, 25 g cheese sample were aseptically transferred in a sterile stomacher bag and homogenized with 225 mL of 0.1% (w/v) buffered peptone water (BPW) in a stomacher (Lab Blender 400, Seward, London, UK) for 60 s at room temperature. Serial decimal dilutions in 0.1% BPW were prepared, and duplicate 1 mL or 0.1 mL samples of appropriate dilutions were poured or spread on the total or selective agar plates. All diluents, microbiological enumeration agar media and supplements were purchased from Neogen Culture Media (formerly Lab M; Heywood, Bury, UK).

Microbial quantification analyses were conducted as reported by Pappa et al. (2022b). Selection of appropriate enumeration agar media and incubation conditions were according to procedures justified during our previous microbiological ecology studies on traditional Greek Galotyri PDO and Galotyri-like cheeses (SamelisandKakouri 2019; Samelis*et al.,* 2021). Total viable bacteria (TVC) were counted on Milk Plate Count agar (MPCA), incubated at 37^{o}C for 48-72 h. Total mesophilic and thermophilic LAB populations were separated on MRS agar incubated at 30^{o}Cfor 48-72 hand at 45^{o}Cfor 24-48 h, respectively. Mesophilic and thermophilic dairy (lactose-fermenting) LABwere counted on M17 agar incubated at 22^{o}C for 48-72 h and at 42^{o}C for 48 h, respectively. Enterococci were selectively enumerated on Slanetz and Bartley (SB) agar, incubated at 37^{o}C for 48 h. Coliforms were counted by pouring 1 mL samples into melted (45^{o}C) Violet Red Bile (VRB) agar, overlayed with 5 mL of the same medium and incubated at 37^{o}C for 24 h. Total staphylococci were enumerated on Baird-Parker (BP) agar base with egg yolk tellurite, incubated at 37^{o}C for 48 h.Yeasts and molds were selectively enumerated on Rose Bengal Cloramphenicol (RBC) agar, incubated at 25^{o}C for 5 days. The lowest detection limit of all microbial quantifications was set at 100 CFU/g, except of the coliform bacteria quantified by pouring at 10 CFU/g. When required, the electivity of the SB, BP and RBC agar media was checked, according to previously described procedures (SamelisandKakouri 2019; Samelis*et al.,* 2021; Pappa et al., 2022b).

Statistical analysis

One way analysis of variance was carried out to determine the effect of ripening and storage time on the different parameters studied in the six batches of Tsalafouti cheese. Least square difference (LSD) test was applied for comparison of means at the 95% confidence level ($P<0.05$) using software Statgraph-

ics Plus for Windows v. 5.2 (Manugistics, Rockville, Maryland, USA).

RESULTS AND DISCUSSION

Biochemical analyses

The physicochemical characteristics of Tsalafouti cheese at different sampling dates are shown in Table 1. From this table it can be seen that moisture content was <75% and fat >10% therefore according to the Greek legislation it is classified as a fresh, soft and spreadable cheese (Greek Codex Alimentarius, 2009).

All physicochemical characteristcs remained stable (P>0.05) during storage (Table 1). The pH values ranged from 3.85-4.13 and acidity from 2.02-1.88% at day-15 and day-90, respectively. Similar pH values were observed for XygaloSiteiasspreadable soft cheese (Danezis*et al.,* 2020).

Moisture content ranged from 69.06-68.37%; similar values were found for Anevato(68%) soft cheese. Fat content ranged from 14.03-14.28% and FDM from 45.19-45.09% at the beginning (15th day) and at the end (90th day) of storage. Lower fat values were found at Galotyri (10.9%), KatikiDomokou(11.3%) spreadable cheeses but higher at Anevato(16.5%) soft cheese and PhichtogaloChanion (19.6%). Similar FDM values to this study were found at KatikiDomokou(45.3%),lower at Galotyri(41.9%) and XygaloSiteias (40.6%) (Danezis*et al.,* 2020) and higher at PichtogaloChanion (Papageorgiou et al 1998). The different technological parameters may explain the above differences. The salt content ranged from 1.81-1.59% and was similar to that of XygaloSiteias (1.62%) and Galotyri (1.6%) cheese (Katsiari*et al.,*2009).

The ash content was similar to that of fresh goat's milk cheese (Asteri*et al.,* 2010) and Galotyri cheese (Katsiari*et al.,* 2009). The protein content were 10.92-11.08%; similar to Quark-type (10.97-12.18%) as reported by Lepesioti*et al.* (2021) and to Kopanisti soft cheese, but higher to Galotyri(8.24%) and KatikiDomokou(8.54%) as found by Danezis*et al*. (2020).

The viscosity values for artisanal Tsalafouti that was produced without the addition of cultures or rennet ranged from 12-47 (mPa*s)x1000 (Pappa *et al.,* 2022a), whereas the values for semi-industrial Tsalafouti manufacture with the starter culture were 140-133 (mPa*s)x1000 (Pappa *et al.,* 2022b) at 10 and 90 days, respectively. In the present work, with the addition of rennet, the respective values were 340-392 (mPa*s) x 1000 (Table 1). It is known that when a small amount of rennet is used in the production, it gives firmer texture of fresh acid-curd cheeses (Guinne*et al.,*1993).

Table 2 shows the mean values for TN, WSN, nitrogen soluble in 12% TCA (TCA-N) and nitrogen soluble in 5% PTA (PTA-N) expressed as a % of TN during storage of Tsalafouti cheese. The formation of soluble nitrogen compounds during cheese ripening

Table 1. Physicochemical parameters of industrial Tsalafouti cheese during ripening and storage(Means of six manufacturing trials ± Standard Error)

Age (days)	pH	moisture, %	fat, %	fat in dry matter, %	salt, %	salt in moisture,%	acidity, %	viscosity, cP (mPa*s) × 1000	ash, %
15	3.85±0.16a	69.06±1.09a	14.03±0.91a	45.19±1.38a	1.81±0.13a	2.62±0.15a	2.02±0.18a	340±71a	2.43±0.21a
30	4.05±0.11a	68.06±1.04a	14.9±0.75a	46.52±1.03a	1.55±0.23a	2.26±0.31a	1.92±0.19a	385±42a	2.4±0.14a
45	4.08±0.13a	68.20±1.10a	15.05±0.79a	47.19±1.12a	1.33**±0.**08a	1.96**±0.**08a	1.80±0.16a	397±62a	2.42±0.14a
60	4.16±0.12a	68.84±1.11a	14.68±0.70a	47.07±1.2a	1.39±0.18a	2.01±0.27a	1.77±0.16a	354±33a	2.38±0.15a
90	4.13±0.14a	68.37±0.88a	14.28±0.56a	45.09±0.75a	1.59±0.09a	2.32±0.10a	1.88±0.17a	392±33a	2.42±0.13a

a: Means of each parameter within the same column without different letters do not differ significantly (LSD test, p>0.05)

Table 2. Proteolysis of industrial Tsalafouti cheese during ripening and storage (Means of six manufacturing trials ± Standard Error)

Age (days)	TN*, %	WSN %TN	TCA %TN	PTA %TN	Proteins, %
30	1.71±0.05a	8.04±0.76a	6.64±0.52a	1.97±0.15a	10.92±0.29a
60	1.67±0.04a	9.39±0.98a	7.90±0.66a	1.85±0.09a	10.63±0.26a
90	1.74±0.03a	9.55±0.83a	7.58±0.54a	1.90±0.21a	11.08±0.19a

a: Means of each parameter within the same column without different letters do not differ significantly (LSD test, p>0.05)
*TN: Total nitrogen, WSN: Water soluble nitrogen, TCA: nitrogen soluble in 12% trichloroacetic acid, PTA: nitrogen soluble in 5% phosphotungstic acid

and storage is an index of the rate and extent of proteolysis. The WSN contains serum proteins, medium molecular mass peptides and amino acids (Christensen*et al.,*1991). High and medium molecular mass peptides and caseins are gradually broken down by rennet and starter culture enzymes to lower molecular mass peptides and amino acids (O' Keeffe*et al.,* 1976) which are soluble in 12% TCA. Low molecular mass peptides (<600 Da) and amino acids (except lysine and arginine) present in cheese are soluble in 5% PTA (Jarrett*et al.,* 1982). From table 2, it can be seen that no significant differences were observed during storage. The TN ranged from 1.71-1.74%, the WSN from 8.04-9.55 %TN, the TCA from 6.64-7.58%TN and the PTA from 1.97-1.90%TN at the 30th and 90th day respectively.Lower WSN values were reported for Galotyri cheese (Kondyli*et al.,* 2008) and for Quark-type cheese (Lepesioti*et al.,* 2021). Also similar WSN values to this study but lower TCA and PTA were observed for fresh, soft, acid-curd Xinotyri cheese (Pappa *et al.,* 2017). The above differences could be attributed to differences in processing prameters.

The thirty seven volatile components identified in the headspace of the 30-day Tslafouticheese and classified by chemical families (ketones, esters, alcohols, aldehydes, hydrocarbons, free fatty acids and terpenes) are listed in Table 3. From this table, it can be seen that alcohols, followed by ketones, esters and aldehydes were found in abundance.The compounds 3-methyl butanol, acetoin ethanol, acetone, were found in high levels of Tsalafouti, at 30 days of ripening and storage. Other compounds found in significant levels were ethylacetate, phenylethyl alcohol, hexanal and heptanal (Table 3). It is known that ethanol is produced by the fermentation of lactose or citrate (Marilleyand Casey, 2004) or from alanine catabolism (Castillo *et al.,* 2007). The 3-methyl bu-

Table 3. Volatile compounds (peak area x10^3) of industrial Tsalafouti cheese at 30-day of ripening and storage (Means of six manufacturing trials ±Standard Error)

KETONES	
Aceton	22578.4±7347.66
Acetoin	30611.6±10832.3
2 Nonanone	2612.23±841.99
Total ketones	55802.23±15761.6
ESTERS	
EthylAcetate	19305.5±9005.61
Butanoicacidethylester	630.22±158.30
1 Butanole 3 methylacetate	2261.83±650.74
Octanoicacidethylester	1385.08±303.68
Pentanoic acid 2 hydroxy 4 methyl , methyl ester	12945.6±351.66
Aceticacid 2 phenylethylester	8646.3±2572.56
Total esters	45174.53±5065.12
Alcohols	
Ethanol	29396.5±10518.5
1 propanol 2 methyl	7160.5±547.26
1Penten 3 ol	515.6±63.61
1 Butanol 3methyl	88850.1±22645.7
1 Pentanol	6437.28±1084.53
2 Heptanol	1756.02±456.93
2,3 Butanediol	1350.4±125.9
1 Hexanol	4134.85±785.31
1 Octen 3 ol	1236.87±411.64
1 Heptanol	1888.47±124.56
1 Hexanol 2 ethyl	1126.02±163.67
Citronellol	881.87±275.37
Menthol	616.52±82.09
Octanol	695.15±204.12
Phenylethylalcohol	13788.9±889.22
Total alcohols	159835.05±36255.9

ALDEHYDES	
Butanal 3 methyl	4016.85±1259.68
Hexanal	17271.4±6036.75
Heptanal	16609.7±5369.19
Octanal	930.95±311.18
Nonanal	1813.68±427.62
Total aldehydes	40642.58±12740.3
HYDROCARBONS	
Dodecane	687.3±128.5
Tetradecane	545.63±175.92
2 Undecanone	503.67±15.79
Total hydrocarbons	1736.6±265.90
FREE FATTY ACID	
Acetic acid	1173.4±780.48
Hexanoic acid	4287.68±335.82
Octanoic acid	2378.13±210.0
Total FFA	7839.21±575.30
TERPENES	
D Limonene	1647.08±389.38
Caryophyllene	475.95±32.03
Total terpenes	2123.03±32.03
TOTAL VOLATILE COMPOUNDS	313153.2±37185.3

tanol may be derived through reduction of 3-methyl butanalformed via Strecker degradation from leucine (McSweeney and Sousa, 2000). Acetoin (3-hydroxy, 2-butanone) is formed from diacetyl (Fox and Wallace, 1997) or it may be synthesized from pyruvate, lactose or citrate by lactic acid bacteria(Crow, 1990). Acetone generally originates either from milk or is produced from the thermal degradation of β-ketoacids (McGugan *et al.,* 1968).The volatile compounds identified in the present study have been previously reported in other cheeses of the same variety (Kondyli*et al.,* 2013; Pappa *et al.,* 2017;Karali*et al.,* 2013).

Organoleptic evaluation

The results from the organoleptic evaluation of industrial Tsalafouti cheese are shown in Table 4. From this table it can be seen that from day-15 to day-45 scores for appearance, taste and total scores remained stable (P>0.05); whereas a slight variation was observed in texture from day-30 to day-45. Until day-45,Tsalafouti had a smooth appearance, thick consistency-texture, pleasant and refreshing, mildly acidic taste. Panelists also evaluated Tsalafouti cheese after the day-45 and observed that generally it was deteriorated a lot, especially regarding the taste having a bitter, metallic and unclean taste.

Microbiological analyses

The results of the microbiological analyses are shown in Table 5. The following points should be considered: (i) specific details regarding the cheese-making technology were not provided by the dairy plants,(ii) all industrial Tsalafouti samples had already been ripened in each dairy company for 15 days,(iii) all samples were well-acidified soft acid-curd cheese products with an average pH as low as 3.85 on day 15 (Table 1),(iv) thus, all industrial Tsalafouti samples were *a priori* safe, according to the current microbiological criteria for *Listeria monocytogenes*growth which is not supported in all ready-to-eat (RTE) foods with a pH equal or below 4.4, regardless of their water activity and moisture content values (EC Regulation 2073/2005).

The TVC of all industrial Tsalafouti samples on day 15 were well above 7.5 log CFU/g and consisted mainly of typical mesophilic LAB capable for growth on MRS/30°C and M17/22°C agar plates (Table 5). Dairy (lactose-fermenting) LAB capable for growth on M17/42°C agar plates, including presumptive thermophilic cocci (e.g.,*Streptococcus thermophilus*) and/or presumptive lactococci (e.g., several starter strains of *Lc. lactis*subsp. *lactis*) or mesophilic lactobacilli (e.g., starter or non-starter strains of *Lactiplantibacillusplantarum* and *Lacticaseibacilluscasei/*

Table 4. Organoleptic characteristics of industrial Tsalafouti cheese during ripening and storage (Means of six manufacturing trials ± Standard Error)

Age (Days)	Appearance(10)*	texture(40)	flavour(50)	total(100)
15	8.81±0.03a	35.09±0.11ab	41.23±0.37a	85.19±0.22a
30	8.86±0.06a	34.18±0.42a	37.74±2.47a	80.78±2.88a
45	9.02±0.07a	35.67±0.46b	40.42±2.74a	85.12±3.24a

**In each organoleptic characteristic, values in brackets show the maximum scores*

a-b: Mean values of each parameter in the same column with different letters are statistically different (LSD test, P<0.05).

Table 5. Microorganisms of industrial Tsalafouti cheese during ripening and storage(Means of six manufacturing trials ± Standard Error)

Cheese age (days)	Total viable count (TVC) (37°C)*	Total mesophilic LAB (30°C)	Total thermophilic LAB (45°C)	Total mesophilic dairy LAB (22°C)	Total thermophilic dairy LAB (42°C)	Enterococci (37°C)	Total staphylococci (37°C)	Yeasts (25°C)
15	7.84±0.49a	6.87±0.28a	6.26±0.87a	6.88±0.30a	6.43±0.83a	4.92±1.27a	2.21±0.13a	5.17±0.51a
30	7.54±0.40a	8.10±0.23b	6.99±0.71a	8.07±0.25b	7.31±0.68a	6.03±1.04a	2.85±0.11b	5.71±0.42ab
45	7.81±0.40a	8.10±0.23b	6.41±0.71a	8.20±0.25b	7.21±0.68a	5.87±1.04a	2.09±0.11a	6.82±0.42bc
60	7.96±0.40a	8.53±0.23b	5.62±0.71a	8.43±0.25b	6.78±0.68a	5.88±1.04a	2.00±0.11a	7.39±0.42c
90	7.75±0.40a	8.09±0.23b	5.32±0.71a	8.25±0.25b	7.11±0.68a	5.90±1.04a	2.00±0.11a	7.52±0.42c

a-c: Mean values of each parameter in the same column with different letters are statistically different (LSD test, P<0.05).
**Temperature values in bracket indicate the incubation/growth temperature of each different LAB or other microbial group enumerated on the corresponding agar media reported in the microbiological analyses section of the Methods.*

paracasei groups), as well as, thermophilic dairy lactobacilli able to grow on MRS/45°C,also were numerous (>6.0 to 7.0 log CFU/g) in most Tsalafouti samples on day-15(Table 5). On the contrary, mean levels of *Enterococcus* spp. were by 1.5 to 3.0 log CFU/g lower than other LAB and TVC counts. All industrial Tsalafouti samples contained very low viable levels (mean 162 CFU/g) of total staphylococci. Pathogenic staphylococci forming lecithinase zones on typical BP/37°C agar plates and coliform bacteria on VRB/37°C agar plates were below 100 CFU/g and 10 CFU/g, respectively, and were suppressed below their lowest detection limits during storage (data not tabulated). Conversely, on day 15, most industrially ripened Tsalafouti samples contained high levels of yeasts (mean 5.17 log CFU/g) (Table 5).

The most noteworthy microbiological finding was that only typical mesophilic LAB enumerated on MRS/30°C and M17/22°C managed significant growth increases (i.e., by at least 1.5 log CFU/g) during storage; their counts exceeded 8 log CFU/g on day 30 and remained viable at populations well above this high level throughout storage of industrial Tsalafouti samples for 90 days (Table 5). In contrast, neither the TVC on the skim-milk containing MP-CA/37°Cmedium nor any of the thermophilic LAB groups, inclusive of enterococci, managed significant growth increases during storage. The TVC on MP-CA/37°Cand the total dairy (lactose-fermenting) LAB on M17/42°C showed minor population changes, a result suggesting that most were thermophilic or probably mesophilic LAB species that could survive well, but had negligible or limited growth potential at low (<10°C) cheese ripening and storage temperatures. Conversely, typical thermophilic LAB on MRS/45°C showed moderate 10-fold decreases, whereas typical enterococci on SB/37°C showed moderate 10-fold increases during storage (Table 5); these findings suggested that all colonies on the MRS/45°C agar plates on days 30 to 90 of storage were enterococci, able for slow growth at low (<10°C) temperatures. Total staphylococci were suppressed at an approximate 100 CFU/g level throughout storage (Table 5). Yeasts showed major ($P < 0.05$) growth increases in all samples during storage; their final counts reached or exceeded the 7.5 log CFU/g level by late storage (day 60 to 90). Yeasts were beneficial for the organoleptic cheese attributes up to day 45, but were the primary microbial spoilage agent that reduced the sensory quality of industrial Tsalafouti after day45 of storage.

The above findings are in general agreement with the main microbiological findings reported in our

previous studies (Pappa *et al.,* 2022a, b). In specific, the indigenous microbiota of artisanal Tsalafouti was similarly dominated by mixed mesophilic LAB types whose numbers exceeded 8 log CFU/g on day-10 of ripening and remained high during storage (Pappa *et al.,* 2022a). Enterococci also increased at an approximate level of 6.5 log CFU/g, whereas thermophilic dairy lactobacilli were not found in artisanal Tsalafouti (Pappa *et al.,* 2022a). In this study, the high levels of mesophilic LAB were most likely representative of the commercial multi-strain cultures of the primary mesophilic starter species *Lactococcus lactis*used for industrial Tsalafouti production in both dairy companies. Indeed, Pappa *et al.* (2022b) clearly demonstrated an exceptionally high ability of several commercial starter strains of *Lc. lactis* subsp. *lactis* to grow competitively and predominate during semi-industrial production of Tsalafouti at low (10°C) fermentation and ripening temperatures. Notably, because of the very strict hygienic process conditions in our pilot plant, the semi-industrial Tsalafouti product was practically free (<100 CFU/g) of enterococci, staphylococci, gram-negative bacteria and mainly yeasts (Pappa *et al.,* 2022b) and generally it was of very limited biodiversity as compared to the present industrial samples, which contained much higher levels of enterococci and yeasts, most probably due to environmental contamination. However, based on the LAB quantification results in Table 5, neither the addition of yeasts in industrial Tsalafouti cheese milks as 'pre-ripening' agents nor the use of complex commercial mesophilic starters, consisting of industrial strains of *Lc. lactis* plus *Lactiplantibacillus plantarum* or *Lacticaseibacillusrhamnosus* with ability to grow at a wide temperature range of 10-45°C, or mixed mesophilic lactococcal strains with commercial *Streptococcus thermophilus*or *Pediococcus*starter strains, can be excluded. Samelis and Kakouri (2019) isolated a great diversity of the above LAB genera or species from commercial artisan-type GalotyriPDO cheeses and suggested that many of them could be commercial starter or protective culture strain constituents, as well as, members of natural cheese starters or indigenous non-starter LAB strains. Therefore, additional research based on LAB isolation and identification studies or metagenomic culture-independent approaches are required to study the microbial ecology and LAB species diversity in industrial Tsalafouti. Nevertheless, the results of the present study corroborate with those of previous studies regarding standardization of Tsalafouti and Galotyri PDO acid-curd cheese productions from 'boiled' or industrially pasteurized milk. Production of these two traditional cheeses should be primarity based on milk fermentation and ripening by mesophilic commercial starter LAB species, primarily *Lc lactis* subsp. *lactis* and*Lc lactis* subsp. *cremoris*(Katsiari*et al.,* 2009; Kondyli*et al.,* 2013; Samelis and Kakouri 2019; Pappa *et al.,* 2022b). In particular, Katsiari*et al.* (2009) compared four commercial starter cultures [two mesophilic (MA01 1 and Probat 222), one thermophilic (CH-1) and one mixed thermophilic/mesophilic (CHOOZIT MT 1)] for a similar semi-industrial production of Galotyri-type cheeses. The above authors concluded that high quality Galotyri could be produced by using any of the four commercial cultures, although the plain mesophilic MA01 1 culture, consisting of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, gave cheese with the most consistent flavor and overall quality during storage (Katsiari*et al.,* 2009). The predominance and/or preferential use of mesophilic *Lc. lactis* starter strains has also been reported for other types of traditional acid-curd Greek cheeses, like AnevatoPDO (Xanthopoulos*et al.,* 2000) and soft Xinotyri (Pappa *et al.,* 2017) cheeses. The preferential use of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*starter strains with a profound ability for growth in the temperature range of 10-15°C is particularly important in industrial Tsalafouti production because the milk for making this cheese is traditionally fermented and ripened at slow rates at lowered temperatures.

CONCLUSION

In this work, Tsalafouti cheese was manufactured industrially and its biochemical, organoleptic and microbiological characteristics were determined. At 30 days of storage, its moisture content was 68.06%, fat 14.9%, salt 1.55%, ash 2.4%, proteins 10.92%. Its level of proteolysis did not change significantly during storage. The volatile compounds 3-methyl butanol, acetoin ethanol, acetone, were found in abundance. During the organoleptic evaluation it was very much appreciated until the 45th day. Typical mesophilic starter or non-starter LAB types predominated in all industrial samples, which all were microbiologically safe and stable for 45 days of storage. The results of the present study may provide consumers with useful information regarding Tsalafouti cheese and can represent a valid tool for its promotion, label protection and probable certification as a food of Protected Designation of Origin.

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CONFLICT OF INTEREST STATEMENT

We have no conflict of interest to declare.

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