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Original article

Technological, microbiological and chemical characteristics of Pannerone, a traditional Italian raw milk cheese

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Abstract – Pannerone cheese is a cow raw milk cheese obtained without starter and salt addition, which can be classified as a semi-soft cheese with a high fat content. Prolonged holding of moulded curds at warm room temperatures (~ 30 °C) was identified as having a key role in the cheese microbiological and chemical composition. Microbial populations were numerous and diverse with high numbers of lactic acid bacteria and coliforms. Pathogenic bacteria were not detected, while propionic bacteria and spores of lactate-fermenting clostridia were detected in all samples. Changes in microbial populations corresponded with key changes in chemical composition. During the first 2 days in the warm room at 30 °C, pH decreased to 4.9 and lactose was completely fermented to lactic acid. Over the next 4 days, metabolism of lactate to butyric (7.5 $g \cdot kg^{-1}$) and propionic (1.7 $g \cdot kg^{-1}$) acids with a consequent increase in pH to 5.7 occurred. The importance of the warm room temperature was demonstrated. The incubation of moulded curd had to be carried out at temperatures of about 30 °C to obtain the typical fermentation profile. The addition of lysozyme to the vat milk prevented the formation of high level of butyric acid typical of Pannerone cheese, thus demonstrating the involvement of lactate-fermenting clostridia in the production of butyric acid.

Pannerone cheese / clostridia / butyric acid / propionic acid / cheese-making technology

摘要 – 传统意大利生乳干酪 Pannerone 的加工、微生物及化学特性。 Pannerone 干酪是一种用生鲜牛乳经自然发酵不加盐的高脂肪半软质干酪。有资料显示成型的凝块长期放置在温暖的室温 (~ 30 °C) 下是干酪微生物和化学成分变化的关键原因。其中含有大量的、种类繁多的乳酸菌和大肠菌群。没有致病菌检出，但所有产品中均含有丙酸菌和梭状芽孢菌。微生物菌群的变化与主要化学成分的变化直接相关。 在最初的 2 天里，在室温下 pH 值下降到 4.9，乳糖完全发酵成乳酸。 接下来的 4 天中，乳酸代谢为丁酸 (7.5 $g \cdot kg^{-1}$) 和丙酸 (1.7 $g \cdot kg^{-1}$)， pH 值持续升高到 5.7。 同时，还证实了温暖室温的重要性。 凝块必须放置在 30 °C 下，才能获得典型的发酵特性。 将溶菌酶添加到原料乳中阻止了高含量典型的 Pannerone 干酪丁酸的形成，由此也证实了梭状芽胞杆菌能发酵乳酸盐产生丁酸。

Pannerone 干酪 / 梭状菌 / 丁酸 / 丙酸 / 干酪制作技术

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Résumé – Caractéristiques technologiques, microbiologiques et chimiques du Pannerone, un fromage italien traditionnel au lait cru. Le Pannerone est un fromage au lait de vache cru obtenu sans ajout de levain ni de sel, qui peut être classé comme fromage à pâte mi-molle à teneur élevée en matière grasse. Le maintien prolongé des caillés moulés en chambre chaude (~ 30 °C) a été identifié comme ayant un rôle clé sur la composition microbiologique et chimique du fromage. Les populations microbiennes étaient nombreuses et diverses avec des nombres élevés de bactéries lactiques et de coliformes. Les bactéries pathogènes n'étaient pas détectées alors que les bactéries propioniques et les spores de clostridia fermentant le lactate étaient détectés dans tous les échantillons. Les changements dans les populations microbiennes correspondaient à des changements clés dans la composition chimique. Au cours des 2 premiers jours en chambre chaude à 30 °C, le pH descendait à 4,9 et le lactose était complètement fermenté en acide lactique. Les 4 jours suivants, le métabolisme du lactate en acide butyrique (7,5 $g \cdot kg^{-1}$) et en acide propionique (1,7 $g \cdot kg^{-1}$) et une augmentation conséquente du pH à 5,7 avaient lieu. L'importance de la température en chambre chaude était démontrée. L'incubation du caillé moulé devait se faire à des températures d'environ 30 °C afin d'obtenir le profil de fermentation typique. L'addition de lysozyme au lait de fabrication empêchait la formation de niveaux élevés d'acide butyrique typique du fromage Pannerone, démontrant ainsi l'implication des clostridia fermentant le lactate dans la production de l'acide butyrique.

fromage Pannerone / clostridia / acide butyrique / acide propionique / technologie fromagère

1. INTRODUCTION

Pannerone cheese is traditionally produced near Lodi (Italy), nowadays only in two dairies. This cheese has a cylindrical shape, with a diameter of 28–30 cm, a height of about 20 cm and a weight of 10–13 kg. Pannerone is an unsalted cheese obtained with unrefrigerated cow raw milk and without the use of lactic starter culture. Its production is characterized by milling of the curds by hand before moulding and by a prolonged holding of the moulded curd in a warm room at a temperature of about 29 ± 2 °C up to 7 days. This is then cooled to 4–10 °C, and it is ready for consumption after 10–12 days from the start of cheese-making, but it can be stored for at least 2 months [22, 26]. The interior of the cheese has a sponge-type structure, with a large number of eyes of different sizes produced by heterofermentative lactic acid bacteria (LAB), yeasts, *Enterobacteriaceae* [23] and propionic acid bacteria (PAB) [5]. Ottogalli et al. [23] did not isolate butyric clostridia from Pannerone cheese, but their study did show that within 8 days of cheese-making the butyric acid was 79.4% of the sum of the volatile-free fatty acids.

The chemical composition of Pannerone cheese is characterized by moisture, fat, protein and ash contents of about 50%, 26%, 21% and 2.4%, respectively [26, 29]. The pH value ranges from 5.4 to 5.8 [5, 23], but it is unknown if these pH values are due to cessation or inhibition of lactic acid fermentation or due to lactate utilization by non-LAB microflora, for example PAB, yeasts or moulds. No data are known about the carbohydrate and organic acids contents of Pannerone cheese.

The aim of this work was to relate the microbiological and chemical composition of Pannerone cheese to its particular cheese-making technology, demonstrating both the key role of the prolonged stay of the curd in the warm room and that of lactate-fermenting clostridia in the complex fermentation of this cheese.

2. MATERIALS AND METHODS

2.1. Pannerone cheese samples

Twenty-six Pannerone cheeses ready for consumption were sampled directly at the dairy or from retail and were analysed for microbiological and chemical compositions.

2.2. Microbiological analyses

Ten grams of cheese were mixed in 90 mL of sterile 2% trisodium citrate buffer, pH 7.5, and blended for 2 min with a Stomacher 400 Circulator (PBI, Milan, Italy). Decimal dilutions of the stomacher fluid were made using sterile quarter-strength Ringer's solution as diluent. The dilutions were used for the enumeration of bacteria by conventional microbiological methods.

Lactococci were enumerated on M17 agar (Merck, Darmstadt, Germany) after incubation at 37 °C for 48 h. Lactobacilli were enumerated on deMan Rogosa Sharpe (MRS) agar (Merck), under anaerobic conditions, after incubation at 37 °C for 48 h. Heterofermentative LAB were determined on MRS broth with Durham's tubes at 37 °C for 48 h, on the basis of the last dilution which showed gas formation in the tube. Enterococci were enumerated on kanamycin aesculin azide (KAA) agar (Merck) after incubation at 42 °C for 48 h. Yeasts and moulds were enumerated on yeast glucose chloramphenicol (YGC) agar (Merck) after incubation at 25 °C for 5 days. Lipolytic bacteria were enumerated on tributyrin agar (Oxoid, Milan, Italy) at 30 °C for 72 h. Coliforms and *Escherichia coli* were enumerated on plates of Coli-ID agar (Biomérieux, Rome, Italy), after incubation at 37 °C for 24–48 h. Coagulase-positive staphylococci were enumerated on plates of Baird Parker RPF agar (Biomérieux) after incubation at 37 °C for 48 h. PAB were enumerated on Pal Propiobac agar (Laboratoires Standa, Caen, France) after incubation at 30 °C for 6 days under anaerobic conditions [27]. Enumerations of anaerobic lactate-fermenting clostridia were carried out on tubes of Bryant Burkey Modified Broth (BBMB) (Biolife, Milan, Italy) using the most probable number counting technique. After heat treatment at 80 °C for 15 min, to kill vegetative cells and activate spores, 1-mL aliquots of sample or dilution were inoculated into each of the three tubes containing 9 mL of BBMB broth. After inoculation, the tubes were sealed by addition of a layer of paraffin (about 15 mm thick), incubated at 37 °C for 7 days and observed for gas production [8]. Detection of pathogens were carried out on 25 g of sample. *Salmonella* spp. were detected by (i) pre-enrichment in 225 mL buffered peptone water (Merck) at 37 °C for 24 h, (ii) selective enrichment of 0.1 mL of pre-enrichment culture in 10 mL of Rappaport-Vassiliadis (RV, Merck) broth at 42 °C for 24 h and (iii) selective isolation by streaking 10 μL of RV in duplicate onto plates of Rambach agar (Merck) at 37 °C for 24 h. *Listeria monocytogenes* was detected by (i) primary enrichment in 225 mL of buffered Listeria enrichment broth (BLEB, Oxoid) at 30 °C for 24 h, (ii) secondary enrichment of 0.1 mL of the primary enrichment culture in 10 mL of fresh BLEB at 30 °C for 24 h and (iii) selective isolation by streaking 10 μL of primary and secondary enrichment in duplicate onto plates of Agar Listeria according to Ottaviani and Agosti (ALOA, Biolife) at 37 °C for 24–48 h. *E. coli* O157:H7 was detected by (i) enrichment in 225 mL of tryptone soya broth (TSB, Oxoid) with 20 $mg \cdot L^{-1}$ of novobiocin at 41 °C for 24 h and (ii) selective isolation by streaking 10 μL of enrichment in duplicate onto plates on O157:H7 ID with cefixime-tellurite supplement (Biomérieux) at 37 °C for 24 h.

2.3. Chemical analyses

The moisture, ash, fat and protein contents of the cheeses were determined according to IDF Standards 25/1964, 27/1964, 105/1981 and 4A/1982 [14–17], respectively. The lactose, galactose, citric acid, lactic acid, acetic acid, propionic acid and butyric acid contents of the cheeses were determined according to the HPLC method described by Bouzas et al. [6] for the simultaneous analysis of sugars and organic acids in cheese, using an Aminex HPX-87 column (Bio-Rad Laboratories, Richmond, CA, USA) and ultraviolet and refractive index detectors in series. Samples for HPLC analysis were prepared according to Bouzas et al. [6]. Twenty-five millilitres of 0.009 N H_2SO_4 were added to 5 g of ground cheese and extracted for 1 h while mixing using a magnetic stirrer. The extract was centrifuged at 3000× *g* for 10 min. The supernatant was filtered through a 0.45-μm regenerated cellulose membrane syringe filter (Econofilter, Agilent Technologies, Germany). The pH was measured by means of a pH meter (Portamess 913, Knick Elektronische, Berlin, Germany).

2.4. Monitoring of technological parameters

Ten cheese-makings were performed at the Carena Dairy (Caselle Lurani, Lodi, Italy) and the main technological parameters used were recorded. To better understand their dynamics, for two of ten cheese productions temperature and pH were measured during the first 8 days of production with a frequency of one measure every 30 min, by means of a pH meter (Portamess 913) and temperature data logger (Testo 177-T3, Testo SpA, Settimo Milanese, Italy) equipped with a negative temperature coefficient thermistor probe (Testo 0613 2211). Samples of milk, curd before moulding and cheese after 1, 4, 8, 12 and 32 days from cheese-making were sampled to determine their microbiological and chemical characteristics.

2.5. Influence of the warm room step on Pannerone cheese quality

Cheese-making trials were performed at the experimental dairy of CRA-FLC using an incubator to simulate warm room temperatures, able to contain two moulds of Pannerone, with the air temperature regulated at 4, 20, 25, 30 (control) and 38 °C. Each trial was carried out in duplicate for all the temperatures of incubation. Temperature and pH of cheese were monitored. Samples of milk and curd were analysed after 24 h and 6 days to assess their microbiological and chemical characteristics.

2.6. Influence of lactate-fermenting clostridia on Pannerone cheese quality

To demonstrate the hypothesis that butyric acid is mainly produced by lactate-fermenting clostridia, duplicate cheese-making trials were undertaken in which lysozyme was added to milk at a rate of 50 $mg \cdot L^{-1}$ (Chr. Hansen Ltd., Parma, Italy). The cheese was produced according to its traditional procedure and the curd was held at 30 °C for 6 days. Temperature and pH of cheese were monitored every 30 min and samples were analysed after 6 days to assess their chemical characteristics.

2.7. Statistical analyses

The chemical composition of cheese according to different warm room temperatures and lysozyme addition was analysed using one-way analysis of variance via Microsoft Office Excel 2003 (Microsoft Corp., USA). Differences between treatments were considered significant at the level of $P < 0.05$.

Table I. Microbiological characteristics (CFU·g^{-1}) of 26 samples of Pannerone cheese.

Bacteria	Mean	SE[a]	Median	SD[b]
Coliforms	6.9×10^6	4.7×10^6	1.9×10^3	2.4×10^7
Escherichia coli	4.5×10^6	4.0×10^6	3.7×10^4	2.1×10^7
Coagulase-positive staphylococci	1.6×10^2	1.3×10^2	1.0×10^{-1}	6.8×10^2
Enterococci	3.8×10^6	1.7×10^6	7.4×10^5	8.7×10^6
Cocci-shaped LAB	7.9×10^7	3.1×10^7	2.5×10^7	1.6×10^8
Rod-shaped LAB	1.7×10^8	5.3×10^7	1.2×10^8	2.7×10^8
Heterofermentative LAB	2.1×10^7	1.1×10^7	1.0×10^7	5.5×10^7
Yeasts	3.7×10^5	2.3×10^5	1.8×10^3	1.1×10^6
Moulds	5.4×10^5	4.8×10^5	1.8×10^2	2.5×10^6
Lactate-fermenting clostridia spores	1.9×10^5	8.9×10^4	1.6×10^4	4.0×10^5
Propionic acid bacteria	9.0×10^5	5.3×10^5	3.6×10^5	2.1×10^6
Lipolytic bacteria	Absent in 1 g sample			
Listeria monocytogenes				
Salmonella spp.	Absent in 25 g sample			
E. coli O157:H7				

[a] SE, standard error.
[b] SD, standard deviation.

3. RESULTS AND DISCUSSION

3.1. Microbiological characteristics of Pannerone cheese

The microbiological analyses of 26 Pannerone cheeses (Tab. I) ready for consumption showed that pathogenic bacteria, i.e. *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7, were absent. Forty-six *E. coli* strains isolated from six Pannerone cheeses of the present study were also studied by Zago et al. [30] to determine the presence of genes codifying for virulence factors. This study confirmed the absence of both O157:H7 and other enteropathogenic strains. Total coliforms were mostly *E. coli*, with median values, respectively, of 1.9×10^3 and 3.7×10^4 CFU·g^{-1} but with a very large variability. This large variability is common in artisanal raw milk cheeses as described by Freitas and Malcata [13]. Coliforms were considered important for the sensorial properties of Pannerone cheese [23], because of their ability to produce gas and aromatic compounds such as formic and acetic acids, and 2,3-butanedione and to utilize citrate [9]. Counts of coagulase-positive staphylococci were usually low. The predominant microflora were LAB, mainly lactobacilli, present at levels of 10^8 CFU·g^{-1}, while the counts of cocci were 1 log lower. Among the other gas-producing bacteria that can be involved in the formation of the typical sponge-like structure of Pannerone cheese, heterofermentative LAB showed the highest counts, followed by PAB and lactate-fermenting clostridia spores, in that order (Tab. I). Yeasts, traditionally considered an important flora of Pannerone cheese [5, 23], showed a lower count than expected (mean value of 3.7×10^5 CFU·g^{-1} and median value of 1.8×10^3 CFU·g^{-1}). No lipolytic bacteria were detected in Pannerone cheeses.

3.2. Chemical composition of Pannerone cheese

The mean chemical composition of Pannerone cheese (Tab. II) showed that total solids content (592.3 ± 9.4 g·kg^{-1}) of the

Table II. Mean chemical composition ($g \cdot kg^{-1}$) of 26 samples of Pannerone cheese.

	Mean	SE[a]	Median	SD[b]
Total solids	592.3	1.9	590.6	9.4
Fat	334.0	2.9	336.0	15.0
Protein	235.6	1.9	235.4	9.5
Ash	22.6	0.5	23.4	2.7
Lactose	0.00	0.00	0.00	0.00
Galactose	0.01	0.01	0.00	0.03
Lactic acid	1.27	0.35	0.74	1.79
Acetic acid	0.69	0.07	0.72	0.37
Citric acid	0.00	0.00	0.00	0.00
Propionic acid	1.71	0.10	1.77	0.50
Butyric acid	7.49	0.29	7.64	1.49
pH	5.61	0.03	5.59	0.13

[a] SE, standard error.
[b] SD, standard deviation.

cheeses were higher than the value of 500 $g \cdot kg^{-1}$ previously reported [26, 29], and similar to that reported for Pannerone cheese ripened for 30 days at a temperature ranging from 8 to 10 °C instead of 4 °C [26]. Lactose, galactose and citric acid were detected but at negligible levels. The amount of lactic acid was very low (1.27 ± 1.79 $g \cdot kg^{-1}$) in comparison to cheeses other than mould ripened cheeses [22], with an average pH value of 5.61. High amounts of butyric acid (7.49 ± 1.49 $g \cdot kg^{-1}$) were detected. Butyric acid can be derived from lactate fermentation by butyric clostridia or from lipolysis, mainly by moulds or lamb paste rennet. Usually, butyric acid is present in cheese in lower amounts, even in defective cheeses such as hard cheeses cracked by an intensive late blowing. Bacci et al. [3] measured up to 1.8 $g \cdot kg^{-1}$ of butyric acid in blown Parmigiano Reggiano cheese. Experimental Emmental cheeses produced by adding *Clostridium tyrobutyricum* to milk had a butyric acid content of about 2.7 $g \cdot kg^{-1}$ [20]. Values of butyric acid similar to those measured in Pannerone were only found in a Turkish pickled white cheese after more than 6 months of ripening [2], but its origin was related to lipolysis. The amount of butyric acid in sheep raw milk Fiore Sardo cheese, obtained using lamb paste rennet ranged from 1.0 to 4.0 $g \cdot kg^{-1}$ [1, 24]. The butyric acid content was usually not higher than 2.6 $g \cdot kg^{-1}$ in Gorgonzola or other blue cheeses, produced with lipolytic *Penicillium roqueforti* as a starter [7, 10, 25]. In ripened Portuguese Serra ewe's cheese, the amount of butyric acid did not exceed 3.2 ± 1.8 $g \cdot kg^{-1}$ [21].

The level of propionic acid of Pannerone cheese (1.7 ± 0.5 $g \cdot kg^{-1}$, i.e. 4.2 ± 1.2 $g \cdot kg^{-1}$ when calculated on its moisture basis) was lower than that of ripened Swiss cheese (4.0–6.0 $g \cdot kg^{-1}$ of cheese on moisture basis) [4] or Emmental Grand Cru cheeses (8.0 $g \cdot kg^{-1}$ in the extracted aqueous phase). This level is similar to that of Comté cheese (4.9 $g \cdot kg^{-1}$ in the extracted aqueous phase), but higher than that produced by PAB in some industrial French Emmental (1.0–2.0 $g \cdot kg^{-1}$ in the extracted aqueous phase) [28]. The amount of acetic acid of Pannerone (minimum 0.17 $g \cdot kg^{-1}$ and maximum 1.83 $g \cdot kg^{-1}$) was similar to that

of other raw milk cheeses obtained without starter addition to milk, but significantly less than that recoverable in Emmental [28].

3.3. Pannerone cheese technology

Unrefrigerated raw milk was collected immediately after milking and transported to the dairy, where it was immediately transformed following the traditional technology (Tab. III) described by Savini [26] and Del Forno [11], with a few differences: a shorter renneting time (about 20 min compared to 45 min applied in the past [26]) and a shorter period in the warm room at 30 °C (6 days compared to 7–10 days previously described [11, 26]). Moreover, today the cheese is at retail within 1 week from cheese-making, while in the past the cheese was ripened at 8–10 °C for 8–10 days and it was considered mature after 20 days [26].

3.3.1. Monitoring of pH, temperature, microbial counts and chemical composition during Pannerone cheese-making

The changes of pH during the first 8 days from Pannerone cheese-making showed a specific behaviour (Fig. 1) characterized by a first phase where pH was substantially unchanged for 10 h, followed by a sharp decrease to pH 5.1–5.2 in the successive 10 h. The drop in pH continued up to 48 h from cheese-making reaching a minimum value of 4.9, and then the pH remained around that value for 40 h. After the fourth day from cheese-making, the pH started to rise up to 5.7. The pH drop and the successive increase occurred because the temperature of the cheese (28.4 ± 1.4 °C) firstly remained favourable to the growth of LAB and then, when milk sugars had been depleted, the same high temperature allowed the growth of lactate-fermenting bacteria, such as butyric clostridia and PAB.

The growth kinetics of the different microbial populations originating from raw milk and from the environment are summarized in Table IV. The lack of LAB starter was responsible for the delay in the start of acidification (Fig. 1). After 1 day from curd moulding, when the pH dropped to 5.1, cocci-shaped LAB were the most represented microflora, followed by total coliform bacteria. Heterofermentative LAB and enterococci grew at different speeds, according to the initial count in raw milk. *E. coli* reached their maximum values in the curd after 1 day (i.e. 10^8 CFU·g^{-1}) and remained substantially stable up to 30 days of storage of the cheese at 4 °C. Yeasts grew to 10^5 CFU·g^{-1}. During the step in the warm room, the cheese temperature of about 28 °C allowed the growth of homofermentative LAB, mainly rod-shaped, to continue until there was a complete depletion of lactose, galactose and citric acid and a maximum production of lactic acid (Tab. V) and pH drop (Fig. 1). After the eighth day from cheese-making, the count of coagulase-positive staphylococci decreased. On the contrary, at the same time PAB started their growth reaching their maximum value after coming out of the warm room. The level of PAB was substantially unchanged during the storage of cheese at 4 °C. The presence of the lactate-fermenting clostridia spores was detected only after more than 12 days from cheese-making, despite the large butyric acid amount present in the cheese after being in the warm room (Tab. V).

The growth kinetics of the different microbial groups are similar to those described previously by Ottogalli et al. [23], although they never found PAB and butyric clostridia in milk or in cheese. However, Bodini et al. [5] detected high levels of PAB both in the curd (6.8×10^7 CFU·g^{-1}) and in Pannerone cheese (3.8×10^8 CFU·g^{-1}).

The changes of the chemical composition of Pannerone cheese during the

Table III. Pannerone cheese-making technology.

	Mean	SD[a]
Milk acidity in the vat (pH)	6.6	0.0
Temperature of milk in the vat (°C)	30.0	0.0
Starter addition	None	
Liquid calf rennet (g·kg^{-1} of milk) (chymosin minimum 70%)	0.04	0.0
Renneting time (min)	20.2	4.0
Holding time between renneting and curd breaking – gel hardening (min)	5.4	1.7
Time of curd breaking (min)	20.2	7.5
Acidity of the whey at the curd extraction (°SH/50 mL)	2.6	0.0
Curd extraction from the vat	By means of linen cloths, each containing about 6 kg of curd, forming bundles superimposed one on top of the other	
Time used for curd extraction by hand with linen cloths (min)	15.7	2.6
Temperature of the extracted curd (°C)	27.3	0.5
Holding time of the curd in cloths for the first whey drainage, before transport in another room for the 2nd drainage (min)	9.7	4.1
Holding time of the curd before moulding (min)	23.7	5.7
Time used for curd milling by hand and moulding (min)	13.3	2.9
Temperature of the curd in the mould (°C)	25.2	0.4
Holding time of the moulded curd in the room for the 2nd whey drainage (h)	11.6	1.2
Temperature of the curd at the end of the holding time (°C) before the entry in warm room	27.7	1.5
Temperature of the warm room (°C)	29.8	0.8
Temperature of the curd after 48 h of rest in warm room (°C)	29.5	0.6
Extraction of the curd from the mould and wrapping with a wooden band	Usually after 48 h in warm room	
Holding time in the warm room (h) cheese wheels are turned upside down	111.4	14.1
	Every day	
Temperature of Pannerone cheese at the exit from the warm room (°C)	27.6	1.1
Temperature of the room for the pre-cooling of the cheese (°C)	18.2	1.5
Holding time for the pre-cooling step (h)	14.4	6.4
Temperature of Pannerone cheese at the end of pre-cooling (°C)	18.2	1.7
Holding time in refrigerated room at 5 ± 1 °C (h)	20.9	6.4
Salting of Pannerone (brining/dry)	None	
Storing of the cheese in the warehouse of the dairy at 6 ± 1 °C	< 1 week	
Shelf life	Up to 60 days	

[a] SD, standard deviation.

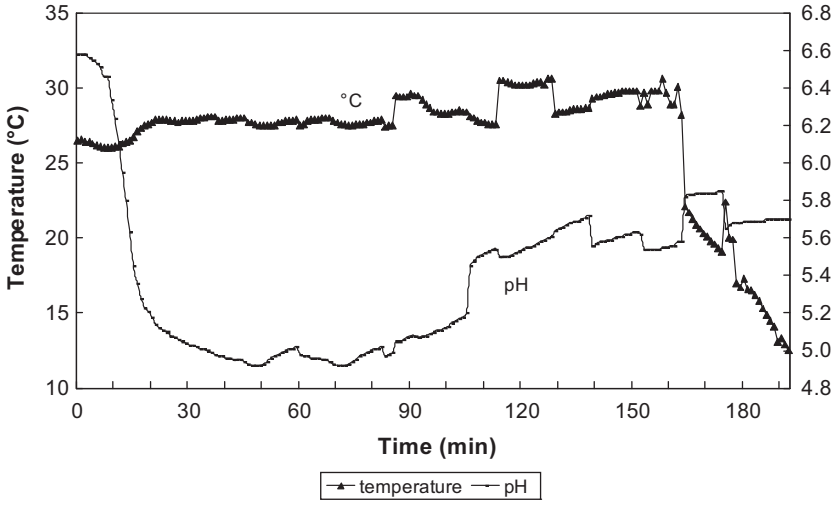

Figure 1. Evolution of temperature and pH during the first 8 days from cheese-making. *Note*: The fluctuations of pH and temperature values, mainly after the fourth day, correspond to the different positions of the probes when the cheese was turned upside down.

cheese-making and shelf life are summarized in Table V. The balance between lactose, galactose and lactic acid showed fast depletion of lactose and galactose by the fourth day from cheese-making and increase of lactic acid up to 15 $g{\cdot}kg^{-1}$. By the end of the warm room time, on the eighth day, citric acid was totally consumed, lactates were progressively metabolized to $< 2\ g{\cdot}kg^{-1}$ and the maximum production of butyric and propionic acids was observed. Lactate utilization was concomitant with increase in the pH of the cheese to values higher than 5.45 (Fig. 1). Acetic acid concentration showed a more complicated behaviour: it reached a maximum value during the warm room step, corresponding to the maximum growth of heterofermentative LAB and coliforms, then it decreased concurrently to butyric acid production, and finally it increased a second time. Differently from the other works on Pannerone cheese that underlined the importance of heterofermentative LAB and coliforms [5, 23], our data demonstrate that there was a fundamental role played by the lactate metabolism, and specifically by the lactate-fermenting bacteria, mainly butyric clostridia [18], whose growth was favoured by the maintenance of the cheese at 30 °C.

3.3.2. *Influence of the warm room step on Pannerone cheese quality*

While Swiss-type cheeses are usually kept at warm room temperatures (22–24 °C) after brining to enable the propionic fermentation [19], prolonged holding of unsalted curd at similar or higher temperatures is typical of few raw milk cheeses, among them Pannerone and Castelmagno. After extraction from the cheese vat the curd of Castelmagno is cut into large pieces and dipped into whey at room temperature up to 6 days, then it is milled, dry salted and moulded [12, 22]. To demonstrate that the specific time and temperature combination applied in the warm room is essential for Pannerone cheese, incubation trials were performed

Table IV. Microbial population changes (CFU·g^{-1}) during traditional Pannerone cheese production and storage. Trials were performed in duplicate.

Bacteria		Milk	Curd	Cheese				
				Warm room at 30 °C			Storage at 4 °C	
			Before moulding	1 day	4 days	8 days	12 days	30 days
Coliforms	Mean	7.2×10^2	3.6×10^3	2.6×10^7	4.0×10^4	1.4×10^4	1.0×10^4	1.0×10^1
	SD[a]	8.7×10^2	9.1×10^3	3.7×10^7	6.5×10^5	1.2×10^6	1.3×10^5	9.5×10^4
E. coli	Mean	2.0×10^2	5.0×10^1	2.9×10^5	1.7×10^5	1.5×10^5	5.0×10^4	7.3×10^4
	SD	1.4×10^2	1.4×10^4	3.4×10^5	4.7×10^5	9.8×10^4	7.8×10^4	1.5×10^5
Coagulase-positive staphylococci	Mean	1.5×10^2	1.5×10^2	6.9×10^4	8.8×10^3	1.0×10^2	1.0×10^1	1.0×10^1
	SD	2.0×10^2	1.3×10^4	6.8×10^4	1.4×10^4	6.4×10^2	6.0×10^2	1.3×10^1
Enterococci	Mean	1.7×10^1	1.3×10^3	2.8×10^5	5.5×10^5	1.1×10^6	1.4×10^6	2.5×10^5
	SD	4.7×10^2	1.2×10^4	4.3×10^6	6.4×10^6	3.0×10^7	4.5×10^7	4.3×10^6
Cocci-shaped LAB	Mean	1.2×10^4	2.4×10^5	4.7×10^8	1.2×10^9	3.5×10^8	1.7×10^8	2.0×10^7
	SD	8.2×10^5	1.1×10^8	1.4×10^9	1.4×10^9	8.9×10^7	8.5×10^8	6.0×10^7
Rod-shaped LAB	Mean	4.5×10^3	1.2×10^4	6.7×10^5	1.8×10^8	1.9×10^8	1.2×10^8	1.1×10^7
	SD	6.1×10^3	1.5×10^4	2.1×10^6	1.7×10^7	1.1×10^8	4.2×10^7	2.5×10^8
Heterofermentative LAB	Mean	1.0×10^4	1.0×10^5	1.0×10^7	1.0×10^7	1.0×10^7	1.0×10^7	1.0×10^6
	SD	1.2×10^4	1.3×10^5	1.4×10^7	1.1×10^7	1.3×10^7	4.2×10^7	5.7×10^6
Lactate-fermenting clostridia spores	Mean	2.4×10^2	3.6×10^0	3.6×10^0	2.3×10^1	2.3×10^1	7.5×10^1	2.1×10^4
	SD	3.4×10^2	3.8×10^0	7.9×10^0	0.0×10^0	6.2×10^2	2.9×10^2	2.1×10^4
Propionic acid bacteria	Mean	3.0×10^1	1.0×10^3	nd[b]	7.0×10^3	nd	nd	1.9×10^6
	SD	4.1×10^1	1.4×10^3	–	9.9×10^3	–	–	2.0×10^6
Yeasts	Mean	3.5×10^3	1.4×10^4	2.6×10^5	4.4×10^5	9.7×10^5	3.0×10^6	2.6×10^5
	SD	1.8×10^3	1.3×10^3	5.2×10^5	3.8×10^5	1.3×10^6	4.2×10^6	3.5×10^5
Moulds	Mean	4.8×10^2	1.4×10^3	3.5×10^2	6.0×10^4	5.0×10^4	4.1×10^5	2.6×10^5
	SD	4.7×10^2	1.1×10^3	2.3×10^4	3.5×10^4	8.5×10^3	4.7×10^5	3.6×10^5
Lipolytic bacteria				Not detected in 1 g of cheese				

[a] SD, standard deviation.
[b] nd, not determined.

Table V. Chemical composition ($g \cdot kg^{-1}$) during traditional Pannerone cheese production and storage. Trials were performed in duplicate.

		Milk	Curd	Cheese				
				Warm room at 30 °C			Storage at 4 °C	
			Before moulding	1 day	4 days	8 days	12 days	30 days
pH	Mean	6.60	6.55	5.18	5.10	5.70	5.69	5.68
	SD[a]	0.01	0.02	0.04	0.03	0.06	0.04	0.05
Total solids	Mean	130.4	461.5	516.1	572.5	579.9	588.6	609.4
	SD	5.8	43.7	23.5	3.2	0.7	2.1	10.1
Lactose	Mean	52.58	27.42	13.20	0.00	0.00	0.00	0.00
	SD	3.07	1.94	0.94	0.00	0.00	0.00	0.00
Citric acid	Mean	1.82	1.80	1.59	0.31	0.00	0.00	0.00
	SD	0.21	0.29	1.07	0.22	0.00	0.00	0.00
Galactose	Mean	0.00	0.02	0.17	0.00	0.00	0.00	0.00
	SD	0.08	0.02	0.02	0.09	0.03	0.08	0.04
Lactic acid	Mean	0.00	0.14	6.74	15.03	1.04	1.24	1.12
	SD	0.00	0.10	1.94	0.01	4.38	4.47	3.72
Acetic acid	Mean	0.00	0.00	0.41	1.16	0.33	0.48	0.83
	SD	0.00	0.00	0.54	0.34	0.16	0.28	0.71
Propionic acid	Mean	0.00	0.00	0.00	0.24	1.75	1.57	1.61
	SD	0.00	0.00	0.10	0.07	0.16	0.12	0.05
Butyric acid	Mean	0.00	0.25	0.24	0.92	8.12	7.66	7.53
	SD	0.00	0.18	0.01	0.38	1.36	0.89	0.61

[a] SD, standard deviation.

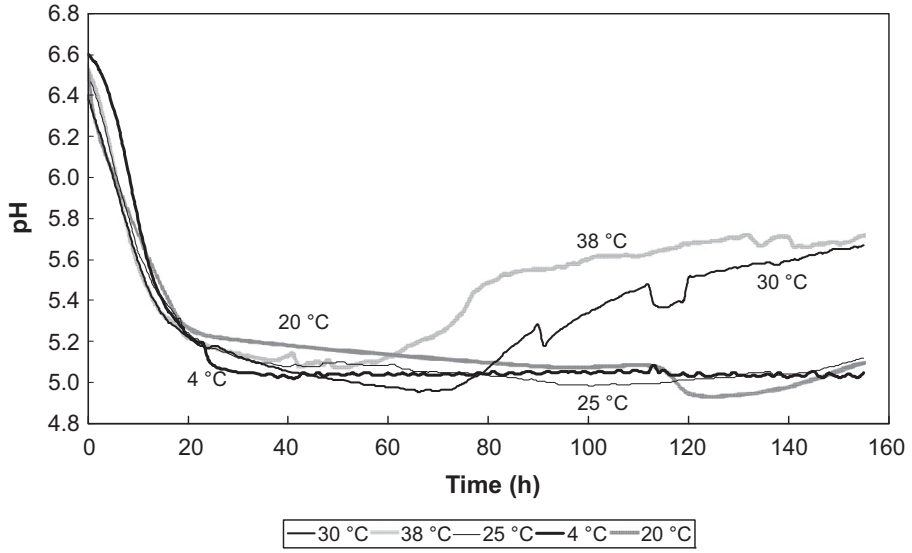

Figure 2. Evolution of pH during the first 6 days from cheese-making of experimental Pannerone cheeses incubated at 4, 20, 25, 30 (control) and 38 °C.

changing the temperature of the warm room. The curd, obtained on different days always using the same cheese-making technology, remained for about 7 h at a room temperature of 26 °C to allow as much draining of the whey as possible. Then the moulds were stored at different air temperatures (4, 20, 25 and 30 as control and 38 °C) for 6 days. Before incubating at 4 °C, the curd was kept at 30 °C for 32 h to allow lactic acid fermentation. The temperature of the curds reached equilibrium with the room temperature within 24 h from the start of the incubation. The pH dropped to about 4.95 (Fig. 2) within the first 60 h and the rate of acidification was substantially the same, in spite of the different temperatures. After this time, the pH changed greatly according to temperature: the pH increased to 5.5 or even to higher values when the cheese was stored at 30 or 38 °C. When the cheese was incubated at 4 °C, the usual ripening temperature for Italian soft or semi-soft cheeses that are not mould surface ripened, like Crescenza, Quartirolo or Italico cheeses [22], the pH did not increase. Cheese stored at 30 and 38 °C showed a significant pH increase compared to that observed at lower temperature. The higher pH values after 6 days corresponded to significant lower lactic acid and to higher propionic and butyric acid contents (Tab. VI).

The amounts of residual lactose, galactose and lactic acid after the first day from cheese-making were not significantly different in all the curds. Acetic acid was present at levels of about 1 $g \cdot kg^{-1}$, probably produced by the high number of coliform bacteria [9] (Tab. VII). Propionic and butyric acids were detected, but in small amounts. After 6 days, lactose was depleted in all the cheeses with the exception of that stored at 4 °C, and galactose was still present in traces. The concentration of lactic acid was inversely correlated to the temperature of the warm room, while that of propionic and butyric acids was positively correlated

Table VI. Chemical composition ($g \cdot kg^{-1}$) of Pannerone cheese after 1 day and 6 days of storage at different temperatures. Number of trials at temperature of 4, 20, 25, 30 and 38 °C were respectively 3, 3, 5, 3 and 3.

	4 °C		20 °C		25 °C		30 °C		38 °C	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 day										
Total solids	510.2[a]	3.1	516.5[a]	3.0	512.4[a]	19.7	540.5[b]	12.5	574.8[b]	12.5
Lactose	11.40	0.42	8.45	3.46	11.88	2.42	11.10	4.81	6.85	2.19
Galactose	0.30	0.00	0.20	0.14	0.25	0.06	0.15	0.07	0.30	0.14
Lactic acid	8.75	1.20	7.50	1.13	7.70	1.51	6.80	3.96	7.90	0.99
Acetic acid	0.75	0.35	1.10	0.14	1.03	0.37	1.20	0.00	1.20	0.28
Citric acid	0.60	0.85	0.00	0.00	0.30	0.60	0.05	0.07	0.00	0.00
Propionic acid	0.20	0.14	0.20	0.14	0.10	0.08	0.20	0.14	0.10	0.14
Butyric acid	0.05	0.07	0.20	0.14	0.28	0.24	0.25	0.07	0.30	0.42
pH	5.24	0.04	5.27	0.07	5.26	0.06	5.22	0.05	5.27	0.01
6 days										
Total solids	546.1[a]	7.1	552.9[a]	5.9	566.5[b]	11.0	579.8[b]	2.0	598.0[c]	9.5
Lactose	4.05[a]	0.49	0.00[b]	0.00	0.00[b]	0.00	0.00[b]	0.00	0.00[b]	0.00
Galactose	0.30	0.14	0.17	0.06	0.15	0.08	0.07	0.06	0.00	0.00
Lactic acid	13.15[a]	1.20	15.27[a]	1.58	9.40[a]	5.09	3.09[b]	1.84	0.60[c]	0.51
Acetic acid	0.90[a]	0.28	0.90[a]	0.26	0.81[a]	0.48	0.40[b]	0.17	0.29[b]	0.12
Citric acid	0.60[a]	0.85	0.00[b]	0.00	0.00[b]	0.00	0.00[b]	0.00	0.00[b]	0.00
Propionic acid	0.10[a]	0.14	0.30[a]	0.10	0.71[a]	0.73	1.27[b]	0.51	1.61[b]	0.50
Butyric acid	0.35[a]	0.07	1.14[b]	0.37	3.43[b]	2.60	6.29[c]	1.21	6.40[c]	1.18
pH	5.28[a]	0.25	5.27[a]	0.08	5.36[a]	0.36	5.69[b]	0.10	5.72[b]	0.08

[a, b, c] Different letters in the same row mean that the values are significantly different ($P < 0.05$).
Rows without superscripts did not present significant differences.

Table VII. Microbial counts (CFU·g^{-1}) of Pannerone cheese after 1 day and 6 days of storage at different temperatures. Number of trials at temperature of 4, 20, 25, 30 and 38 °C were respectively 3, 3, 5, 3 and 3.

Bacteria	4 °C		20 °C		25 °C			30 °C			38 °C	
	Mean*	SD[a]	Mean*	SD	Mean	Median	SD	Mean	Median	SD	Mean*	SD
1 day												
Coliforms	3.3×10^7	2.2×10^7	9.8×10^7	8.2×10^7	1.2×10^8	1.3×10^8	5.0×10^7	1.8×10^8	8.2×10^7	2.4×10^8	9.6×10^7	4.0×10^7
E. coli	9.8×10^5	7.4×10^5	3.6×10^8	5.0×10^8	1.3×10^7	3.3×10^6	2.2×10^7	1.3×10^8	1.3×10^6	2.6×10^8	2.6×10^7	3.6×10^7
Coagulase-positive staphylococci	5.1×10^2	7.0×10^2	1.1×10^4	1.4×10^4	1.7×10^6	1.1×10^5	3.3×10^6	5.9×10^3	1.4×10^3	1.0×10^4	4.3×10^6	5.7×10^6
Cocci-shaped LAB	2.4×10^8	3.4×10^8	1.1×10^9	3.9×10^8	9.9×10^8	8.9×10^8	6.5×10^8	6.8×10^8	6.3×10^8	6.0×10^8	6.0×10^8	8.8×10^7
Rod-shaped LAB	4.1×10^4	5.5×10^4	1.6×10^6	2.1×10^6	1.7×10^5	1.2×10^5	1.3×10^5	6.4×10^5	1.9×10^5	1.0×10^6	2.1×10^6	2.9×10^6
Heterofermentative LAB	1.0×10^4	0.0×10^0	4.1×10^5	5.6×10^5	2.8×10^6	5.5×10^5	4.8×10^6	4.6×10^5	2.5×10^5	6.2×10^5	5.1×10^6	7.0×10^6
Yeasts	1.6×10^5	6.0×10^4	7.0×10^6	7.9×10^6	4.6×10^5	3.4×10^4	8.7×10^5	3.7×10^6	4.2×10^5	6.8×10^6	4.0×10^4	2.7×10^4
Moulds	1.0×10^3	0.0×10^0	1.3×10^2	3.5×10^1	1.3×10^2	1.0×10^2	5.8×10^1	4.2×10^3	1.0×10^2	8.3×10^3	5.5×10^1	6.4×10^1
Lactate-fermenting clostridia spores	7.0×10^0	5.7×10^0	2.3×10^0	1.8×10^0	1.0×10^0	1.0×10^0	0.0×10^0	5.6×10^0	5.1×10^0	5.3×10^0	2.3×10^0	1.8×10^0
Propionic acid bacteria	1.3×10^3	4.2×10^2	1.4×10^3	2.0×10^3	1.5×10^3	9.0×10^2	1.7×10^3	1.1×10^3	8.5×10^2	1.2×10^3	4.0×10^3	4.5×10^3
6 days												
Coliforms	4.1×10^7	5.2×10^7	2.6×10^7	2.5×10^7	4.9×10^5	4.9×10^5	2.8×10^5	4.2×10^7	4.1×10^7	4.6×10^7	5.1×10^1	7.0×10^1
E. coli	1.2×10^6	1.1×10^6	2.2×10^7	3.1×10^7	2.4×10^7	2.0×10^6	4.5×10^7	2.2×10^7	1.2×10^6	4.2×10^7	8.5×10^1	2.1×10^1
Coagulase-positive staphylococci	1.4×10^7	1.9×10^7	1.4×10^4	1.6×10^4	1.7×10^6	9.4×10^5	1.9×10^6	6.9×10^6	7.8×10^2	1.4×10^7	5.1×10^1	7.0×10^1
Cocci-shaped LAB	1.3×10^9	5.6×10^8	6.3×10^8	8.7×10^8	6.3×10^8	5.9×10^8	2.6×10^8	8.8×10^8	7.2×10^8	5.6×10^8	5.7×10^5	6.6×10^5
Rod-shaped LAB	2.9×10^6	2.4×10^6	5.8×10^7	1.2×10^7	1.2×10^8	1.0×10^8	8.2×10^7	9.1×10^7	4.8×10^7	1.2×10^8	6.2×10^7	1.6×10^7
Heterofermentative LAB	1.0×10^7	0.0×10^0	5.7×10^7	2.3×10^7	5.5×10^6	5.5×10^6	5.2×10^6	1.1×10^8	8.3×10^7	1.3×10^8	5.5×10^6	6.4×10^6
Yeasts	6.2×10^6	8.7×10^6	1.7×10^6	2.3×10^6	2.6×10^5	1.3×10^5	3.3×10^5	3.2×10^6	1.3×10^5	6.1×10^6	5.5×10^1	6.4×10^1
Moulds	7.5×10^2	3.5×10^2	1.8×10^5	2.1×10^5	1.3×10^5	1.3×10^5	8.7×10^4	1.4×10^4	3.8×10^2	2.8×10^4	5.5×10^1	6.4×10^1
Lactate-fermenting clostridia spores	3.0×10^0	0.0×10^0	3.3×10^1	1.4×10^1	4.2×10^1	2.3×10^1	4.4×10^1	1.5×10^2	7.6×10^1	2.2×10^2	5.6×10^2	7.7×10^2
Propionic acid bacteria	8.3×10^3	1.0×10^4	1.4×10^4	6.0×10^3	9.5×10^4	7.9×10^4	8.7×10^4	1.7×10^6	1.6×10^4	2.9×10^6	1.3×10^3	1.1×10^3

* Mean value is equivalent to median value.
[a] SD, standard deviation.

Table VIII. Effect of lysozyme addition to milk on Pannerone cheese fermentations: content of organic acids ($g \cdot kg^{-1}$) after 6 days of warm room at 30 °C. Trials were performed in duplicate.

	Control		Lysozyme (50 $mg \cdot kg^{-1}$)	
	Mean	SD*	Mean	SD
Total solids	579.8	2.0	589.4	8.5
Lactose	0.00	0.00	0.00	0.00
Galactose	0.07	0.06	0.00	0.00
Lactic acid	3.09[a]	1.84	14.99[b]	0.86
Acetic acid	0.40[a]	0.17	1.64[b]	0.20
Citric acid	0.00	0.00	0.00	0.00
Propionic acid	1.27[a]	0.51	0.10[b]	0.00
Butyric acid	6.29[a]	1.21	0.49[b]	0.00
pH	5.69[a]	0.10	5.11[b]	0.08

Different letters in the same row mean that the values are significantly different ($P < 0.01$).
* SD, standard deviation.
Rows without superscripts did not present significant differences.

to temperature. Butyric acid concentration was significantly the lowest at 4 °C. To have amounts of butyric acid similar to those usually found in Pannerone cheese from retail, it is necessary to maintain the temperature of the warm room at about 30 °C. The storage of Pannerone cheese at a temperature higher than 30 °C did not affect the lactate fermentations, but increased significantly the total solid contents of the cheese. In order to obtain a cheese with traditional characteristics, the holding of cheese to correct temperature for the time necessary to accomplish the specific fermentations is a critical point. The length of the holding time of Swiss cheese into the warm room at 25 °C was related to its propionic acid content [19]. The holding of the curd of Castelmagno for 3 days under whey at uncontrolled room temperature allowed for a sharp decrease of pH to 4.78 and a high formation of lactic acid. However, propionic and butyric acid contents were < 1.5 and 0.2 $g \cdot kg^{-1}$, respectively, by the end of ripening [12].

The microbial counts confirmed that the microflora of raw milk was comparable in almost all the trials and the cocci-shaped LAB were the most numerous (data not shown). After the first day from cheese-making (Tab. VII), cocci-shaped LAB, *E. coli*, other coliform bacteria and coagulase-positive staphylococci were the more represented bacteria, in the order mentioned. However, the results were characterized by a large expected variability among the replicated trials as shown by the differences between the mean value and the median value. On the contrary, after 6 days an important reduction of coagulase-positive staphylococci count was observed (Tab. VII). The higher the temperature of the warm room, the higher the reduction of viable cell number of staphylococci. Coliforms and *E. coli* counts were substantially stable, with the exception of the cheese at 38 °C where an important count reduction was measured. The highest counts of heterofermentative and rod-shaped LAB were observed at 30 and 25 °C, respectively, PAB counts were higher at cheese temperature between 25 and 30 °C, according to what happens during the warm room period for the Emmental cheese [28]. The number of lactate-fermenting clostridia was generally low, according to the limit of the analytical method employed that counted only the spores.

3.4. Influence of butyric clostridia on Pannerone cheese quality

Butyric acid can originate from animal, i.e. lamb paste rennet, or microbial lipase activity or from lactate fermentation. The absence of lipolytic bacteria and the numbers of moulds (Tabs. I and IV) minimized the role of microbial lipases. However, it is not possible to exclude the activity of the native milk lipoprotein lipase, because Pannerone is made using raw milk. To demonstrate the involvement of the lactate-fermenting clostridia, in particular the vegetative cells, in the production of butyric acid during the warm room step, lysozyme was added to the raw milk used for Pannerone manufacture. Lysozyme is usually and successfully used in Grana Padano cheese-making to prevent late blowing. While spores are resistant to lysozyme, lysozyme lyses the cell wall of the mature vegetative cells of *C. tyrobutyricum* and *Clostridium butyricum* or that of the new vegetative cell during the outgrowth from the spore [7, 22]. The amount of lysozyme used in Swiss-type cheese to prevent lactate fermentation by clostridia without blocking the propionic fermentation is usually lower than the 20 $mg \cdot L^{-1}$ added to milk for Grana Padano [7]. Hence, considering the favourable conditions for clostridia in Pannerone cheese-making and the need to allow lactic acid fermentation, the lysozyme amount used was limited to 50 $mg \cdot L^{-1}$. Lysozyme, in spite of the high amount used, did not inhibit lactic acid fermentation by the heterogeneous indigenous flora of raw milk, as shown by a lactic acid content of 15 $g \cdot kg^{-1}$ measured after 6 days at 30 °C, similar to that observed in control cheeses after 4 days in warm room (Tab. V), and by the concurrent depletion of lactose and galactose. The butyric and propionic acid contents (0.5 and 0.1 $g \cdot kg^{-1}$, respectively) were significantly lower ($P < 0.01$) than those of the control cheese made without lysozyme (Tab. VIII). The pH of the curd during the warm room time decreased to about 4.9, but this is not inhibitory for butyric clostridia [7]. Their growth and the consequent utilization of lactate contributed to raise the pH value of the cheese. The pKa value of butyric acid (4.86) is lower than that of lactic acid (3.86). The negative relation between lactate and butyrate content in lysozyme cheese demonstrated that inhibiting lactate-fermenting clostridia by the addition of high amount of lysozyme also inhibits butyric acid production.

Propionic acid content was also significantly lower (Tab. VIII) in cheeses made using lysozyme. This could be due to an inhibitory effect on PAB of the high dose of lysozyme used. Moreover, the inhibition of butyric clostridia growth and the lack of lactate fermentation maintained the curd at a low pH which is unfavourable for the growth of PAB.

The amount of acetic acid was higher in cheese made using lysozyme than that in the control. This result could be explained both by the low activity of lysozyme against gram-negative bacteria and by the lack of acetate utilization as a consequence of inhibition of germination of butyric clostridia spores [7].

4. CONCLUSIONS

Pannerone is a cheese obtained from raw milk, without starter addition and without salting, using a traditional technology unchanged over the years. This study demonstrated that the most important step to preserve the characteristics of Pannerone cheese is the period when the cheese is in the warm room at 30 °C up to 6 days. The presence and the role of coliform bacteria giving the typical characteristics of the cheese is confirmed, but it is shown for the first time that butyric clostridia and PAB are fundamental and their growth is favoured by the unique prolonged time of warm room. The presence of high amounts

of butyric and propionic acids is important for the characteristics of the cheese. Fermentation of lactose to lactate firstly, and the following lactate conversion to butyric and propionic acids are responsible for the pH changes of the curd during the warm room time. Like all the raw milk cheeses with a short ripening time, Pannerone cheese may be considered as potentially hazardous. However, pathogenic bacteria always are absent in all the 26 samples analysed. It can be hypothesized that holding the curd at low pH value (< 5.0) during the first 4 days of warm room at a temperature of about 30 °C, and the presence of a mixture of organic acids (lactic, butyric, propionic and acetic) could contribute to control the pathogenic bacteria. Lowering the temperature of the warm room at or below 25 °C, the fermentation profile of Pannerone cheeses deeply changes.

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