PAPER

EFFECT OF ARTISANAL RENNET PASTE ON THE CHEMICAL, SENSORY AND MICROBIOLOGICAL CHARACTERISTICS OF TRADITIONAL GOAT'S CHEESE

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ABSTRACT

In a study using three replicates, Marzolina goat cheese made with artisanal rennet paste from goat kid was compared with cheese made with commercial liquid rennet from calf. Samples of fresh cheese were subjected to chemical and microbiological analyses. Samples of ripened cheese collected after 50 days of ripening were submitted to chemical and sensory analysis. Results of this study show that cheese made with artisanal rennet pastes did not contain pathogenic micro-organisms and that this kind of rennet provided the enzymatic content necessary to achieve the typical characteristics of traditional cheeses.

- Keywords: rennet paste, chemical and sensory characteristics -

INTRODUCTION

In recent years, there has been an increased interest in the safety and promotion of cheeses prepared according to local traditional processes. The renewed attention is given to artisanal rennet, which is considered one of the most important factors affecting the characteristics of some typical Mediterranean cheeses. ARPs usually originate from lamb or kid abomasa, and farmers directly prepare them for use during cheese making. According to current legislation, the use of ARPs is allowed after a special derogation from Regulation (EC) n. 852/2004 for foods with traditional characteristics (EC Reg. n. 2074/2005). To obtain this derogation, it has been necessary to study their safety and hygienic characteristics. Results of a number of studies indicate that traditional cheese-making does not compromise health and hygiene (COSSEDDU and PISANU, 1980; DEIANA *et al.*, 1980; PISANU and COSSEDDU, 1982; BARZAGHI *et al.*, 1997; CALANDRELLI *et al.*, 1997; IRIGOYEN *et al.*, 2001; MOATSOU *et al.*, 2004; MOSCHOPOULOU *et al.*, 2007; TRIPALDI *et al.*, 2012). Determining the role and influence of ARPs on the sensory characteristics of cheeses is also necessary.

Marzolina is a traditional Italian cheese made from the milk of local goat breeds of the Latium region, in the Centre of Italy and from artisanal kid rennet pastes. Marzolina is characterised by a weigth of about 150 g and high salt content that preserves the cheese for long periods even in a natural room. ARP is traditionally used in the process for MARZOLINA cheese and its particular flavor has been attributed to the use of rennet pastes (Addis *et al.*, 2005). However factors such as the time and the effort required to prepare the rennet at the farm, as well as the lower demand for strongly flavoured cheeses, have contributed to the rapid replacement of ARP with commercial liquid rennet (CLR).

The aim of this trial was to study the hygienic and health characteristics of cheese from ARP so contributing to the approval of the derogation for using artisanal rennet pastes. Moreover this trial was finalised to evaluate the effect of rennet pastes, as compared to liquid rennet, on the chemical, and sensory characteristics of Marzolina goat cheese.

MATERIALS AND METHODS

Marzolina cheese made with ARP from kid was compared with that made with CLR from calf. Both cheeses were produced by farmers. Two kinds of rennet were used and three replicates were performed. The trial was carried out according to the process usually employed by farmers in a small cheese farm. Thirty litres of milk for each kind of rennet in each replicate were processed. Abomasa were removed from suckling kids slaughtered at the age of 30–45 days and they were submitted to a drying phase preceded by a salting phase. Ten abomasa were ground, merged and then utilised in all replicates of the trials. The commercial liquid calf rennet (Naturen®), used in the trial was produced by Chr. Hansen's (Denmark).

Data specifying proteolytic activity of CLR were supplied by Chr. Hansen's. Enzimatic characteristics of ARP were determined according to the following methods.

Total milk clotting activities of the artisanal rennet pastes were determined according to ISO 23058 IDF 199: 2006 method known as REMCAT method. To determine the chymosin and pepsin content, test samples were prepared by dissolving 25 g of rennet paste in 100 g of buffer solution (CH3COOH/CH3COONa) at pH 5.5; the samples were centrifuged at 3000 rpm (2189 g, refrigerated centrifuge ALC 4237R, ALC, Milano, Italy) for 30 min at 4°C. The supernatant was analysed as described in the International IDF Standard 110B: 1997. Chymosin and pepsin enzymes were expressed as a percentage of the sample's total milk clotting activity. Lipase activity was analysed as described in the Food Chemical Codex (1981).

Characteristics of both types of rennet are shown in Table 1. During the study, raw goat milk was utilised and no starter cultures were added. After coagulation at a temperature below 35°C, the curd was cut, reduced to small granules and then packed in a small cylindrical mould. After about 12 hours from the start of cheese-making, the cheeses were subjected to dry salting and then air-dried in a natural room for one week. Finally, they were packaged under vacuum and stored at 4°C.

During each experiment, samples of fresh cheese were collected before dry salting. Sam-

Table 1 - Enzymatic activity of artisanal rennet pastes and commercial liquid rennet.

Rennet	g or mL of rennet on 100 l of milk	total IMCU × g^{-1} or mL^{-1} of rennet	total IMCU on 100 l of milk on 100 l of milk	chymosin (% RU)	chymosin IMCU	ILU × g^{-1} of rennet
ARP	19	129	2493	95,34	2377	35,76
CLR	20	162	3229	80,00	2583	

ARP: Artisanal Rennet paste; CLR: Calf Liquid Rennet.
IMCU: International Milk Clotting Unit; RU: Rennet Unit;ILU: International Lipase Units.

ples of fresh cheese were subjected to chemical analyses, which were conducted in duplicate. Analyses consisted of measurements of moisture (IDF, 1986), total nitrogen (TN) (FIL-IDF, 1993), soluble nitrogen (SN) (FIL-IDF, 1991), fat (FIL-IDF, 2001), salt (IDF, 1988), ash (AOAC, 2000), and free fatty acids (FFAs). FFAs (mmol/kg) were analysed by capillary gas chromatography (De Jong and Badings, 1990) and expressed as mmol/kg to assess each individual FFA independent of its molecular weight. Samples of fresh cheese were also subjected to microbiological analysis. All samples were subjected to qualitative tests for *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157, and to quantitative analyses for *L. monocytogenes*, sulphite-reducing clostridia, total microbial count at 30°C, coagulase-positive staphylococci, and β-glucuronidase-positive *E. coli* (Tripaldi *et al.*, 2012). Samples of cheese ripened for 50 days were also collected and then subjected to chemical and sensory analyses.

Descriptive sensory analysis was performed to determine the differences in sensory characteristics of the two kinds of cheeses. Ten panelists (5 males and 5 females; between 20 and 50 years of age) were selected and trained in accordance with ISO 8586-1:1993 e ISO 8586-2:1994 standards.

For the laboratory conditions, UNI ISO 8589 standard was followed. The test was carried out according to UNI 10957:2003 standard based on triplicate analysis of each sample.

Twenty-two descriptors were identified, as follows: 3 visual (color intensity, color omogeneity, rind color), 5 olfactory (smell intensity, stable straw smell, lactic smell, vegetable smell, other smell), 5 basic taste and trigeminal sensations (salty, sweet, sour, bitter, piquant), 6 retro-olfactory (flavour intensity, stable straw flavour, lactic flavour, vegetable flavour, other flavour, persistence), and 3 tactile descriptors (adhesiveness, moisture, firmness). Attributes were rated on a continuous scale of values from 0 to 10 (0 = absence of the intensity, maximum intensity = 10). Sensory descriptors and their rating scale were defined according to UNI 10957:2003 and European Guide for the sensory evaluation of hard and semi-hard cheese standard (BÉRODIER e al.1997; LAVANCHY *et al.*1994).

The GLM procedure of SAS software (SAS Institute Inc., 2007) was used for statistical analysis of chemical parameters by using the model

$$Y_{ijl} = \mu + A_i + B_j + A_i \times B_j + E_{ijl}$$

where

Y_{ijl} = qualitative characteristics of the cheese

A_i = fixed effect of the kind of rennet (i = 1 for ARP; i = 2 for CLR)

B_j = fixed effect of days of ripening (j = 1: 1*d*; j = 2: 50*d*)

E_{ijl} = residual of error

Data processing of sensory evaluations was carried out according to UNI 10957:2003 standard.

RESULTS AND DISCUSSION

Results of proteolytic and lipolytic activity of ARPs and CLR are summarised in Table 1. The clotting characteristics were different: 129.38 total International Milk Clotting Units (IMCU) × g^{-1} and 95.34% of chymosin for ARP and 162.00 total IMCU × ml^{-1} and 80.00% of chymosin for CLR. The latter was subjected to a preliminary test and then added to milk in quantity of 19.93 mL/100 l of milk. This led to coagulation in approximately 60 minutes, the clotting time used by farmers.

During the experiment, the addition of rennet to 100 l of milk resulted in 2493 and 3229 total IMCU for ARP and CLR, respectively. Chymosin IMCU values, 2377 and 2583 per 100 l of milk for ARP and CLR, respectively, were more similar than that of total IMCU. . The lower activity of total clotting of ARP could be due to dilution of enzymes caused by the presence of milk in the abomasa used to produce the rennet (PIRISI *et al.*, 2007). The high chymosin percentage (95.34 and 80.00) in the rennet paste could be attributed to the completely milk-based diet and/or to complete filling of the stomach with milk when the kid was killed (BUSTAMANTE *et al.*, 2000; ADDIS *et al.*, 2005). We observed that in our study the kind of diet given to the kid and the status of the abomasum before the slaughtering agreed with the conditions found by other Authors (BUSTAMANTE *et al.*, 2000; ADDIS *et al.*, 2005). The international lipase units (ILU) of the rennet paste, 35.76 ILU × g^{-1}, was similar to the average value (36.18 ILU × g^{-1}) obtained from other samples of rennet paste from the same region (TRIPALDI *et al.*, 2012).

The chemical characteristics of fresh and ripened goat cheese made using ARP and CLR are reported in Table 2. The moisture values were not largely affected by the kind of rennet in both fresh and ripened cheese (69.65 and 42.30% in ARP cheese and 69.81 and 40.87% in CLR cheese). Generally, the cheese moisture depends on the temperature and relative humidity conditions of cheese-making and the ripening conditions (IRIGOYEN *et al.*, 2002); thus, it is difficult to find differences between the two cheeses in which the only change is the type of rennet.

We can observe slight differences in protein content between the two kinds of cheeses (10.60 *vs* 11.13 and 20.98 *vs* 21.30 in ARP- and CLR-treated fresh cheese and in ARP- and CLR-treated ripened cheese, respectively). The fat content of the two kinds of cheese also differed slightly (13.29 *vs* 14.03% in ARP and CLR of fresh cheese and 28.19 *vs* 30.31% in ARP- and CLR-treated ripened cheese, respectively). On the contrary, SANTORO and FACCIA (1998) observed a significant difference in fat content in CanestratoPugliese cheese

Table 2 - Composition of fresh and ripened Marzolina goat cheese made using artisanal rennet paste (ARP) and commercial liquid rennet (CLR) at 1 day and 1 month of ripening.

	Fresh cheese						Ripened cheese						P		
	ARP	+	++	CLR	+	++	ARP	+	++	CLR	+	++	SE	rennet	ripening
moisture (%)	69,65	ns	a	69,81	ns	a	42,30	ns	b	40,87	ns	b	1,15	NS	*
protein (%)	10,60	ns	b	11,13	ns	b	20,98	ns	a	21,30	ns	a	0,60	NS	*
soluble protein (% of total protein)	4,96	ns	b	3,98	ns	b	9,12	ns	a	10,11	ns	a	0,13	NS	*
fat (%)	13,28	ns	b	14,03	ns	b	28,19	ns	a	30,31	ns	a	0,73	NS	*
NaCl (% of moisture)	0,79	ns	b	0,82	ns	b	7,40	a	a	6,01	b	a	0,44	*	*
ash (%)	1,66	ns	b	1,71	ns	b	5,09	ns	a	4,36	ns	a	0,26	NS	*

ARP: Artisanal Rennet paste; CLR: Calf Liquid Rennet.
+ kind of rennet; ++ ripening time.
a, b, *: $p<0.05$.

made with rennet having different characteristics. They attributed this result to the different aggregation states of the casein micelles in the curd.

The salt in moisture (S/M) content of samples of fresh cheese was similar (0.79% in ARP-treated cheese and 0.82% in CLR-treated cheese), while S/M content was higher in ARP-treated than in CLR-treated ripened cheese (7.40 *vs* 6.01%; $P < 0.05$). The difference in salt content in the two kinds of ripened cheese is probably due to manual dry salting, a practice that is subject to large variations. Our results show that ripened Marzolina cheese has higher salt content in comparison with the majority of ripened cheeses (2.67 in ARP-treated cheese and –3.13% in CLR-treated cheese), corresponding to about 5% of dry matter.

Soluble protein as a percentage of total protein was higher in ARP-treated fresh cheese than in CLR-treated fresh cheese (4.96 *vs* 3.98%). On the contrary, higher values of this proteolysis index were found in ripened CLR-treated cheese in comparison with those obtained in ripened ARP-treated cheese (10.11 *vs* 9.12%). During cheese ripening, the higher salt content of ARP relative to the one of CLR cheese may influence negatively the proteolytic process, as observed in Romano type cheese (GUINEE and FOX, 1984).

Similar values of soluble N/total N at pH 4.6 were found in Protected Designation of Origin (PDO) sheep cheese Canestrato Pugliese (CORBO *et al.*, 2001) at 1 and 35 days of ripening (5.59-6.78% and 8.65-11.50%, respectively). Values of the soluble N/total N at pH 4.6 (7.87%) of the traditional Italian cheese Piacentinu Ennese at 2 months of ripening (FALLICO *et al.*, 2006) are lower than those of ripened Marzolina cheese.

Differences in all parameters between fresh and ripened samples of both kinds of cheese were observed. With ARP and CLR, the moisture of ripened cheese significantly decreased compared with that of fresh cheese. The other cheese components protein, fat, S/M and ash increased significantly during cheese ripening as a result of the decrease in moisture. The soluble protein/total protein ratio was significantly higher in ripened cheese as result of increased proteolysis (UPADHYAY *et al.*, 2004).

Table 3 shows the individual and total FFA (TFFA) content of cheese made with ARP compared with cheese made with CLR. The TFFA content was higher in ARP-treated cheese than in CLR-treated cheese: 8.94 versus 4.09 mmol× kg^{-1} and 39.51 versus 36.56 mmol× kg^{-1} in fresh and ripened cheese, respectively. The difference between fresh and ripened cheese of ARP- and CLR-treated cheeses was significant.

Short chain free fatty acids (SCFFAs) are the most abundant FFAs in ARP and CLR. Similar to TFFAs, SCFFA was present at higher levels in ARP-treated cheese (5.58 *vs* 2.06 mmol× kg^{-1} and 21.46 *vs* 15.75 mmol× kg^{-1}, in fresh and ripened cheese, respectively). The difference was significant between ripened cheeses made with ARP and with CLR and between fresh and ripened cheese of both ARP- and CLR-treated cheeses.

Butyric and capric acids were the most abundant FFAs. Levels of butyric acid were the highest in both kinds of fresh cheese. Levels of capric acid were the highest in both kinds of ripened cheese.. Butyric acid levels were significantly higher in fresh or ripened ARP cheese than in CLR cheese (2.27 *vs* 0.81 mmol × $kg^{-1}$and 4.89 *vs* 3.85 mmol × kg^{-1}, $P \leq 0.05$). Caproic acid only in ripened cheese was significantly higher in ARP-treated cheese than in CLR-treated cheese (4.85 *vs* 3.31 mmol × kg^{-1}). Also Capric acid was higher in ARP ripened cheese than in CLR ripened cheese (8.39 vs 6.05 mmol × kg^{-1}, $P \leq 0.06$)

The content of all individual fatty acids of both groups of ripened cheeses were higher than that of both groups of the fresh ones ($P \leq 0.01$).

Levels of medium-chain FFAs (MCFFAs) and long-chain FFAs (LCFFAs) were higher in fresh cheese made with ARP (1.91 *vs* 1.02 mmol × $kg^{-1}$and 1.44 *vs* 1.00 mmol ×kg^{-1} for MCFFAs and LCFFAs, respectively) and lower in ripened cheese made with ARP (10.58 vs 12.04 mmol × kg^{-1} and 7.48 *vs* 8.77 mmol × kg^{-1}, respectively). The dif-

Table 3 - Free fatty acids (mmol x kg^{-1}) in fresh and ripened Marzolina goat cheese made using artisanal rennet paste (ARP) and commercial liquid rennet (CLR) at 1 day and 1 month of ripening.

	Fresh cheese						Ripened cheese						P		
	ARP	+	++	CLR	+	++	ARP	+	++	CLR	+	++	SE	rennet	ripening
C4:0	2,27	a	B	0,81	b	B	4,89	a	A	3,85	b	A	0,33	*	**
C6:0	1,28	ns	B	0,37	ns	B	4,85	a	A	3,31	b	A	0,31	*	**
C8:0	0,60	ns	B	0,24	ns	B	3,29	ns	A	2,50	ns	A	0,40	NS	**
C10:0	1,42	ns	B	0,62	ns	B	8,39	ns	A	6,05	ns	A	0,80	NS	**
SCFFAs	5,58	ns	B	2,06	ns	B	21,46	a	A	15,75	b	A	1,45	*	**
C11:0	0,01	ns	B	0,00	ns	B	0,04	a	A	0,03	b	A	0,00	*	**
C12:0	0,44	ns	B	0,20	ns	B	2,42	ns	A	1,81	ns	A	0,22	NS	**
C14:0	0,47	ns	b	0,22	ns	B	2,75	ns	a	3,13	ns	A	0,43	NS	*
C15:0	0,03	ns	b	0,02	ns	B	0,18	ns	a	0,23	ns	A	0,03	NS	*
C16:0	0,94	ns	b	0,56	ns	B	5,05	ns	a	6,69	ns	A	0,84	NS	*
C16:1	0,02	ns	b	0,01	ns	b	0,13	ns	a	0,15	ns	a	0,03	NS	*
MCFFAs	1,91	ns	B	1,02	ns	B	10,58	ns	A	12,04	ns	A	1,55	NS	**
C17:0	0,02	ns	b	0,01	ns	B	0,10	ns	a	0,13	ns	A	0,02	NS	*
C18:0	0,45	ns	b	0,33	ns	B	2,15	ns	a	2,73	ns	A	0,34	NS	*
C18:1	0,88	ns	b	0,63	ns	b	4,57	ns	a	5,05	ns	a	0,89	NS	*
C18:2	0,06	ns	b	0,03	ns	B	0,41	ns	a	0,56	ns	A	0,10	NS	*
C18:3	0,03	ns	b	0,01	ns	B	0,23	ns	a	0,30	ns	A	0,05	NS	*
LCFFAs	1,44	ns	b	1,00	ns	B	7,48	ns	a	8,77	ns	A	1,40	NS	*
TFFAs	8,94	ns	B	4,09	ns	B	39,51	ns	A	36,56	ns	A	4,24	NS	**

ARP: Artisanal Rennet paste; CLR: Calf Liquid Rennet.
+ kind of rennet; ++ ripening time.
SCFFAs = short chain free fatty acids MCFFAs = medium chain free fatty acids LCFFAs = long chain free fatty acids. TFFAs = total free fatty acids.
a, b, *: p<0.05; A, B, **: p<0.01

ference was significant for both groups of FFAs only between fresh and aged cheeses.

The higher butyric acid content of cheese made with ARPs compared with cheese made with CLR is confirmed by the high specificity of the enzymatic activity of pregastric lipase for SCFAs, especially butyric acid, esterified to the *sn*-3 position of triglycerides (Pitas and Jensen, 1970; Kim and Lindsay, 1993). FONTECHA *et al*. (2006) found higher butyric acid content in Spanish goat cheese made with rennet paste compared with cheese made with CLR.

The higher concentration of capric acid compared with butyric acid in ripened cheeses was observed in other goat cheeses (BUFFA *et al*., 2001; POVEDA and CABEZAS, 2006; ATASOY and TURKOGLU, 2009). According to BUFFA *et al*. (2001), the capric acid content of cheese from goat milk increased during ripening while butyric acid varied slightly from the start to the end of ripening. The small increase in butyric acid content was probably due to its metabolic conversion to aromatic compounds (BUFFA *et al*., 2001).

Sensory attributes of each kind of cheese are shown in Fig. 1. Mean values of the following sensory descriptors were significantly different

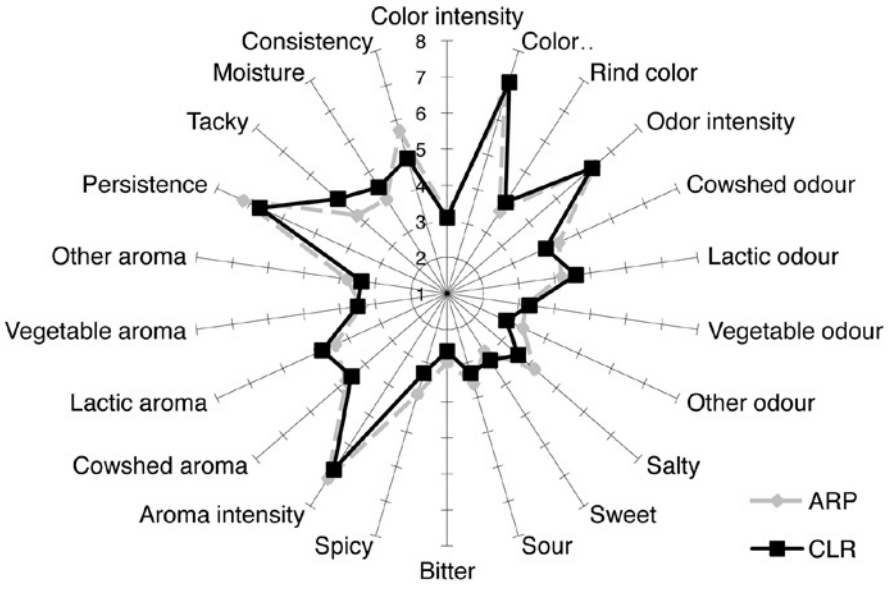

Fig. 1 - Sensory profile of ripened Marzolina goat cheese made using artisanal rennet 14 pastes (ARP) and commercial liquid rennet (CLR).

Table 4 - Microbiological characteristics of fresh Marzolina goat cheese made using artisanal rennet paste (ARP) and commercial liquid rennet (CLR).

Sample	Replicate	*Salmonella* spp (in 25 g)	*Listeria monocytogenes* (in 25 g)	*E. coli* (cfu x g^{-1})	*E. coli* O:157 (in 25 g)	Coagulase positive staphylococci (cfu x g^{-1})	Enterobacteriaceae (cfu x g^{-1})	Sulphite reducing clostridia (cfu x g^{-1})	Total mesophilic count (cfu x g^{-1})
ARP	1	negative	negative	<10	negative	<10	<10	<2	$2.6x10^7$
CLR	1	negative	negative	<10	negative	<10	<10	<2	$3.0x10^8$
ARP	2	negative	negative	<10	negative	<10	$3.5x10^3$	<2	$9.3x10^5$
CLR	2	negative	negative	<10	negative	<10	$4.8x10^3$	<2	$1.3x10^6$
ARP	3	negative	negative	100	negative	<10	$6.2x10^3$	<2	$4.9x10^6$
CLR	3	negative	negative	<10	negative	<10	<10	<2	$1.5x10^5$

(P<0.05) in the two kinds of cheese (ARP vs CLR, respectively): color omogeneity (6.6 vs 7.1), rind color (3.7 vs 4.0), stable straw smell (4.4 vs 4.0), lactic smell (4.2 vs 4.6), other smell (3.3 vs 2.8), salty (4.2 vs 3.6), sweet (2.9 vs 3.2), sour (3.6 vs 3.3), bitter (2.9 vs 2.6), piquant (3.9 vs 3.3), flavour intensity (7.1 vs 6.8), stable straw flavour (4.7 vs 4.5), lactic flavour (4.4 vs 4.8), other flavour (3.8 vs 3.4), persistence (7.2 vs 6.7), adhesiveness (4.3 vs 5.0), moisture (4.1 vs 4.5) and firmness (5.7 vs 4.9).

There was no significant interaction between assessor and replicate, suggesting high repeatability of panellist assessment in the three replicates. Sample-replicate and sample-panellist interactions were not significant, showing either homogeneity of samples in the three replicates or good agreement among panellist assessments during sensory evaluation. Analysis of the eyes and slits in cheese showed a higher number of samples made with ARPs having these defects compared with those in cheese samples made with CLR (82 vs 57).

The basic tastes salty, acid, bitter and piquant were more pronounced in cheeses made with ARP than in cheeses made with CLR. In addition, the smell and flavour of cowshed were more marked in cheese made with ARP than in cheese made with CLR. Its texture was firmer, less tacky and less moist than the latter kind of cheese. The odour and flavour given by lactic acid to cheeses made with CLR are more dominant than in cheeses made with ARP. Generally, the sweet, salty and sour attributes of taste were less pronounced. The texture was tackier, more moist and less firm than that of cheese made with ARP.

It is noteworthy that basic tastes salty and piquant, which are more pronounced in cheese made with ARP than in cheese made with CLR, agree with the chemical results. As reported by Addis *et al*. (2005), cheese made with ARPs has a major amount of butyric acid, which may be responsible for piquancy in cheese (Rennet paste has been associated with piquancy or pungency and with characteristic flavours of certain cheeses from the Mediterranean basin (ANIFANTAKIS, 1976; NELSON *et al*., 1977; WOO and LINDSAY, 1984; BATTISTOTTI and CORRADINI, 1993; BARZAGHI *et al*., 1997; CALANDRELLI *et al*., 1997). In a study on Idiazabal cheese (ETAYO *et al*., 2006), cheese made with lamb rennet pastes showed higher butyric acid content and received higher scores compared to cheese made with commercial liquid lamb rennet. .

A larger number of eyes and slits in cheese made using ARPs was also observed by FERRANDINI *et al*. (2012). This could be attributed to different textural properties (FERRANDINI *et al*., 2011) of cheese made with the two kinds of rennet. In fact, results of microbiological analyses carried out on ARP used in this study (TRIPALDI *et al*., 2012) exclude the presence of microorganisms markers of hygiene characteristics including germs causing microbiological spoilage in cheese.

Table 4 displays the microbiological characteristics of the cheese. *Salmonella* and *L. monocytogenes*, pathogens considered as health markers (Reg. CE 2073/2005), were undetected by qualitative analyses. β-Glucuronidase-positive *E. coli* and coagulase-positive staphylococci, which include *Staphylococcus aureus*, are considered as hygiene markers (Reg CE 2073/2005). The maximum count tolerated for coagulase-positive staphylococci in cheese from raw milk is 10^5cfu × g^{-1}. Samples with more elevated counts must be

analysed for staphylococci enterotoxins. Counts of coagulase-positive staphylococci in all samples were lower than the detection limit of the method (10 cfu $\times$ g^{-1}). The count of β-glucuronidase-positive *E. coli* was 100 cfu $\times$ g^{-1} in only one sample, but lower than the detection limit in other samples (10 cfu$\times$ g^{-1}). Qualitative analysis for *E. coli* O157 in our samples gave negative results. Sulphite-reducing clostridia is another group of microbial pathogens considered as hygiene markers, but legislation has not established permissible levels for these in food. Their levels in all samples were lower than the detection limit of the method (2 cfu $\times$ g^{-1}).

The mean total mesophilic count in our samples was 5.6×10^7cfu$\times$ g^{-1}. Microbiological analyses of the cheese samples confirmed the results obtained during the monitoring of some ARPs collected in the same region of Marzolina production (TRIPALDI *et al.*, 2012). Similar results were obtained in three PDO raw ewe milk cheeses from Spain, as Manchego, Idiazabal and Zamorano cheese (ETAYO *et al.*, 2006), where the hygienic quality of cheeses made with lamb rennet paste is comparable to that of cheeses manufactured with non-paste commercial rennet. Another study on Idiazabal did not detect *E. coli*, *Clostridium*, *Salmonella* or *L. monocytogenes*, and levels for other microorganisms were below the limits of the European legislative standards for cheese manufactured with raw milk (GIL *et al.*, 2007).

The results of our study show that treatment with ARPs did not favour the growth of microbial pathogens and that ARPs provided the enzymatic content necessary to achieve the typical characteristics of traditional cheeses.

CONCLUSIONS

Butyric acid was the main marker of cheeses made with ARPs because of the high specificity of enzymatic activity of pre-gastric lipase for butyric acid. Results of sensory evaluation show that the piquant flavours as well as the odour and flavour of cowshed were more pronounced in cheeses made with ARP, confirming the results for other Mediterranean cheeses made with rennet paste. Therefore, the use of ARPs provided the enzymatic content necessary to achieve the typical characteristics of traditional cheeses. At the same time, this kind of rennet did not favour the growth of microbial pathogens in cheese.

ACKNOWLEDGEMENTS

This work was funded by the Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricultura del Lazio, Italy (ARSIAL) and to the Ruggieri farm from Itri (LT) for providing the Marzolina cheese samples.

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Paper Received May 29, 2014 Accepted December 1, 2014