ORIGINAL RESEARCH

Chemical composition and microbial evaluation of Argentinean Corrientes cheese

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The chemical and microbial composition of an artisanal cheese made from raw cow's milk produced and consumed in the province of Corrientes (north-eastern Argentina) was evaluated using standard methods. Corrientes cheese has high moisture content (50–60%), normal protein and fat contents (21–27 and 22–26% respectively), and is low in salt (0.5–2.0% w/w). Microbial counts also varied significantly between samples (colony-forming units per gram ranges covering logs of 5–11), probably due to environmental contamination in the raw material. These results will help produce higher quality Corrientes cheeses with well-defined characteristics.

Keywords Artisanal cheese, Microbiology, Physicochemical analysis, Raw milk.

INTRODUCTION

The province of Corrientes (north-eastern Argentina) has a very long tradition of artisanal cheese production. The climate in this region is considered tropical all year round, characterized as being hot and humid from October to March, with maximum daily temperatures of 35–45°C in this period. Corrientes artisanal cheeses have been manufactured by local farmers on a small scale for decades using raw milk from Argentine Criolla cows and traditional techniques passed down from generation to generation, and are now commonly consumed by most of the north-eastern population of Argentina. Typically, the cheeses are cylindrical or rectangular flat shaped (5–8 cm high), weighing about 1.00–1.50 kg, and having a compact interior appearance (without holes) with a creamy-pasty consistency, and a slightly acidic sour flavour.

Commercial starter cultures are not used in the production of Corrientes cheese; instead the cheese maker relies on the lactic acid bacteria (LAB) naturally present in the raw unpasteurized milk as adventitious contaminants. These beneficial microorganisms produce lactic acid as a result of the fermentation of sugars present in milk (principally lactose) which acidifies the milk and helps the whey expulsion from the curd necessary for cheese production. Bacterial biodiversity coming from the raw milk and environmental contamination (from the farm and production practices) constitute the principal source of the microorganisms which are necessary for the development of the typical features (taste, flavour, consistency) of this traditional cheese product. The microbial diversity originating from environmental exposure during cheese manufacture and maturation and the initial natural diversity of the microbiota present in the raw milk all play a role in fermentation processes and are important in the final development of traditional dairy products (Garabal 2007).

In Latin America, there are regions where fermented products are still manufactured traditionally. In specialized markets throughout the world, these fermented products are highly appreciated and are considered to be of premium value because of their flavour characteristics, which are not normally present in commercially produced cheeses. Although Corrientes cheese does not yet have a standard of identity, it plays an important role in the local farmer's economy. Until now, Corrientes cheese studies have been focused on contaminating microorganisms (Pereira *et al*. 1995) and no information is available on either the cheese microflora or their chemical, rheological and sensory characteristics.

The aim of this work was to characterize an artisanal cheese produced in the province of Corrientes, Argentina. The chemical and microbial evaluation of this artisanal cheese is beneficial for not only the consumers but also the manufacturers, since it will help them to standardize their production

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practices and allow them to produce higher quality and more stable products with well-defined characteristics.

MATERIALS AND METHODS

Cheese production and sampling

Corrientes artisanal cheese is manufactured principally in the hot and humid period (October to March) because of the increased availability of milk during the spring and summer. Although no statistics are currently available, the volume of production of Corrientes cheese is very important. It is elaborated using crude methods that have been transmitted from generation to generation. Fresh whole milk is subjected to spontaneous fermentation by the microbiota naturally present in the milk (6 h at 30–37°C), then curdling (2 h at 30–35°C) is performed by addition of calf rennet obtained from local farmers. The curd is then manually cut into small grains (1–3 cm), a small amount of salt (1–4%, w/v) is then added, and the salty curd mixture is placed in stainless steel moulds and pressed by hand. The mould is left incubating overnight under refrigeration (4–6°C), the cheese is then demoulded and sold fresh in local markets within 48 h of manufacture without any type of packaging.

Thirty whole cheeses from several regions of Corrientes province (Argentina) were collected fresh from the manufacturer during the main production period (October to March) and transported to the laboratory under refrigerated conditions (4–6°C). Samples (50–100 g) were taken from each cheese in accordance with International Dairy Federation (IDF) Standards (1985) and analysed immediately (within 24 h of their extraction).

Chemical analysis

All chemical analyses were determined according to the methods of the Association of Official Analytical Chemists (AOAC 1990) unless otherwise stated. Cheese pH was measured with a Crison model 2002 pH meter by direct readings using an Ingold insertion electrode (Crison Instruments, Barcelona, Spain). Titrable acidity was determined according to AOAC method 920.124 (1990) by quantifying the volume of 0.11 N NaOH required to raise the pH value to 8.4, and expressed as Dornic degrees. Total solids (TS) were analysed according to AOAC method 926.08 (1990). Fat content was determined according to IDF (reference method 5B, 1986). Sodium chloride and protein contents were determined according to Case *et al*. (1985). Total nitrogen (TN) was determined using Kjeldhal method as described by Case *et al*. (1985). Water-soluble nitrogen (W-SN) determination was according to Jin and Park (1995), the soluble fraction in trichloroacetic acid (12%) (TCA-SN) was prepared following Kuchroo and Fox (1982), while the soluble fraction in (5%) phosphotungstic acid (PTA-SN) was determined following the procedure of Jarrett *et al*. (1982) as modified by Aston *et al*. (1993). All determinations were conducted three times.

Microbiological analysis

Cheese samples (15 g) were taken according to AOAC method 955.30 (1990). A portion (10 g) of each cheese sample (taken from the interior of each block of cheese) was homogenized with 90 mL of a 2% (w/v) sterile sodium citrate solution at 45°C for 1 min using a Stomacher 400 Laboratory Blender (Tekmar Co., Cincinnati, OH, USA). Decimal dilutions were prepared in sterile NaCl (0.9%) and inoculated in specific media in duplicate. All media were purchased from Merck (Darmstadt, Germany) unless otherwise stated.

Coliforms and *Escherichia coli* numbers were determined on McConkey agar incubated at 30°C for 48 h and BRILA agar incubated at 42°C for 24 h, respectively, according to the Most Probable Number Method (IDF, Reference Method 73, 1974); *Staphylococcus aureus* on Baird–Parker agar according to IDF methods (Standard 145, 1990a) confirmed by a positive coagulase and catalase test; and moulds and yeasts on acidified potato-dextrose agar (IDF Standard 94B, 1990b).

Total lactic acid bacteria were counted in LAPTg_{10} agar (Raibaud *et al*. 1961) after incubation to 30°C during 72 h; lactococci in M17 agar after incubation at 30°C for 48 h; lactobacilli in MRS agar, pH 5.4, after 72 h of incubation at 30°C in an enriched CO_2 atmosphere using the BBL GasPack System (Becton Dickinson, Cockeysville, MD, USA); leuconostoc on the surface of MSE agar (Biokar Diagnostics, Beauvais, France) containing 30 μg/mL vancomycin after 3–5 days of incubation at 25°C. Enterococci were scored after 48 h of incubation at 44°C in KF *Streptococcus* agar containing 0.1 g/L triphenyltetrazolium chloride.

From the LAPTg_{10}, M17, MRS and MSE agar plates, a total of 306 colonies representative of all different morphologies were chosen at random and stored at –20°C in 10% (wt/vol) reconstituted skim milk powder containing 0.5% (wt/vol) yeast extract, 1.0% (wt/vol) glucose and 10% (wt/vol) glycerol. Working cultures were prepared after three successive transfers in the specifics media mentioned above.

After microscopic examination, Gram-positive and catalase-negative rod-shaped bacteria were tested for their ability to grow in MRS broth at 15°C for 7 days and at 45°C for 48 h, and cocci for their ability to grow in M17 broth at 10°C for 10 days and at 45°C for 48 h under both anaerobic and aerobic conditions. Microorganisms were also tested for CO_2 production from D-glucose and ability to grow at initial pH 9.6 and in 6.5% NaCl broth. Lactic acid bacteria genus was determined according to the methods and criteria of Axelsson (1998).

Statistical analysis

Data were expressed as means of three independent determinations of the 30 cheese samples ($n = 90$) ± standard deviation (SD). Calculation of mean ($x^1/_2$), SD, minimum and maximum values, variation coefficient, range and frequency distribution of chemical results and log counts of microbial groups were performed using Infostat software version 1.1 (Grupo Infostat, F.C.A., Universidad Nacional de Córdoba, Argentina).

RESULTS AND DISCUSSION

The chemical composition of Corrientes cheese is shown in Table 1. Corrientes cheese may be characterized as a soft (50–60% moisture), high fat (22–26%) cheese with 21–27% protein and low salt (0.5–2.0%) content, with a final pH in the range of 5.0–5.5. Cheeses were samples from several regions of this province. In general, physicochemical parameters varied significantly in all cheese samples as was shown by wide ranges and important standard deviations of mean values; however, no interregion variations were observed. This result could be attributed to: (i) variations in the chemical composition of the raw milk used in cheesemaking since milk was obtained by different suppliers: and (ii) differences in the elaboration, distribution and marketing of cheeses since no standardized protocols or controls are used in Corrientes cheese production. For a better description of the behaviour of the evaluated variables, the frequency distribution of the chemical parameters was determined (Table 2).

Table 1 Biochemical composition of artisanal cheese marketed in Corrientes

Parameter	*Mean ± SD*[a]	*Minimum*	*Maximum*	*Range*
Total solid[b]	46.71 ± 4.53	42.45	58.26	15.81
Fat[b]	24.43 ± 2.13	21.08	29.56	8.49
NaCl[b]	1.28 ± 0.58	0.56	2.44	1.88
pH	5.24 ± 0.70	3.90	5.98	1.99
Acidity[c]	1.23 ± 0.79	0.22	2.89	2.67
Protein[b]	24.24 ± 3.32	17.87	29.30	11.43
W-SN[d]	5.79 ± 1.60	3.63	9.63	6.00
TCA-SN[d]	4.65 ± 1.33	2.84	7.71	4.87
PTA-SN[d]	3.51 ± 0.96	1.54	4.88	3.34

[a]Means of three independent trials ± standard deviation (SD).

[b]Expressed as percentage.

[c]Expressed as percentage of lactic acid.

[d]Expressed as percentage of total nitrogen.

W-SN, water-soluble nitrogen; TCA-SN, trichloroacetic acid-soluble nitrogen; PTA-SN: phosphotungstic acid-soluble nitrogen.

Table 2 Frequency distribution of biochemical composition of artisanal cheese marketed in Corrientes

	Interval									
	1		*2*		*3*		*4*		*5*	
Parameter	*Range*	n	*Range*	n	*Range*	n	*Range*	n	*Range*	n
TS[a]	42.45–45.65	16	45.66–48.86	8	48.87–52.07	2	52.08–55.23	2	55.24–58.49	2
Fat[a]	21.08–22.78	8	22.78–24.48	8	24.49–26.19	10	26.19–27.89	2	27.90–29.60	2
NaCl[a]	0.56–0.94	10	0.94–1.32	8	1.32–1.69	6	1.70–2.07	0	2.08–2.45	6
pH	3.90–4.30	6	4.40–4.70	0	4.80–5.10	2	5.20–5.50	12	5.50–5.90	10
Acidity[b]	0.11–0.75	10	0.76–1.29	10	1.30–1.83	2	1.84–2.37	4	2.38–2.91	4
Protein[a]	17.87–20.17	4	20.17–22.47	4	22.47–24.78	10	24.78–27.08	6	27.09–29.39	6
W-SN[c]	3.63–4.83	12	4.84–6.04	4	6.05–7.25	10	7.26–8.46	2	8.47–9.67	2
TCA-SN[c]	2.84–3.81	10	3.82–4.79	6	4.80–5.77	8	5.78–6.75	4	6.76–7.73	2
PTA-SN[c]	1.54–2.21	12	2.22–2.89	6	2.90–3.57	8	3.58–4.25	2	4.26–4.93	2

[a]Expressed as percentage.

[b]Expressed as percentage of lactic acid.

[c]Expressed as percentage of total nitrogen.

n, number of samples; W-SN, water-soluble nitrogen; TCA-SN, trichloroacetic acid-soluble nitrogen; PTA-SN, phosphotungstic acid-soluble nitrogen.

The highest frequency distribution was observed for TS values corresponding to the lowest levels of this parameter (included in the 42.45–45.65% range), indicating that the majority of Corrientes artisanal cheeses have high moisture content, an important spoilage factor. This result could be explained by the fact that these cheeses are placed for sale when they are fresh and, as is the case with many raw milk high-moisture cheeses that are microbially challenged and not refrigerated, they have very short shelf lives.

The highest frequency interval for fat content was found to be in the range of 24.49–26.19%. Conversely, NaCl content exhibited a bimodal distribution, showing two different cheese populations, the most representative (80% accumulated frequency) involving cheeses with a low salt content of ≤ 1.7%: and a minor population (20%) that included the high salty cheeses (2.08–2.45%). Similar results were found when acidity was measured, in which a high accumulated frequency (67%) for values between 0.22 and 1.29% and a low accumulated frequency (27%) for 1.84–2.91% values were obtained. The pH values included in the highest frequency (93%) in the range of 5.20 and 5.90 were similar to those observed in Manchego cheese (Ballesteros *et al*. 2006), or in San Simón cheese (García Fontán *et al*. 2001), although they were lower than those reported by Torres-Llanez *et al*. (2005), who found pH values near to 6.3 and 6.0 for an artisanal Mexican Fresco cheese. Since pH values higher than 5.0 will provide optimal conditions for pathogen growth, with a corresponding decrease of cheese hygienic quality, slightly lowering the pH of Corrientes cheese by selecting highly acidifying starter cultures could be a mode to improve the safety of this artisanal product without significantly altering is particular characteristics.

The frequency distribution for protein content (33%) showed a range from 22.47 to 24.78% and two intervals of 20% frequency for higher protein values (24.78–27.08%). The distribution of W-SN product also showed two different cheese populations with values lower than 4.84%-TN, and the most representative fraction with highest frequency of 33% at 6.05–7.25%-TN interval. The TCA-SN fractions distribution showed the highest frequency (33%) for values lower than 3.82%, and a second group (27% accumulated frequency) for values at the 4.80–5.77%-TN interval. W-SN and TCA-SN are normally used to evaluate the evolution of proteolysis during cheese ripening (Daigle *et al*. 1999). It was not surprising that these levels were relatively low because Corrientes cheese is not ripened and therefore proteolysis is expected to be low.

The levels of PTA-SN/TN may be considered, according to some authors (Hickey *et al*. 1983), as aroma and taste indicators of cheeses. In this study, the PTA-SN fractions showed a frequency of 40% for values ranging from 1.54 to 2.21%-TN and a frequency of 27% for a minor cheese population with values from 2.90 to 3.57%-TN interval. The variability in the chemical composition of milk, the environmental conditions of ripening and the microbial diversity may determine protein degradation during cheese ripening, which is important in defining the final product type. Since Corrientes artisanal cheese is sold as a fresh product, it can be assumed that little protein degradation has occurred, since most microbial proteolysis takes place during cheese resting/ripening and could account for some of the typical organoleptic characteristics of this artisanal cheese.

As was the case for the physicochemical properties, the microbial ecology of Corrientes artisanal cheese varies significantly, as can be observed by the presence of extended intervals with high SDs (Table 3).

Frequency distributions (shown in Table 4) indicate a high incidence of total coliforms (53%), with values ranging between 4.56 and 6.07 log colony-forming units (CFU)/g. When the frequency distribution for *E. coli* was determined, a frequency of 37% was found for cell counts ranging between 2.58 and 3.86 log CFU/g, while in 20% of cheese samples evaluated, this microorganism was

Table 3 Main microbial populations (log colony-forming units per gram (CFU/g)) in Corrientes artisanal cheeses

Microbial population	*Mean ± SD*[a]	*Minimum*	*Maximum*	*Range*
Total coliforms	4.88 ± 1.41	< 0.48	9.04	8.56
Escherichia coli	2.99 ± 1.75	< 0.48	7.70	7.22
Staphylococcus aureus	< 2.00 ± 1.95	< 2.00	6.50	4.5
Moulds and yeasts	5.40 ± 1.62	< 2.00	9.70	7.7
Moulds	4.85 ± 1.83	< 2.00	9.4	7.40
Yeasts	5.36 ± 1.62	< 2.00	9.4	7.40
Total lactic acid bacteria	9.05 ± 1.43	6.00	11.11	5.11
Lactococci	8.79 ± 1.54	5.81	10.96	5.15
Leuconostoc	8.20 ± 1.59	5.20	10.72	5.52
Lactobacilli	8.27 ± 1.52	5.30	10.38	5.30

[a]Means of three independent trials (log CFU/g) ± standard deviation (SD).

Table 4 Frequency distribution of microbiological composition of the artisanal cheeses marketed in Corrientes

Microbial population	*Interval*											
	1		*2*		*3*		*4*		*5*		*6*	
	Range[a]	n	*Range*	n	*Range*	n	*Range*	n	*Range*	n	*Range*	n
Total coliforms	< 1.51	1	1.51–3.03	2	3.04–4.55	6	4.56–6.07	16	6.08–7.59	4	7.60–9.11	1
Escherichia coli	< 1.28	6	1.28–2.57	3	2.58–3.86	11	3.87–5.15	7	5.16–6.44	2	6.45–7.73	1
Staphylococcus aureus	< 2.00	17	2.00–2.90	1	2.91–3.81	4	3.82–4.72	4	4.73–5.63	3	5.64–6.54	1
Moulds and yeasts	< 2.00	1	2.00–3.48	2	3.49–4.97	9	4.98–6.46	11	6.47–7.95	5	7.96–9.74	2
Total lactic acid bacteria	6.00–6.85	1	6.86–7.71	2	7.72–8.57	8	8.58–9.43	6	9.44–10.29	5	10.30–11.15	3

[a]Log colony-forming units per gram. *n*, number of samples.

detected at a low level (1.28 log CFU/g). The raw milk and the artisanal rennet used in Corrientes cheese production, as well as the high moisture and low salt content found, could promote the growth of coliforms and *E. coli* (found in all cheese samples) which could pose a significant risk to consumers. However, it is positive to note that the enterotoxigenic *S. aureus* was detected (in low levels) in less than 3% of cheese samples.

Moulds and yeasts counts, which are environmental contamination indicators, reached a maximum frequency (37%) at levels of 4.98–6.46 log CFU/g. When these populations were compared, mould numbers were found to be 0.5 logarithmic cycles lower than those of yeasts (Table 3). Yeast counts in this artisanal cheese were higher than those reported by other authors who have studied the microbiology of bovine cheeses made from raw milk without addition of starter cultures. In fact, mean yeast counts per gram of cheese ranging from 2.7 to 6.4 log CFU/g were observed in an artisanal Portuguese ewes' cheese (Pereira-Dias *et al*. 2000); Hatzikamari *et al*. (1999) published yeast counts from 10^3 in curd to 10^5 CFU/g in 1-month-old Greek Anevato cheese. The high number for yeast found in this study may account for the typical organoleptic characteristics of Corrientes cheese, since recent investigations have shown that some lipolytic and proteolytic enzymes produced by these microorganisms contribute to the development of aroma and flavour compounds (Marino *et al*. 2003).

LAB constituted the predominant bacterial group, with a population levels higher than 7 log CFU/g with a maximum incidence (27%) within the interval from 7.72 to 8.57 log CFU/g (Table 4), these numbers being lower than those obtained in other artisanal raw cow milk cheeses (Estepar *et al*. 1999; Menéndez *et al*. 2001). Among LAB, lactococci were found in higher numbers (8.79 ± 1.54) when compared with leuconostocs and lactobacilli (Table 3). These results indicate that lactococci constituted the predominant bacterial group. Even when enterococci appear to be ubiquitous in the artisanal cheese environment (Torres-Llanez *et al*. 2005; Ballesteros *et al*. 2006), they were not detected in Corrientes cheese. It was established that their major contribution to the ripening process is the utilization of lactic acid which, by increasing the pH, promotes the growth of bacteria sensitive to acidic environments, thus helping to initiate ripening (Gardini *et al*. 2003). High LAB concentrations could cause an early ripening of cheese and be at least partially responsible for the 'mature (ripened) flavour' of Corrientes cheese even though this product is sold fresh without ripening.

From these microbial results and those obtained previously by Pereira *et al*. (1995), Corrientes artisanal cheese could be considered unsafe from a hygienic point of view and would be categorized as

unacceptable by the Technical General MERCOSUR Regulations (Mercosur 1993). From a microbiological point of view, the high bacterial counts caused by deficient handling practices and environmental contamination should be improved for the safety of consumers. Selection of suitable LAB starter cultures with required technological traits to produce the specific characteristics of this interesting cheese in addition to antipathogenic properties (high acidifying capacities, antimicrobial production, etc.) could be an ideal solution to improve the hygienic condition of Corrientes cheese.

The chemical and microbial evaluation of this artisanal cheese reported in this study is beneficial for not only consumers but also manufacturers, since it will help them to standardize their production practices and allow them to produce higher quality and more stable products with well-defined characteristics.

Further knowledge of the natural microbial communities present in this artisanal cheese may help to prevent the loss of microbial diversity associated with local and regional traditions. International legal regulations have been developed to protect biodiversity throughout the world. In this regard, the preservation of autochthonous strains is of major interest since they are important biological and genetic resources. Since Corrientes artisanal cheese has not yet received a Designation of Origin classification, it is not protected under international laws. The isolation and conservation of the native microbiota found in this product must be well characterized and preserved in order to prevent modifications and losses of important microbial species.

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