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Chemical and microbiological characteristics of traditional homemade fresh goat cheeses from Northern Morocco

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ABSTRACT

A chemical and microbiological survey was performed on 28 fresh raw goat's milk cheeses from Northern Morocco. Cheeses were characterized by their low pH values (3.81–4) and low dry matter contents (24.9–31.2 g/100 g). Fat, protein and ash contents were 14. 4–17.1, 8.3–10.3, and 1.12–1.75 g/100 g, respectively. High levels of FFA were determined (3260–3507 mg/kg), the most abundant being oleic, stearic, palmitic, myristic and capric acid. Lactic acid bacteria (LAB) were the dominant microbiota (7.9–9.7 log cfu/g), and yeasts and total coliforms were also present in high numbers (5–7 log cfu/g). *Salmonella* spp. was not detected in any of the cheese samples. *Listeria monocytogenes* was detected in four cheeses. A total of 294 LAB isolates were identified by PCR as *Enterococcus* spp. (249 isolates), *Lactococcus lactis* (36), *Lactobacillus plantarum* (7) and *L. paracasei* (2).

1. Introduction

In the mountainous regions of Northern Morocco, the sectors of agriculture and stockbreeding are poorly developed and show a low productivity. The populations of the area usually live under precarious conditions. Therefore, goat's breeding has been the main occupation of many people since ancient times, and goat's milk and cheese have always been a fundamental part of their economy, diet and cultural heritage. In Northern Morocco, cheese is produced traditionally in rural households, and these artisanal products are usually sold in local open markets and supermarkets.

Due to their specific composition, organoleptic characteristics and healthy attributes, the production of goat's milk and goat cheese has attracted growing interest over recent years (Silanikove et al., 2010). Goat's milk fat and protein are more easily digestible than those of cow's milk and it contains higher levels of vitamin A, thiamine and niacin (Haenlein, 2001). In addition, the lower allergenic capacity compared to cow's milk (Guo et al., 1998) makes goat's milk an alternative for people who cannot tolerate cow's milk.

Cheeses made from goat's milk are greatly appreciated because of their particular organoleptic characteristics. The composition of the lipid fraction plays an essential role in the sensory attributes of these products. The fatty acids hexanoic (caproic), octanoic (caprilic) and decanoic (capric), together with certain branched-chain free fatty acids, are responsible for the characteristic 'goaty flavour' of goat cheeses (Salles et al., 2002). Nevertheless, fresh and soft cheeses made with raw goat's milk constitute a suitable medium for the growth of many pathogens and have frequently been associated with several foodborne diseases in many countries. It has been pointed out that food infections caused by the consumption of raw milk cheese represent a threat to public health, leading to big economic losses (Milani et al., 2014).

Manufacture of homemade North-Morocco fresh goat cheese has not yet been standardized. Raw goat's milk is obtained from indigenous breeds of low milk production. The milk is filtered through a cloth filter in order to remove undesirable particles. No starter is added to the milk, which is usually curdled without cooling after milking. Commercial calf rennet is usually employed to coagulate the milk, although in some cases vegetable rennet (crude aqueous extract from *Cynara cardunculus* flowers) is used. The coagulation time varies between 1 and 24 h. The curd is usually cut by hand into 3–12 cm pieces, mixed and salted (usually 1–2 g salt per litre of milk) in the cheese vat. In order to remove the whey, the curd

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is sieved for 2–3 h without pressure or with light pressure. Finally, cheeses are moulded or shaped, usually like a parallelepiped or block, and stored at 10–15 °C during 1–5 days. Traditionally, the cheeses are moulded and kept in braided palm-leaf moulds until they are sold.

To our knowledge and up to now, no scientific data have been published concerning goat's milk cheeses from Northern Morocco. So, the aims of the present work were: (i) to study some compositional and chemical data of these particular dairy products, and (ii) to characterize the cheeses from a microbiological point of view, including the determination of the most common bacterial pathogens and the identification of the main lactic acid bacteria (LAB) isolated from these naturally fermented products.

2. Materials and methods

2.1. Collection of the cheeses

Twenty-eight raw goat's milk block-shaped cheeses (1000 ± 200 g in weight) manufactured in the winter–spring season were acquired from different small domestic producers from Northern Morocco. Cheeses were collected immediately after their manufacture, and stored during 5 days at 6 ± 1 °C before being analyzed. The cheeses were classified into three groups in accordance with their manufacturing conditions (Table 1): (i) process 1: 14 cheeses made with calf rennet without milk cooling after milking, which is the most habitual practice; (ii) process 2: 7 cheeses made with calf rennet with milk cooling after milking (milk is cooled to 4–5 °C in approximately 6 h in cold storage chambers); and (iii) process 3: 7 cheeses made with vegetable rennet without milk cooling after milking.

2.2. Chemical analyses

Two slices of 50 ± 2 g were cut from one edge and from the middle of the cheese block, and placed in a plastic waterproof bag (Polyskin X, Amcor Flexibles Hispania S.L., Barcelona, Spain). The pieces were thoroughly kneaded by manual pressing to a homogeneous paste and portions were taken for chemical and free fatty acid (FFA) analyses.

Cheeses were analyzed following the International Dairy Federation (IDF) Standards for moisture content (IDF, 1982), fat content (IDF, 1997), protein content (IDF, 1986), and ash content (IDF, 1964). The pH was determined on 10 g samples weighted in a blender bag and suspended in 20 mL of distilled water previously heated at 70 °C. The mixture was homogenized by means of a laboratory peristaltic blender (Masticator, IUL, Barcelona, Spain) for 60 s, and the pH of the resultant slurry was measured at room temperature using a digital pH-metre (pH 211, Hanna Instruments, Padova, Italy). All the analyses were performed in triplicate samples.

2.3. Free fatty acid analysis

Samples for gas chromatographic analysis were processed as described by Poveda and Cabezas (2006). Lipid extraction was carried out on an acidified cheese (10 g) slurry using diethyl ether; methylation of the fatty acids was performed with 20% trimethylanilinium hydroxide (TMAH) in methanol. The TMAH soaps (in the lower layer) were neutralized before injection and pyrolised to methyl esters in the chromatograph injector.

Free fatty acids (FFA) were determined by gas chromatography with flame ionization detection (FID), as described by De la Fuente et al. (1993) with some modifications. Analyses were carried out in a Varian model 3800 instrument (Varian Inc., Palo Alto, CA, USA) equipped with an automatic sampler (CP Wax 52CB, Varian) and a programmable temperature vaporizer (PTV) injector. Free fatty acids were separated on a fused-silica capillary column (DB-FFAP; 30 m × 0.25 mm i.d. × 0.25 μm, Agilent Technologies, Wilmington, DE, USA), with helium as carrier gas for 1 mL/min (split/splitless ratio 1:20). Oven temperature was held at 60 °C for 2 min, then raised to 180 °C at a rate of 5 °C/min, and held at 180 °C for 60 min. The PTV programme settings were: 60 °C initial temperature, 300 °C at 0.05 min, and 25 °C from 4 min to the end. Detector temperature was 250 °C. Quantification of FFA was carried out with pentanoic, nonanoic and heptadecanoic acids as internal standards added to the cheese sample at the time of extraction. Analyses were performed on duplicate samples.

2.4. Microbiological analyses

Microbiological analyses were carried out on representative 10 g samples removed aseptically from the inner part of the cheese block. Cheese samples were transferred to sterile blender bags, then 90 mL of a sterile 2% (w/v) sodium citrate (Panreac, Barcelona, Spain) solution prewarmed at 45 °C was added, and the mixture was homogenized in a laboratory blender (Masticator, IUL, Barcelona, Spain) for 60 s at room temperature. Sample preparation and serial decimal dilutions were carried out in accordance with the IDF Standard 122C (IDF, 1996).

The following microbial groups were investigated by mixing 1 mL of the corresponding dilutions with the appropriate culture media, or by spreading aliquots of 100 μL on the selective media (yeasts and moulds, enterococci and coagulase-positive staphylococci): (a) mesophilic aerobic bacteria on plate count milk agar (Oxoid, Basingstoke, UK) incubated at 30 °C for 72 h; (b) psychrotrophic aerobic bacteria on plate count milk agar (Oxoid), incubated at 7 °C for 10 days; (c) lactic acid bacteria (LAB) on pH 5.7 MRS agar (Merck GmbH, Darmstadt, Germany), incubated under anaerobic conditions (Anaerocult® A, Merck) at 30 °C for 5 days; (d) yeasts and moulds (presumptively differentiated by colony appearance) on oxytetracycline-glucose-yeast extract agar (OGYE agar) (Oxoid), incubated at 25 °C for 5 days; (e) enterococci on Slanetz and Barley agar (Merck), incubated at 44 °C for 48 h; (f) total coliforms on violet red bile (lactose) agar (VRBA) (Oxoid), overlaid and incubated at 37 °C for 24 h; (g) faecal coliforms on VRBA (Oxoid), overlaid and incubated at 44 °C for 24 h; and (h) (lecithinase-) coagulase-positive staphylococci on Baird–Parker agar base supplemented with egg yolk tellurite emulsion (Oxoid), incubated at 37 °C for 48 h. All analyses were performed in duplicate.

Detection of the pathogens *Salmonella* spp. and *Listeria monocytogenes* was carried out on 25 g samples taken as described previously. Cheese samples for *Salmonella* spp. detection were added to 225 mL of pre-enrichment solution (1% buffered peptone water, Merck) and then homogenized and incubated (37 °C for 24 h) according to IDF Standard 93B (IDF, 1995). Rappaport-Vassiliadis broth (Merck) and Rambach agar (Merck) were used for selective enrichment (42 °C for 24 h) and isolation (37 °C for 24 h), respectively. *L. monocytogenes* was determined according to Barancelli et al. (2011). Three to five typical colonies taken from the selective agar medium of Ottaviani and Agosti (ALOA) (Biolife, Milano, Italy) were confirmed by the API *Listeria* identification kit (BioMerieux, Marcy L'Etoile, France).

2.5. Isolation, maintenance and phenotypic identification of LAB

Four to six colonies from pH 5.7 MRS agar and plate count milk agar (incubated at 30 °C) plates corresponding to the highest dilutions at which growth occurred were randomly selected and sub-cultured to purity by streaking on MRS agar. Gram-positive, catalase-negative cultures were maintained at −20 °C and −80 °C in MRS broth containing 20% glycerol (v/v). Working cultures were prepared by two consecutive transfers in MRS broth at 30 °C. Isolates were phenotypically assigned to the genus level as described by Garabal et al. (2008). Criteria followed for phenotypic identification were those compiled by Wood and Holzapfel (1995).

2.6. PCR-identification of LAB isolates

Isolates of presumptive lactococci, lactobacilli and enterococci were assigned to the genus or species level by PCR, with the use of specific primers (Table 2).

The total DNA for PCR reactions was obtained by a freeze/unfreeze shock method, as described by Garabal et al. (2008). Lysed crude extracts were stored at −20 °C until being used as templates (1.2 μL) for PCR. The PCR primers employed in the present study were synthesized by Invitrogen™ Custom DNA Oligos (Life Technologies Corporation, Carlsbad, CA, USA). In general, PCR was performed in a 15 μL mixture containing 1.2 μL DNA template, 50 pM – 0.5 μM primer, 0.2 mM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 75 mM Tris–HCl, 20 mM $(NH_4)_2SO_4$, 0.01% Tween® 20 and 0.625 units of Thermoprime Taq DNA polymerase (2× Ready mix™ PCR Master Mix, ABgene, UK).

The PCR was performed in a DNA-Thermal cycler (BioRad 580 BR 7779, Applied Biosystems, Foster City, CA, USA) with different programmes to ensure experimental specificity (Table 2). The PCR-products were resolved by electrophoresis, at 110 V, on 1.5 g/100 mL agarose containing 0.5 μg/mL ethidium bromide. Defined type strains of each species, obtained from the Colección Española de Cultivos Tipo (CECT, Valencia, Spain), were included in each reaction as positive or negative controls.

2.7. Statistical analysis

Chemical and microbiological data obtained for the different cheese groups were subjected to analysis of variance (ANOVA) and where statistical differences were noted, differences among the distinct groups were determined by the Duncan's test. Differences were considered significant at $P < 0.05$. Statistical procedures were performed with the software package SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Chemical parameters

The results obtained for the chemical parameters and FFA contents determined in the three cheese groups are shown in Table 3. The mean pH values ranged from 3.81 (process 3; cheeses made with vegetable rennet) to 4.00 (process 1; cheeses made with calf rennet and without milk cooling). No significant differences were found among the different manufacturing processes. These