Preliminary microbiological and chemical characterisation of edible goat’s rennet, a unique product of Sardinian food tradition

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ABSTRACT

The edible goat rennet (EGR) namely Caggiu de crabittu, is a traditional Sardinian foodstuff deriving from the stomach of a weaned (breastfed) kid whose edible part is represented by the milk coagulated inside the abomasum that, before consumption, is subjected to a suitable ripening time. In this study, a preliminary investigation into the microbiological characteristics and physicochemical parameters of different EGRs manufactured by distinct Sardinian farms was conducted. Results showed that the edible goat rennet was free of spoilage/pathogenic bacteria and was characterised by a significant presence (6–7 log cfu/g) of mesophilic lactic acid bacteria (i.e. \textit{Lactococcus lactis}, \textit{Lactobacillus plantarum}, \textit{Lactobacillus paracasei}, \textit{Lactobacillus brevis}) and \textit{Enterococci}. Oleic, linolenic, palmitic and myristic acids were the most abundant free fatty acids (FFA) in all samples, while both caprylic and butyric acid contents resulted the lowest. Long chain FFA (\textgreater C18:0) represented about 50% of total FFA. Among the polyunsaturated FFA, high content of linoleic (C18:2n-6) and alpha-linolenic (C18:3n-3) acids have been detected. In this study, EGR is shown to be microbiologically safe, with a high number of live lactic bacteria and an FFA content that is attractive from a nutritional point of view.

HIGHLIGHTS

- Edible goat’s rennet (EGR) resulted microbiologically safe with a high number of viable mesophilic lactic acid bacteria.
- In the EGR an intense process of lipolysis has occurred.
- EGR had a high content of linolenic acid.

Introduction

Kid or lamb rennet paste is commonly used as a coagulant in traditional cheese manufacturing in a number of European Countries (Deiana et al. 1980; Mangia et al. 2011; Fresno et al. 2014). Their employment in cheesemaking confers a characteristic piquant taste to the cheese and preserves its traditional traits (Addis et al. 2008). However, an alternative use of goat rennet has long existed in Sardinia (Italy), where it is consumed as a traditional foodstuff (and listed in the list of Sardinian Traditional Agro-foods (http://www.sardegnaagricoltura.it/index.php?xsl=443&s=45004&v=28c=3595), namely Caggiu de crabittu or ‘edible goat’s rennet’ (EGR).

Currently, EGR is still being produced by goat farmers using artisanal methods, similar to those applied in the production of coagulant rennet (Moschopoulou 2011). Being an artisan process, each manufacturer has developed his/her own methodology, reflecting their own experience; as such, the production process is far from being standardised. It is likely that this has led to a degree of variability in the microbiological and physicochemical features of the foodstuff, as well as its overall safety and quality, which had never been investigated until now.

The main steps involved in the manufacturing of EGR are the following: the kid abomasum, devoid of perivisceral fat and full of suckled milk, is air dried for 20-40 days. If the milk content of the abomasum is insufficient, it is commonly supplemented with raw (freshly milked) goat’s milk. The edible part is the coagulated milk inside the abomasum. Before consumption, this product is subjected to a suitable ripening time (on average 30–60 days) in a cool, dry place, and...
the level of its creaminess depends on the ripening time. The husbandry techniques used to raise the kids also play an important role in EGR production: as soon as they are born, they are removed from their mothers and they are only returned for feeding. This forced isolation may mean that the microorganisms present in the abomasum are only sourced from the suckled milk, from the surface of the teat, or from the teat canal (Gougoulis et al. 2007).

Knowledge about the enzyme complexes responsible for the proteolysis and lipolysis processes in this product can be only deduced from studies on animal rennet used in cheesemaking. The general composition of kid paste rennet is determined by the activity of chymosin, pepsin and lipase (Addis et al. 2008). When the abomasum is removed from the animal, the kid’s pre-stomachs have not yet developed, and the function of the digestive system is similar to that of monogastric animals. Suckled milk quickly coagulates in the extremely acidic environment of the abomasum, where it is partially digested by the action of a specific proteases on casein (Collins et al. 2003) and lipases on fat (Villeneuve et al. 1996), before moving into the duodenum. The lipolytic enzymes release free fatty acids (FFA), especially short- and medium-chain FFA, which contribute directly to cheese flavour (Addis et al. 2005). Due to the peculiarities and the rarity of this foodstuff, no comparable experimental data exist in relation to EGR, and information regarding its safety and nutritional traits are essentially missing. However, to protect and promote this traditional niche product, a microbiological and physicochemical characterisation is essential. Accordingly, a preliminary investigation into the microbiological characteristics and physicochemical parameters of different EGRs manufactured by distinct Sardinian farms was conducted and is reported herein. This first study provides the necessary starting point from which to plan further research into the microbiota and the biochemical processes that characterise EGR production.

**Materials and methods**

**Edible goat rennet origin**

Nine *Caggiu de crabittu* (manufactured as described in the section ‘Introduction’) at 40 days of ripening were collected from three different producer’s representatives of the typical area of production (Ogliastra, Sardinia). These samples were analysed after storage at 5°C per 3 h, from a microbiological and physicochemical point of view respectively.

**Microbiological analyses**

For microbiological analyses, 10 g of EGR samples (three samples for each producer) were homogenised in 90 mL sterile Ringer’s solution for 2 min in a Stomacher Lab Blender 80 (PBI). Aliquots (1 mL) were 10-fold diluted in Ringer’s solution and plated/inoculated on the specific media used to quantify different microbial groups. In particular, microbiological analyses targeted the presence of the following microbial groups: total microbial count, lactococci and lactobacilli, enterococci, staphylococci, yeasts, total and faecal coliforms and spores of sulphite-reducing clostridia. The following media and incubation conditions were used for the enumeration of the different microbial groups: aerobic mesophilic bacteria in Plate Count Agar (Oxoid, Milan, Italy) incubated at 30°C for 48 h; presumptive mesophilic streptococci in M17 agar incubated at 22°C for 72°C, presumptive thermophilic streptococci in M17 incubated at 45°C for 48 h in anaerobic conditions (Gas-Pack; Oxoid); presumptive mesophilic and thermophilic lactobacilli in MRS agar (Oxoid) acidified to pH 5.4 incubated in anaerobic conditions (Gas-Pack; Oxoid) at 22°C and 45°C for 72 h respectively; presumptive enterococci in Kanamycin Aesculin Azide Agar Base (Biolife) incubated at 37°C for 48 h in anaerobic condition; staphylococci in Baird Parker Agar (Oxoid) supplemented with Egg Yolk Tellurite Emulsion (Oxoid) incubated at 37°C for 48 h; yeasts in YPDA (1% w/v yeast extract, 2% w/v dextrose, 2% w/v peptone, 1.5% w/v agar, pH 4.5) incubated at 25°C for 48 h; total and faecal coliforms (MPN method) in Brilliant Green Bile Broth (Oxoid) incubated at 37 and 44°C, respectively for 48 h; spores of sulphite-reducing clostridia (MPN method) in DRCM broth (Oxoid) after heat treatment (80°C for 10 min) of the samples, and incubation at 37°C for 48 h in anaerobic conditions.

**Isolation and phenotypic characterisation of LAB isolates**

To analyse the lactic acid bacteria (LAB) population of each sample, 10 colonies were randomly picked from M17, MRS and KAA agar plate, and purified by successive streaking in the same medium. All isolates were preliminarily characterised by determining their Gram stain, catalase production and shape morphology (phase contrast microscopy; Zeiss, Gottingen, Germany) and stored at −80°C in MRS (rods) or M17 (cocc) broth with 30% (v/v) glycerol.

Gram positive and catalase negative coccal-shaped LAB were presumptively identified according to the
following test: growth capability at 10 and 45; growth capability at pH 9.6 and in the presence of 4.0 and 6.5% NaCl; Gram positive and catalase negative rod-shaped LAB were tested for growth capability at 15 and 45°C and gas production from glucose. All tests were performed according to the methods reported by Mangia et al. (2011). Carbohydrate fermentation patterns of rod-shaped were determined by using API 50 CHL test galleries (API System bioMerieux, Marcy l’Etoile, France), while for both coccal-shaped LAB and enterococci API 20 STREP galleries were used.

**Physicochemical characteristics**

The following physicochemical parameters were determined for each EGR: total solids (TS) (IDF 1982), fat (IDF 1986), proteins (Butikofer et al. 1993), pH (Crison Instruments SA, Barcelona, Spain).

The FFAs content of the EGR samples was analysed by gas chromatography according to de Jong and Badings (1990). The method was modified as described by Madrau et al. (2006). Briefly, FFAs (C4–C18:3) were separated using a Nukol capillary column (15 m, 0.53 mm i.d., 0.50 mm Df; Sigma-Aldrich Co., St. Louis, USA) using an HP 5890 series II gas chromatograph (Hewlett-Packard Co.) with an autosampler and a flame ionisation detector. Data acquisition was carried out using the HP Chemstation Rev. A.06.03 software (Hewlett-Packard Co.).

**Statistical analysis**

The results of physicochemical and microbiological analyses (transformed into respective decimal logarithms to fit a normal distribution) were subjected to one-way ANOVA and to the post-hoc Fisher’s least significant difference test to separate means. The statistical analysis was carried out using the Statgraphics Centurion XV software (2005).

**Results and discussion**

Table 1 shows the microbial groups recovered from EGR samples collected from three different farms. The total microbial count was approx. 7 log cfu/g with no significant differences between farms. Similar values were obtained for mesophilic streptococci, although the viable counts were lower in the samples from farm B (p < .05) than from farms A and C. The counts of mesophilic lactobacilli were slightly higher (~0.4 log cfu/g) in the samples from farms A and B compared with samples from farm C. Enterococci were

| Microbial groups | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
|------------------|----|----|----|----|----|----|----|----|----|
| Total microbial count | 7.44 ± 0.26 | 7.47 ± 0.19 | 7.47 ± 0.19 | 7.60 ± 0.12 | 7.71 ± 0.14 | 7.36 ± 0.54 | 7.69 ± 0.41 | 7.75 ± 0.33 | 7.50 ± 0.37 |
| Mesophilic streptococci | 7.47 ± 0.38 | 7.38 ± 0.16 | 7.28 ± 0.08 | 6.49 ± 0.29 | 6.54 ± 0.17 | 6.54 ± 0.17 | 6.54 ± 0.17 | 6.54 ± 0.17 | 6.54 ± 0.17 |
| Thermophilic streptococci | <10 <10 <10 <10 <10 <10 <10 <10 <10 |
| Mesophilic lactobacilli | 6.72 ± 0.22 | 6.65 ± 0.27 | 6.67 ± 0.24 | 6.69 ± 0.28 | 6.69 ± 0.28 | 6.69 ± 0.28 | 6.69 ± 0.28 | 6.69 ± 0.28 | 6.69 ± 0.28 |
| Thermophilic lactobacilli | <10 <10 <10 <10 <10 <10 <10 <10 <10 |
| enterococci | 3.44 ± 0.25 | 4.06 ± 0.36 | 3.84 ± 0.06 | 2.08 ± 0.07 | 1.09 ± 0.15 | 1.09 ± 0.15 | 1.09 ± 0.15 | 1.09 ± 0.15 | 1.09 ± 0.15 |
| yeasts | <10 <10 <10 <10 <10 <10 <10 <10 <10 |
| total coliforms | <10 <10 <10 <10 <10 <10 <10 <10 <10 |
| faecal coliforms | <10 <10 <10 <10 <10 <10 <10 <10 <10 |
| spores of sulphite-reducing clostridia | <10 <10 <10 <10 <10 <10 <10 <10 <10 |

Average values of three determinations ± SD. Values in the same row with different superscripts are statistically different (p < .05). ANOVA method.
present in all samples, but at significantly different concentrations (ranging from 1.09 to 4.57 log cfu/g). In particular, the samples collected from farm C had the highest enterococci counts, whereas the lowest values were obtained in samples from farm B. Neither thermophilic streptococci nor lactobacilli were detected in any samples.

Preliminary characterisation of LAB isolates, from MR5 and M17 plates, allowed us to identify the rods growing at 15 °C (but not at 45 °C). Those not producing gas from glucose were presumptively considered to be mesophilic homofermentative lactobacilli; likewise, rods growing only at 15 °C and producing gas from glucose were considered to be mesophilic heterofermentative lactobacilli. Cocci only growing at 10 °C on media containing 4% NaCl were considered to be lactococci, whereas cocci growing at both 10 and 45 °C on media containing 6.5% NaCl and at pH 9.6 were presumptively considered as enterococci.

The phenotypic identification of LAB isolates showed that *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus paracasei* group, *Lactobacillus brevis*, *Enterococcus faecalis* and *Enterococcus faecium* were present in all samples (Table 2). A similar microbial composition has been found in Fiore sardo – a Sardinian sheep’s cheese – (Mangia et al. 2008), Orinotyri – a Greek sheep’s cheese – (Prodromou et al. 2001) and a Moroccan traditional cheese (Galiou et al. 2015), whose technologies do not involve any heat treatment (milk pasteurisation and/or curd heated).

Our results showed that distribution of microbial species depended on the EGR origin; for example, in samples A and C, *Enterococcus* spp. dominated the LAB populations, whereas in samples from farm B, *L. paracasei* and *L. plantarum* were most predominant.

Table 2. *Lactococcus*, *Lactobacillus* and *Enterococcus* species number isolated from nine edible goat rennet samples from A, B, C farmstead.

|                        | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
|------------------------|----|----|----|----|----|----|----|----|----|
| *Lactococcus lactis*   | 8  | 9  | 9  | 5  | 3  | 4  | 4  | 4  | 3  |
| *Lactobacillus casei*  | 4  | 2  | 3  | 4  | 2  | 4  | 5  | 2  | 6  |
| *Lactobacillus plantarum* | 1 | 3  | 3  | 5  | 5  | 4  | 5  | 8  | 4  |
| *Lactobacillus brevis*  | nd | nd | nd | 1  | 3  | 2  | nd | nd | nd |
| *Enterococcus faecium/durans* | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

|                        | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
|------------------------|----|----|----|----|----|----|----|----|----|
| *Lactobacillus lactis*   | 8  | 9  | 9  | 5  | 3  | 4  | 4  | 4  | 3  |
| *Lactobacillus casei*  | 4  | 2  | 3  | 4  | 2  | 4  | 5  | 2  | 6  |
| *Lactobacillus plantarum* | 1 | 3  | 3  | 5  | 5  | 4  | 5  | 8  | 4  |
| *Lactobacillus brevis* | nd | nd | nd | 1  | 3  | 2  | nd | nd | nd |

Nonetheless, no yeasts or microorganisms considered as indicators of the hygienic status, such as staphylococci, total and faecal coliforms and spores of sulphite-reducing clostridia, were detected in any of the samples. These results sustain the correct implementation of hygiene rules (and, therefore, associated animal welfare measures), in the farms involved in the study, with regard to breeding management and in all phases of the production process (Tormo et al. 2015). However, a degree of antibacterial activity by enterococci and mesophilic LAB cannot be ruled out (Mami et al. 2008; Oldak et al. 2017; Mangia et al. 2019).

Table 3 shows the chemical composition results of the EGR studied. These data reveal wide variability between and within the examined samples made by different manufacturers: total solids ranged from 47.96% to 77.85%; fat from 13.64% to 30.99% and protein from 10.19% to 22.45%. The pH value of EGR samples was approximately 4.9, with no significant differences between samples.

The results of the FFA analysis are reported in Table 4. All samples showed a similar FFA content: ranging from 6045 to 6913 mg/kg. These values were higher than those reported in goat cheeses ripened for a short period (Delgado et al. 2009, 2011; Galiou et al. 2015), suggesting that an intense process of lipolysis had occurred. Moreover, oleic, palmitic and myristic acids were the most abundant FFAs in all samples, in line with Van Nieuwenhove et al. (2009) and Galiou et al. (2015) in relation to fresh goat cheese. Long chain FFAs (≥C18:0) constitute almost 50% in all samples. Caprylic and butyric acid were the least prevalent in all samples. This is interesting since a high content of caprylic and butyric acid has been reported for a number of goat cheeses (Boutoial et al. 2013; Tripaldi et al. 2015). It is known that long chain FFAs do not contribute to cheese flavour as a consequence of their high perception thresholds compared with short and medium chain FAs; short chain FAs are mainly responsible for characteristic cheese flavours. Of the polyunsaturated FFAs (PUFA), linoleic (C18:2) and linolenic (C18:3) acids averaged from 170 to 200 and 127 to 179 mg Kg⁻¹, respectively. Interestingly, the linolenic acid content resulted significantly higher
Table 4. Compositional analysis of free fatty acids (mg Kg\(^{-1}\)) of edible goat rennet samples from A, B, C farms.

| FFAs | A1         | A2         | A3         | B1         | B2         | B3         | C1         | C2         | C3         |
|------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| C4:0 | 90.4 ± 0.45 | 96.31 ± 0.63 | 90.05 ± 0.47 | 96.25 ± 0.35 | 102.38 ± 0.54 | 96.21 ± 0.62 | 102.23 ± 0.42 | 90.51 ± 0.60 | 102.25 ± 0.58 |
| C6:0 | 223.57 ± 0.78 | 230.71 ± 1.40 | 224.75 ± 1.81 | 230.78 ± 0.81 | 236.03 ± 0.72 | 231.78 ± 1.87 | 236.39 ± 1.38 | 229.87 ± 1.72 | 236.30 ± 0.85 |
| C8:0 | 69.57 ± 0.42 | 69.62 ± 0.32 | 70.82 ± 0.60 | 70.22 ± 0.47 | 69.38 ± 0.25 | 70.43 ± 0.35 | 69.47 ± 0.47 | 72.35 ± 0.40 | 69.38 ± 0.28 |
| C10:0| 243.43 ± 2.30 | 237.66 ± 4.13 | 232.97 ± 1.04 | 248.33 ± 1.63 | 235.69 ± 1.24 | 246.69 ± 1.02 | 235.66 ± 1.24 | 253.95 ± 2.28 | 265.49 ± 1.50 |
| C12:0| 804.68 ± 3.24 | 819.74 ± 6.94 | 807.12 ± 4.25 | 819.56 ± 5.74 | 826.86 ± 2.90 | 817.11 ± 2.49 | 827.43 ± 2.97 | 810.50 ± 3.27 | 829.09 ± 5.96 |
| C14:0| 1014.28 ± 6.50 | 979.22 ± 5.38 | 1015.37 ± 5.09 | 976.33 ± 2.98 | 943.86 ± 6.79 | 978.39 ± 6.27 | 943.94 ± 4.97 | 1018.53 ± 9.63 | 942.80 ± 3.91 |
| C16:0| 1305.94 ± 5.42 | 1276.12 ± 9.60 | 1314.65 ± 5.30 | 1267.70 ± 7.41 | 1238.24 ± 8.67 | 1272.93 ± 7.42 | 1238.25 ± 8.67 | 1291.56 ± 2.58 | 1234.94 ± 4.44 |
| C18:0| 894.06 ± 4.02 | 761.04 ± 4.45 | 873.97 ± 2.20 | 771.51 ± 7.30 | 637.48 ± 3.73 | 764.60 ± 4.46 | 638.53 ± 4.59 | 900.86 ± 3.63 | 636.49 ± 1.94 |

Average values of three determinations ± SD. Values in the same row with different superscripts are statistically significantly different (p < .05).
than the values observed for other goat cheeses (Delgado et al. 2009; Galiou et al. 2015).

Analysis of the individual fatty acids highlights a level of variability between samples: for example, C1 and C3, produced by the same manufacturer, have a fatty acid profile similar to sample B2, which was produced by another manufacturer.

These results indicate that fat lipolysis was independent of the producer. It is thus probably that some intrinsic factors vary, even within the same farmstead, that are able to influence the composition and enzymatic activity of the EGR. Addis et al. (2005) reported that the lipase activities of rennet paste used as coagulant could also be attributed to slaughtering conditions, although variations in the procedures applied during the preparation by the same manufacturer cannot be excluded.

In addition, lipolytic enzymes of microbial origin may also be present. The high rate of mesophilic lactobacilli could suggest their involvement in the lipolysis process (Shakeel Ur et al. 2000; Albenzio et al. 2001) and, in particular, in the release of FFAs (Di Cagno et al. 2006). These latter authors concluded that high levels of palmitic and linoleic fatty acids in model cheese were due to L. casei subsp. pseudoplanatarum strains.

From a nutritional point of view, the edible goat’s rennet results are extremely noteworthy because of the high content of linoleic (C18:2n-6; LA) and alphalinolenic (C18:3n-3; ALA) acids – essential FAs for the human body, that is, it is unable to synthesise them (Secchiari et al. 2007). Research in both humans and laboratory animals have revealed many beneficial effects of both these fatty acids. In particular, ALA has been implicated to play an important role in the prevention of cardiovascular disease (Del Gobbo et al. 2016; Barbeau et al. 2017) and associated with enhanced anti-inflammatory activity (Ohue-Kitano et al. 2018) and the retardation of mental disorder (Yamagishi et al. 2017). Moreover, from ALA the human body can synthesise the very long chain omega-3FA, eicosapentaenoic acid, docosahexaenoic acid and stearidonic acid (18:4n-3), for which healthy properties have been documented (Barcelo-Coblijn and Murphy 2009). A beneficial dose-dependent relationship between the intake of linoleic acid and blood LDL cholesterol concentrations has also been acknowledged (EFSA 2010), as well as an effect in hypertension prevention (Tsukamoto and Sugawara 2018). In addition, taking into account that both L. casei and L. plantarum strains can use LA as a substrate for the synthesis of conjugated LA, known for its health benefits (Yadav et al. 2007; Khosravi et al. 2015), the presence of PUFA is a favourable nutritional value of the EGR product.

Conclusions
EGR is a derivative of raw goat’s milk. The properties of this unique food are linked, more than other dairy product, to the breeding activities and the traditional dairy processing techniques of Sardinia.

The peculiarity of this dairy product comes from the fact that the milk coagulation and all the microbiological and biochemical changes that characterise the cheese-making process take place inside the kid’s abomasum, an unusual ‘environment’ that is not used for any other dairy product. It is therefore our opinion that EGR could be fully considered a cheese with features suitable for obtaining a ‘denomination of origin’.

Finally, in this study, EGR is shown to be microbiologically safe, with a high number of live lactic bacteria and an FFA content that is attractive from a nutritional point of view. Further studies into the microbiological and chemical composition of EGR as well as a consumer acceptance evaluation are, however, necessary.

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The authors report no conflict of interest.

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References
Addis M, Cabiddu A, Pinna G, Decandia M, Piredda G, Pirisi A, Molle G. 2005. Milk and cheese fatty acid composition in Sheep fed Mediterranean forages with reference to conjugated linoleic acid cis-9,trans-11. J Dairy Sci. 88:3443–3454.
Addis M, Piredda G, Pirisi A. 2008. The use of lamb rennet paste in traditional sheep milk cheese production. Small Rum Res. 79:2–10.
Albenzio M, Corbo MR, Rehman SU, Fox PF, De Angelis M, Corsetti A, Sevi A, Gobbetti M. 2001. Microbiological and
biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk or by heating the curd in hot whey. Int J Food Microbiol. 67:35–48.

Barbeau PA, Holloway TM, Whitfield J, Baechler BL, Quadrilatero J, van Loon LJC, Chabowski A, Holloway GP. 2017. α-Linolenic acid and exercise training independently, and additively, decrease blood pressure and prevent diastolic dysfunction in obese Zucker rats. J Physiol. 595:4351–4364.

Barceló-Coblijn G, Murphy EJ. 2009. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Prog Lipid Res. 48:355–374.

Boutoial K, García V, Rovira S, Ferrandini E, Abdelkhalek O, López MB. 2013. Effect of feeding goats with distilled and non-distilled thyme leaves (Thymus zygis subp. gracilis) on milk and cheese properties. J Dairy Res. 80:448–456.

Butikofe U, Ruegg M, Ardo Y. 1993. Determination of nitro-

Cocolin L, Foschino R, Comi G, Fortina MG. 2007. Description of the bacteriocins produced by two strains of Enterococcus faecium isolated from Italian goat milk. Food Microbiol. 31:753–758.

Collins YF, McSweeney PLH, Wilkinson MG. 2003. Lipolysis and free fatty acid catalysis in cheese: a review of current knowledge. Int Dairy J. 13:841–866.

Deiana P, Farris GA, Faticchenti F, Carini S, Lodi R, Todesco R. 1980. Impiego di caglio di agnello e di capretto nella fabbricazione di formaggio Fiore Sardo: aspetti microbiologici e tecnologici. Il Latte. 5:191.

Del Gobbo LC, Imamura F, Aslibekyan S, Marklund M, Virtanen JK, Wennberg M, Yakoob MY, Chiuve SE, Dela Cruz L, Frazier-Wood AC, et al. 2016. α-3 Polysaturated fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies. JAMA Int Med. 176:155–1166.

Delgado F J, González-Crespo J, Ladero L, Cava R, Ramírez R. 2009. Free fatty acids and oxidative changes of a Spanish soft cheese (PDO ‘Torta del Casar’) during ripening. Int J Food Sci Tech. 44:1721–1728.

Delgado F J, González-Crespo J, Cava R, Ramírez R. 2011. Free Fatty Acids and Oxidative Changes of a Raw Goat Milk Cheese through Maturation. J Food Sci. 76: C669–C673.

Di Cragno R, Quinto M, Corsetti A, Minervini F, Gobbetti M. 2006. Assessing the proteolytic and lipolytic activities of single strains of mesophilic lactobacilli as adjunct cultures using a Caiciotta cheese model system. Int Dairy J. 16:119–130.

de Jong C, Badings HT. 1990. Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up, and capillary gas-chromatographic analysis. J High Resol Chromatogr. 13:94–98.

European Food Safety Authority (EFSA). 2010. Panel on dietetic products, nutrition, and allergies (NDA). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J. 8:1461.

Fresno M, Álvarez S, Díaz E, Virto M, de Renobales M. 2014. Short communication: sensory profile of raw goat milk cheeses made with artisan kid rennet pastes from commercial-weight animals: alternative to farmhouse goat cheeses. J Dairy Sci. 97:6111–6115.

Galiou OE, Zantar S, Bakkali M, Laglaoui A, Centeno JA, Carballo J. 2015. Chemical and microbiological characteristics of traditional homemade fresh goat cheeses from Northern Morocco. Small Rumin Res. 129:108–113.

Gougoulias DA, Kyriazakis I, Mavrogiani VS, Fragkou IA, Skoufos J, Tzora A, Taitzoglou IA, Kokoli AN, Fthenakis GC. 2007. Patterns of maternal-offspring behaviour of dairy sheep and potential association with mammary health. Can J Anim Sci. 87:469–478.

Giraffa G. 2003. Functionality of enterococci in dairy products. Int J Food Microbiol. 88:215–222.

Holzapfel W, Arini A, Aeschbacher M, Coppolecchia R, Pot B. 2018. Enterococcus faecium SF68 as a model for efficacy and safety evaluation of pharmaceutical probiotics. Beneficial Microbes. 9:375–388.

IDF. 1982. Determination of the total solid content. IDF Standard 4A:1982. International Dairy Federation.

IDF. 1986. Determination of fat content. IDF Standard 58:1986. International Dairy Federation.

Khosravi A, Safari M, Khodaiyan F, Gharizadeh SM. 2015. Bioconversion enhancement of conjugated linoleic acid by Lactobacillus plantarum using the culture media manipulation and numerical optimization. J Food Sci Technol. 52:5781–5789.

Madrau MA, Mangia NP, Murgia MA, Sanna MG, Garau G, Leccis L, Caredda M, Deiana P. 2006. Employment of autochthonous microflora in Pecorino Sardo cheese manufacturing and evolution of physicochemical parameters during ripening. Int Dairy J. 16:876–885.

Mami A, Henri JE, Khal M. 2008. Antimicrobial activity of lactobacillus species isolated from Algerian raw goat’s milk against Staphylococcus aureus. World J Dairy Food Sci. 3:39–49.

Mangia NP, Saliba L, Deiana P. 2019. Functional and safety characterization of autochthonous Lactobacillus paracasei FS103 isolated from sheep cheese and its survival in sheep and cow fermented milk during cold storage. Ann Microbiol. 69:161–170.

Mangia NP, Fancello F, Deiana P. 2016. Microbiological characterization using combined culture dependent and independent approaches of Casizolu pasta filata cheese. J Appl Microbiol. 120:329–345.

Mangia NP, Murgia MA, Garau G, Deiana P. 2011. Microbiological and physicochemical properties of Pecorino Romano cheese produced using a selected starter culture. J Agri Sci Technol. 13:585–600.

Mangia NP, Murgia MA, Garau G, Sanna MG, Deiana P. 2008. Influence of selected lab cultures on the evolution of free amino acids, free fatty acids and Fiore Sardo cheese microflora during the ripening. Food Microbiol. 25:366–377.

Moschopoulou E. 2011. Characteristics of rennet and other enzymes from small ruminants used in cheese production. Small Rum Res. 101:188–195.

Ohue-Kitano R, Yasuoka Y, Goto T, Kitamura N, Park S-B, Kishino S, Kimura I, Kasubuchi M, Takahashi H, Li Y, et al. 2018. α-Linolenic acid-derived metabolites from gut lactic acid bacteria induce differentiation of anti-inflammatory M2 macrophages through G protein-coupled receptor 40. FASEB J. 32:304–318.
Oldak A, Zielińska D, Rzepkowska A, Kołożyn-Krajewska D. 2017. Comparison of antibacterial activity of lactobacillus plantarum strains isolated from two different kinds of regional cheeses from Poland: oscypek and korycinski cheese. BioMed Res Int. 2017:1–10. [PMID][8626762].

Perin LM, Nero LA. 2014. Antagonistic lactic acid bacteria isolated from goat milk and identification of a novel nisin variant Lactococcus lactis. BMC Microbiol. 14:36.

Prodromou K, Thasitou P, Haritonidou E, Tzanetakis N, Litopolulou-Tzanetaki E. 2001. Microbiology of "Orinotyri", an ewe's milk cheese from the Greek mountains. Food Microbiol. 18:319–328.

Schirru S, Todorov SD, Favaro L, Mangia NP, Basaglia M, Casella S, Comunian R, Franco B, Deiana P. 2012. Sardinian goat’s milk as source of bacteriocinogenic potential protective cultures. Food Control. 25:309–320.

Secchiari P, Mele M, Serra A. 2007. Conjugated linoleic acid in meat and milk from ruminants: principal factors of genetic variations in food. Progr Nutr. 9:108–123.

Shakeel Ur R, Fox PF, McSweeney P. 2000. Methods used to study non-starter microorganisms in cheese: a review. Int J Dairy Technol. 53:113–119.

Tormo H, Ali Haimoud Lekhal D, Roques C. 2015. Phenotypic and genotypic characterization of lactic acid bacteria isolated from raw goat milk and effect of farming practices on the dominant species of lactic acid bacteria. Int J Food Microbiol. 210:9–15.

Tripaldi C, Palocci G, Garavaldi A, Bogdanova T, Bilei S. 2015. Effect of artisanal rennet paste on the chemical, sensory and microbiological characteristics of traditional goat’s cheese. Ital J Food Sci. 27:416–423.

Tsukamoto I, Sugawara S. 2018. Low levels of linoleic acid and α-linolenic acid and high levels of arachidonic acid in plasma phospholipids are associated with hypertension. Biomed Rep. 8:69–76.

Van Nieuwenhove CP, Oliszewski R, Gonzalez SN. 2009. Fatty acid composition and conjugated linoleic acid content of cow and goat cheeses from Northwest Argentina. J Food Qual. 32:303–314.

Villeneuve P, Pina M, Graille J. 1996. Determination of pre-gastric lipase specificity in young ruminants. Chem Phys Lipids. 83:161–168.

Yadav H, Jain S, Sinha PR. 2007. Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei during fermentation and storage. Int Dairy J. 17:1006–1010.

Yamagishi K, Ikeda A, Chei CL, Noda H, Umesawa M, Cui R, Muraki I, Ohira T, Imano H, Sankai T, et al. 2017. Serum α-linolenic and other ω-3 fatty acids, and risk of disabling dementia: community-based nested case-control study. Clin Nutr. 36:793–797.