International Dairy Journal 138 (2023) 105538

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Quinolones in goats' milk: Effect on the cheese-making process, chemical and microbial characteristics of acid-coagulated cheeses

M.C. Beltrán [a], A. Sanna [a], P. Quintanilla [a], R. Montes [b], M.P. Molina [a, *]

[a] Institute for Animal Science and Technology, Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain
[b] Advanced Centre for Food Microbiology, Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

ARTICLE INFO

Article history:
Received 11 April 2022
Received in revised form
20 October 2022
Accepted 25 October 2022
Available online 3 November 2022

ABSTRACT

The effect of the presence in goats' milk of enrofloxacin and ciprofloxacin on cheese-making and the characteristics of acid-coagulated cheeses was evaluated. Raw goats' milk was spiked with quinolones at maximum residue limit concentration (100 μg kg^{-1}). For each antibiotic, three batches of cheese were made by acid coagulation (pH 4.6) using a commercial starter culture. Cheese-making process, gross composition and microbial counts in the cheeses were unaffected by the presence of quinolones in milk. However, relatively high amounts of these substances were retained in the cheeses, with residual concentrations of 146.5 ± 4.9 μg kg^{-1} and 150.7 ± 25.7 μg kg^{-1} for enrofloxacin and ciprofloxacin, respectively, after 20 days of maturation. Results suggest that the use of goats' milk containing legally admissible amounts of enrofloxacin and/or ciprofloxacin would have no impact on cheese manufacturing, composition and microflora of acid-coagulated cheese. However, the transfer of these substances to the final products could compromise consumer safety.

1. Introduction

Quinolones are an important group of synthetic antibiotics having a wide range of antibacterial activity and an increasing use in veterinary medicine (Trouchon & Lefebvre, 2016). In dairy goats, fluoroquinolones, especially enrofloxacin, are prescribed for the treatment of gastrointestinal, respiratory and mammary diseases (Clark, 2013; Mavrogianni, Menzies, Fragkou, & Fthenakis, 2011; Papich, 2016). In general, enrofloxacin (ENR) is de-ethylated to its primary metabolite, ciprofloxacin (CPX), and both ENR and CPX residues could be present in products of animal origin such as milk, when improperly applied (López-Cadenas et al., 2013).

The presence of quinolones and other antimicrobial drug residues in milk and related products could be a potential hazard for consumers, causing allergic reactions, intestinal microbiota alterations and leading to the emergence of drug-resistant bacteria (Klimek, Aderhold, Sperl, & Trautmann, 2017; Prestinaci, Pezzotti, & Pantosti, 2015; Redgrave, Sutton, Webber, & Piddock, 2014; WHO, 2021). To protect consumer health, the European Union set maximum residue limits (EU-MRLs) for pharmacologically active substances in foodstuffs of animal origin, milk included. In particular, for the sum of ENR and its metabolite CPX, the EU established an MRL of 100 μg kg^{-1} for milk of all animal species (European Commission, 2010).

It should be noted that quinolones are listed by the World Health Organisation as "critically important antimicrobials" for human medicine in relation to antibiotic resistance (WHO, 2019). Therefore, the European Food Safety Authority suggested that their incidence in animal origin products should be monitored (EFSA, 2021). However, the microbial inhibitor tests routinely applied for screening antibiotics in milk are not able to detect such substances at safety levels, the use of more sensitive specific tests being necessary to ensure compliance with legislation (Beltrán, Berruga, Molina, Althaus, & Molina, 2015; IDF, 2014).

In addition to the adverse effects on human health, the technological impact of drug residues in milk should be also considered as they could interfere with the fermentation processes usually applied in the dairy industry, increasing manufacturing time and affecting the organoleptic characteristics of the final products (Chiesa et al., 2020; Quintanilla, Beltrán, Molina, Escriche & Molina, 2019a). Moreover, safety levels in dairy products have not yet been established and consumers might be exposed to significant amounts of such substances in concentrated milk products like cheese, even reaching drug concentrations higher than those

* Corresponding author.
E-mail address: pmolina@dca.upv.es (M.P. Molina).

https://doi.org/10.1016/j.idairyj.2022.105538

initially present in milk (Cabizza et al., 2017; Quintanilla et al., 2019a).

Research on the transfer of antibiotics from milk to cheese is rather limited, and studies performed have only focused on cheeses coagulated by rennet both on a laboratory scale (Gbylik-Silorska, Gadja, Nowacka-Kozak & Posyniak, 2021; Lupton, Shappell, Shelver, & Hakk, 2018; Shappell et al., 2017) and a pilot plant scale (Cabizza et al., 2017; Quintanilla, Doménech, Escriche, Beltrán & Molina, 2019b; Quintanilla et al., 2019a). However, related studies on acid-coagulated cheeses are currently unavailable.

Acid coagulation is the most ancient cheese-making process with which high quality cheeses, highly appreciated by the consumer, are made. In France, the main producer of goats' milk in the EU, there is a long tradition in the production of acid-coagulated cheese (Raynal-Ljutovac, Le Pape, Gaborit, & Barrucand, 2011), some of them recognised with Protected Designation of Origin (PDO). Also, in other European countries producing goats' milk such as Spain, Greece or Poland, acid-coagulation is a frequent practice in the production of traditional cheeses (Franco, Prieto, Bernardo, González, & Carvallo, 2003; Hatzikamari, Litopoulou-Tzanetaki, & Tzanetakis, 1999; Krajewska-Kamińska, Śmietana, & Bohdziewicz, 2007).

Acid-coagulated cheese comprises a diverse group of varieties produced by the coagulation of milk via acidification with or without the addition of milk-clotting enzymes (McSweeney, Ottogalli, & Fox, 2017). The essential step in the manufacture of acid-coagulated cheeses involves slow quiescent acidification of the milk to pH values close to the isoelectric point of caseins (4.6–4.8). The acidification is generally slow, 12–16 h at 21–23 °C, and is usually brought about by the in-situ conversion of lactose to lactic acid, by an added starter culture generally formed by lactic bacteria (Lucey, 2017). Antibiotic residues in milk for cheese production can inhibit or modify the development of the starter culture and consequently, affect the biochemical processes that occur during cheese-making leading to important technical and economic impacts, without forgetting the variable amounts of the antibiotic that could be transferred from milk to acid-coagulated cheeses, posing a potential risk for consumer health. Therefore, the aim of this study was to evaluate the effect of the presence of ENR, and its main metabolite, CPX, in raw goats' milk on the manufacture, and characteristics of acid-coagulated cheese and to assess the partitioning of these substances during cheese-making.

2. Material and methods

2.1. Experimental procedure

Acid-coagulated cheeses were produced in the pilot plant of Universitat Politècnica de València (UPV, Spain) from goats' milk containing quinolones at EU-MRL concentration (100 μg kg^{-1}). Cheeses from raw goats' milk free of antibiotics were made simultaneously to be used as reference.

Raw milk for cheese production was obtained from the experimental herd of Murciano-Granadina breed goats of UPV. Animals had a good health status and did not receive any veterinary drug before nor during the experimental period.

Antibiotics used in this study were ENR (ref.: 17849) and CPX (ref.: 17850), both provided by Sigma Sigma-Aldrich Química, S.A. (Madrid, Spain). For use, a stock solution (100 mg 100 mL^{-1}) was prepared daily using distilled water, the addition of 3 mL of 5% acetic acid (Fluka, Barcelona, Spain) being necessary to dissolve ENR before adding water. Raw milk was spiked according to the recommendations of the International Dairy Federation (ISO/IDF, 2003).

Three independent trials of acid-coagulated cheeses were carried out for each antibiotic considered. Milk, acid-curd and cheeses ripened for a 20-day period were analysed for gross composition, microbial counts and drug residues. Coagulation time and cheese yield were used to evaluate the technological impact of quinolones on the cheese-making process.

2.2. Cheese manufacture

For each cheese-making trial, 40 kg of raw goats' milk free of antibiotics were divided into two vats of 20 kg each. One vat was spiked with legally admissible amounts of quinolones, while the other one was used as control. Raw goats' milk was then heated to 23–24 °C and inoculated (1 DCU 10 L^{-1} milk) with a predominantly mesophilic commercial starter culture containing *Lactococus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*, and *Streptococcus thermophilus* (CHOOZIT MA4001 LYO 5 DCU, Danisco, Sassenage, France). Animal rennet 1:10,000 (Laboratorios Arroyo, Santander, Spain) was also added at 0.01% (v/v). Milk coagulation was performed at room temperature (20–22 °C) for 12–16 h until reaching a pH lower than 4.60 (isoelectric point of caseins). Then, the acid-curd was transferred to cotton bags, which were left to drain for 24 h at room temperature. After draining, the acid-curd was kneaded, salted with organic sea salt (The MedSalt Co. Jaime Altava S. L. Castellón, Spain) at 1%, and placed in cylindrical moulds (10 cm × 7 cm) to obtain pieces of 250 g. Next, the cheeses were placed in the air chamber for 48 h at 4 °C and 70% relative humidity, and finally, transferred to the ripening chamber where they remained at 10–12 °C and 80–85% relative humidity for a 20 day period.

During cheese-making, the pH of the two milk vats was monitored simultaneously every 15 min using a digital pH-meter provided with two penetration probes (HI 5222, Hanna Instruments, Eibar, Spain). The time required to complete the acidification process, expressed in minutes, was recorded as coagulation time. After draining, acid-curd was accurately weighed to calculate the curd yield, expressed as kg of acid-curd per 100 kg of milk. Acid-curd and 20-day ripened cheeses were sampled for chemical and microbial characterisation.

2.3. Milk and cheeses analyses

2.3.1. Chemical composition

Milk used for cheese production was analysed for gross composition (MilkoScan 6000, Foss, Hillerød, Denmark) and somatic cell counts (Fossomatic 5000, Foss) at the Interprofessional Laboratory of the Valencian Community region (LICOVAL, UPV, Valencia, Spain). The milk pH was measured by a conventional pH-meter (model Basic 20, Crison, Barcelona, Spain) and the microbial screening test Eclipse 100 (Zeulab, Zaragoza, Spain) was applied to rule out the presence of potential inhibitors in milk. The chemical composition of the acid-coagulated cheeses was analysed at different ripening time (0 and 20 days) using a FoodScan Dairy Analyzer (Foss). The pH of the cheese was measured in triplicate.

2.3.2. Microbial counts

Different microbial populations were analysed in milk, acid-curd and ripened lactic cheeses. Sharlau (Barcelona, Spain) provided all the culture media and reagents used for the microbiological analysis, except the gas generation system Anaerocult A (ref: 1.32381) for creating anaerobic environment, which was supplied by Merck (Darmstadt, Germany). Curd and cheeses (10 g) were homogenised in (90 mL) sterile peptone water solution (BPW 0.1%, w/v, ref: 01-412-500) in a Stomacher 400 blender (Seward, London, UK) for 2 min at high speed. Decimal dilutions of raw milk, curd and

cheese homogenates were prepared in sterile BMP and plated in duplicate for counting. Samples were analysed for total mesophilic bacteria on plate count skim milk agar (ref.: 01-412-500) incubated at 30 °C for 48–72 h, acid lactic bacteria on MRS agar (ref.: 01-135-500) incubated a 37 °C for 48 h under anaerobic conditions, and Lactococci on M17 Agar (ref.: 01-245-500) incubated at 37 °C for 48 h. Total coliform and *Escherichia coli* counts were made on Microinstant chromogenic coliforms agar base (CCA) (ref.: 01-695-500) with CV selective supplement for coliforms (ref.: 06-140LYO1) incubated at 37 °C for 24 h, red colonies being counted as coliforms and blue-violet colonies as *E. coli*. For enterococci, Slanetz Bartley agar base (ref. 01-579-500) with 10 mL L^{-1} 1% TTC sterile solution (ref.: 06-023-100) was employed, incubated at 37 °C for 48 h, dark red colonies being counted as enterococci. For verification, at least five colonies were plated in kanamycin esculin azide agar (ref.: 01-263-500) and incubated at 44 °C for 4 h, with enterococci colonies turning black.

2.3.3. Antibiotic residues

Quinolones were quantified in raw milk, acid-curd and 20-day ripening cheeses using a liquid chromatography system consisting of a LC/MS–MS Alliance 2695 with a diode-array detector (Waters Chromatography Division, Milford, MA, USA) and a Micromass Quattro MicroTM triple quadrupole tandem mass spectrometer (Waters Chromatography Division). The method was validated previously as described by Quintanilla et al. (2019a). The typical recoveries were approximately 90–110% and the limit of detection (LOD) was equal to 5 μg kg^{-1} for both ENR and CPX.

2.4. Statistical analysis

Data were analysed using the Statgraphics Centurion XVIII software (Statpoint Technologies, Inc. The Plains, VA, USA). To assess the partitioning of antibiotics during cheese-making, normalised drug distribution rates, expressed as percentage, were determined by applying a mass balance. One-way ANOVA was applied to evaluate the impact of quinolones on cheese-making traits (coagulation time and cheese yield). A multifactor ANOVA test was employed to investigate the effects of the antibiotic concentration (0 and 100 μg kg^{-1}) and the ripening time (0 and 20 days) on the chemical composition and microbial counts of the acid-coagulated cheeses. Interactions between factors were also considered. When significant differences ($p < 0.05$) were found, means were separated by the Least Significance Difference test (LSD).

3. Results and discussion

3.1. Impact of quinolones on the cheese-making process and cheese characteristics

Table 1 summarises the mean quality characteristics of raw goats' milk used in this study; the milk batches used for the manufacture of acid-coagulated cheeses with ENR having similar values ($p > 0.05$) as those employed for the CPX cheese-making trials, for all the quality parameters analysed.

Raw milk used in this study presented typical physicochemical characteristics of the Murciano-Granadina breed goats (Delgado et al., 2017), and hygienic quality with values between ranges indicated by other authors for somatic cells (909–1738 × 10^3 cell mL^{-1}), total mesophilic bacteria (1.2–6.42 × 10^3 cfu mL^{-1}), enterococci (3.5–21 × 10^3 cfu mL^{-1}) and coliform (absence to >3.6 × 10^3 cfu mL^{-1}) counts, in goats' milk used for cheese production in traditional producer countries (Morgan et al., 2003; Picon, Garde, Avila, & Nuñez, 2016).

The cheese-making process was unaffected by the presence of legally admissible amounts of ENR or CPX in goats' milk. As shown in Fig. 1, antibiotics did not significantly affect the acidification kinetics of milk vats inoculated with starters. Therefore, the coagulation time required for cheese production in spiked milk vats was similar ($p > 0.05$) to that of their respective references (ENR: 720 ± 73.7 and 727.5 ± 73.7 min as average for spiked and reference milk vats, respectively; CPX: 815 ± 75.6 and 840 ± 75.6 min). In general, coagulation times were between ranges characteristics in lactic-type cheese manufacture (Lucey, 2017).

Regarding cheese-making efficiency, no significant differences ($p > 0.05$) were found between acid-curd yields from milk containing ENR (22.84 ± 0.23 and 23.03 ± 0.23 kg acid-curd 100 kg^{-1} milk for spiked and antibiotic-free milk vats, respectively) nor CPX (23.94 ± 0.69 versus 25.28 ± 0.69 kg acid-curd 100 kg^{-1} milk for spiked and antibiotic-free milk vats, respectively).

The presence of antibiotics (A) in raw goats' milk neither affect the chemical composition of the acid-coagulated cheeses (Table 2), which was only changed by the ripening time (R) having increased contents of total solids ($p < 0.001$) and fat ($p < 0.01$) after 20 days of maturation. No significant interactions were found affecting the gross composition of the cheeses (A × R, $p > 0.05$).

The experimental cheeses showed a gross composition similar to those lactic ripened cheeses such as Crottin de Chavignol, Sainte Maure, Rocamadur or Picodon (total solids, 50.2 g 100 g^{-1} cheese; fat, 25.8 g 100 g^{-1} cheese; protein, 20.6 g 100 g^{-1} cheese), produced under French PDO (Raynal-Ljutovac et al., 2011). In general, the physico-chemical characteristics of the acid-coagulated cheeses evolved in a similar way during maturation to that reported in other goats' milk cheeses with predominantly lactic curdling, such as Babia-Laciana (Franco et al., 2003) and Sainte-Maure (Gaborit, Menard, & Morgan, 2001) traditionally produced in Spain and France, respectively.

Results herein agreed with those reported by other authors when assessing the effect of the presence of ENR and CPX in goats' milk on the manufacture and chemical composition of fresh (Quintanilla et al., 2019b) and ripened rennet-curd cheeses (Quintanilla et al., 2019a). Thus, no significant differences ($p > 0.05$) were detected in the coagulation time, cheese yield and gross composition of the rennet cheeses from goats' milk containing legally admissible amounts of such substances in milk (100 μg kg^{-1}), nor in the texture profile and colour attributes evaluated in the cheeses at different ripening times (1, 30 and 60 days).

Regarding microbiological characteristics of the cheeses, results showed that the microbial populations counted in the raw milk inoculated with starters and in the lactic cheeses containing antibiotics (Table 3) were similar ($p > 0.05$) to their respective

Table 1
Mean quality characteristics of raw goats' milk (n = 6) used for acid-coagulated cheese production.[a]

Parameter	Mean	SD
pH	6.72	0.077
Total solids (g 100 g^{-1})	13.74	0.337
Fat (g 100 g^{-1})	4.79	0.235
Protein (g 100 g^{-1})	3.74	0.155
Lactose (g 100 g^{-1})	4.51	0.036
Log SCC (cell mL^{-1})	6.21	0.078
TMB (log cfu mL^{-1})	4.98	0.173
LAB (log cfu mL^{-1})	4.41	0.507
Lactococci (log cfu mL^{-1})	4.29	0.273
Enterococci (log cfu mL^{-1})	3.03	0.538
Total coliform (log cfu mL^{-1})	3.38	0.144
Escherichia coli (log cfu mL^{-1})	2.16	0.335

[a] Abbreviations are: SCC, somatic cell count; TMB, total mesophilic bacteria; LAB, lactic acid bacteria.

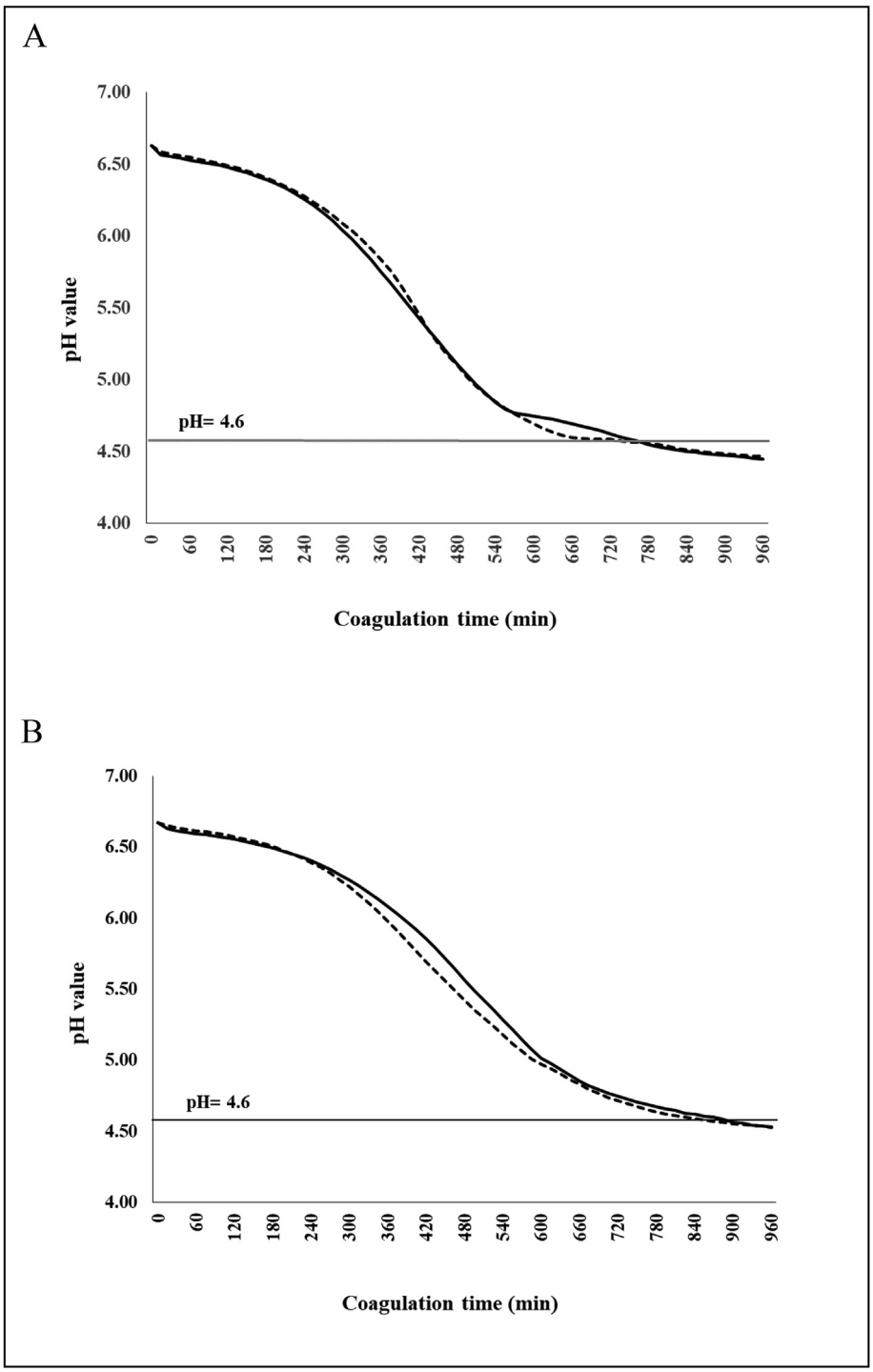

Fig. 1. Acidification kinetics of goats' milk containing (A) enrofloxacin and (B) ciprofloxacin at UE-MRL concentration (100 μg kg^{-1}) for acid-coagulated cheese production (——, reference; ▬ ▬, quinolone).

references. In addition, microbial counts were also highly affected by maturation, with significantly lower counts ($p < 0.01$) for all the populations evaluated in ripened cheeses, except for enterococci having similar counts along time. *E. coli* was not detected in almost all the acid-curd samples regardless of the presence of quinolones in milk. Interactions between the two factors considered were not significant (A × R, $p > 0.05$).

The microbial characteristics of the cheeses agreed to those reported by other authors in different lactic cheeses from goats' milk. Thus, acid-curd samples have similar values for total mesophilic bacteria, acid lactic bacteria, and enterococci counts than those found in Anevato (Hatzikamari et al., 1999; Xanthopoulos, Polychroniadou, Litopoulou-Tzanetaki, & Tzanetakis, 2000) and Crottin (Tamagnini, de Sousa, González, & Budde, 2006) cheeses; the lactic acid bacteria being the dominant flora, and the different microbial populations having lower microbial counts ($p < 0.05$) during ripening and storage. It should be noted that, as indicated by Tamagnini et al. (2006), the competing flora and the unfavourable

Table 2
Effect of the antibiotic concentration (A) and ripening time (R) on the chemical composition of acid-coagulated cheeses from goats' milk containing quinolones.[a]

Parameter	Antibiotic concentration (μg kg^{-1})			Ripening time (days)			ANOVA	
	0	100	SE	0	20	SE	A	R
Enrofloxacin								
pH	4.47	4.46	0.028	4.47	4.45	0.028	0.06^{ns}	0.21^{ns}
Total solids (g 100 g^{-1})	46.37	46.75	0.674	41.61^{a}	51.51^{b}	0.674	0.15^{ns}	107.86***
Fat (g 100 g^{-1})	25.67	26.06	0.840	22.86^{a}	28.87^{b}	0.840	0.11 ^{ns}	25.56**
Protein (g 100 g^{-1})	16.39	16.88	0.535	16.34	16.93	0.535	0.43^{ns}	0.59^{ns}
NaCl (g 100 g^{-1})	2.71	3.05	0.363	2.38	3.38	0.363	0.43^{ns}	3.82^{ns}
Ciprofloxacin								
pH	4.45	4.46	0.031	4.45	4.46	0.031	0.03^{ns}	0.02^{ns}
Total solids (g 100 g^{-1})	46.57	46.00	0.472	40.93^{a}	51.64^{b}	0.472	0.73^{ns}	253.03***
Fat (g 100 g^{-1})	25.93	25.85	0.314	22.61^{a}	29.18^{b}	0.314	0.03 ^{ns}	218.77***
Protein (g 100 g^{-1})	16.07	15.76	0.486	15.47	16.36	0.486	0.20 ^{ns}	1.67 ^{ns}
NaCl (g 100 g^{-1})	2.76	2.57	0.459	2.07^{a}	3.26^{b}	0.459	0.09 ^{ns}	3.40**

[a] Different superscript letters in the same row indicate significant differences ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significantly different ($p > 0.05$).

environmental conditions, might not be sufficient to induce the complete disappearance of the coliforms when present in the cheeses at the beginning of maturation and storage.

3.2. Quinolone residues in acid-coagulated cheeses

As shown in Fig. 2, residual amounts of ENR and CPX detected in acid-curd (ENR: 132.33 ± 2.08 μg kg^{-1}, CPX: 129.67 ± 10.12 μg kg^{-1}) and 20-days ripened cheeses (ENR: 146.5 ± 4.95 μg kg^{-1}, CPX: 150.67 ± 25.73 μg kg^{-1}) were higher than those initially present in raw milk (ENR: 99.33 ± 21.36 μg kg^{-1}, CPX: 98.67 ± 3.05 μg kg^{-1}). To evaluate the transfer of quinolones from milk to acid-curd normalised drug retention rates, expressed as percentage, were calculated considering the residual amounts of antibiotics in the acid-curd samples, and the curd yield obtained in each cheese-making trial. Results indicate that a relatively high percentage of the quinolones present in milk (31.35 ± 6.40% and 31.57 ± 4.36% for ENR and CPX, respectively) was retained in the acid-curd fraction (Fig. 3), with curd concentration ratios of 1.37 ± 0.26 and 1.31 ± 0.11 for ENR and CPX, respectively.

The retention rates calculated in this study for ENR and CPX were lower to those reported by other authors in rennet-curd cheeses. Thus, Lupton, Shappell, Shelver and Hakk (2018) obtained a higher retention rate close to 50% for CPX when assessing at a laboratory scale, the distribution of spiked drugs into the different milk matrices, rennet-curd included. Higher retention rates were also calculated by Quintanilla et al. (2019b) and Quintanilla et al. (2019a) in fresh (ENR: 51.1 ± 8.8%; CPX: 57.3 ± 4.5%) and ripened rennet-curd cheeses (ENR: 39.4 ± 5.7%; CPX: 56.4 ± 3.6%), respectively, obtained at pilot-plant scale from goats' milk containing quinolones at EU-MRL concentration.

Drug distribution into the different milk matrices is highly related to physicochemical properties of such substances (Hakk et al., 2016; Shappell et al., 2017). Thus, the high retention rates found for ENR and CPX in rennet-curd cheeses, with curd concentration ratios ranging from 2.7 to 4.1 (Lupton et al., 2018; Quintanilla et al., 2019a), could be related to their low water solubility, higher affinity for casein associations, and complexation reactions with metal ions like calcium, present in milk forming insoluble quelates (Lizondo, Pons, Gallardo, & Estelrich, 1997; Lupton et al., 2018; Pápai, Budai, Ludányi, Antal, & Klebovich, 2010).

The lower retention rates obtained for ENR and CPX in this study could be related to the predominantly acidic curdling process used for the experimental cheese manufacture, affecting the structure and properties of the caseins micelles and therefore, of the lactic gel obtained after the isoelectric aggregation of caseins (Lucey, 2017). Thus, milk acidification induces the solubilisation of colloidal calcium phosphate from the micelles leading to demineralised curds, with lower calcium (Law & Leaver, 1998; Pawlos, Znamirowska, Zaguła, & Buniowska, 2020), and casein concentration (Guinee, 2016). Moreover, milk acidification increases significantly the ionisation of the quinolones and therefore, their solubility in the aqueous fraction (Blokhina, Sharapova, Ol'khovich, Volkova, & Perlovich, 2016; Lizondo et al., 1997) and might also reduce the complexation reactions with solubilised calcium (Pápai et al., 2010) leading to potential lower drug–curd interaction reactions.

Table 3
Effect of the antibiotic concentration (A) and ripening time (R) on the microbial populations of acid-coagulated cheeses from goats' milk containing quinolones.[a]

Parameter	Antibiotic concentration (μg kg^{-1})			Ripening time (days)			ANOVA	
	0	100	SE	0	20	SE	A	R
Enrofloxacin								
TMB (log cfu g^{-1})	9.04	9.11	0.104	9.59^{b}	8.56^{a}	0.104	0.20 ^{ns}	49.34***
LAB (log cfu g^{-1})	9.09	9.05	0.046	9.68 b	8.46 a	0.046	0.52 ^{ns}	348.28***
Lactococci (log cfu g^{-1})	9.11	9.24	0.061	9.73^{b}	8.63^{a}	0.061	2.22 ^{ns}	162.25***
Enterococci (log cfu g^{-1})	4.54	4.50	0.098	4.58	4.45	0.098	0.05 ^{ns}	0.86 ^{ns}
Total coliform (log cfu g^{-1})	4.47	4.02	0.190	4.56^{b}	3.94^{a}	0.190	2.82 ^{ns}	5.37*
Ciprofloxacin								
TMB (log cfu g^{-1})	8.95	8.98	0.074	9.52^{b}	8.41^{a}	0.074	0.09 ^{ns}	111.22***
LAB (log cfu g^{-1})	9.10	8.89	0.169	9.62^{b}	8.36^{a}	0.169	0.72 ^{ns}	24.88**
Lactococci (log cfu g^{-1})	8.94	9.00	0.072	9.56^{b}	8.38^{a}	0.072	0.31 ^{ns}	134.15***
Enterococci (log cfu g^{-1})	4.47	4.49	0.103	4.56	4.40	0.103	0.02 ^{ns}	1.16 ^{ns}
Total coliform (log cfu g^{-1})	3.77	3.50	0.179	4.94^{b}	2.33^{a}	0.179	0.76 ^{ns}	70.62***

[a] Abbreviations are: TMB, total mesophilic bacteria; LAB, lactic acid bacteria. Different superscript letters in the same row indicate significant differences ($p < 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significantly different ($p > 0.05$).

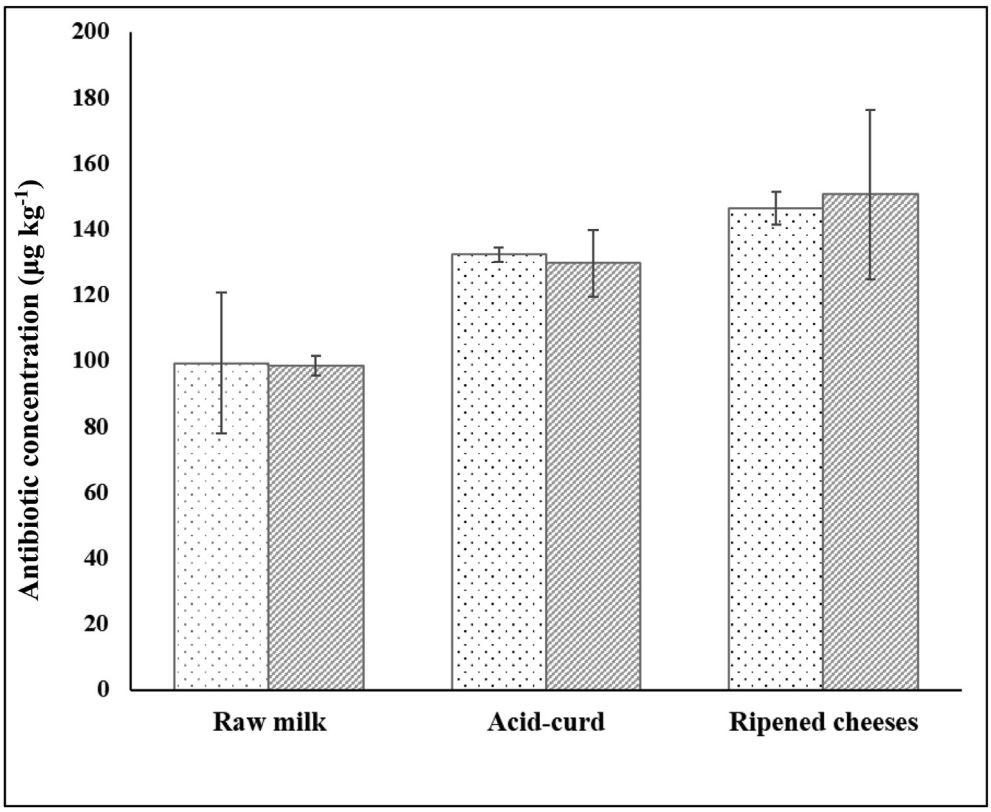

Fig. 2. Antibiotic concentration (mean ± SD) in acid-curd and ripened cheeses from goats' milk containing enrofloxacin ⬚ or ciprofloxacin ▩ at EU-MRL concentration (100 µg kg^{-1}).

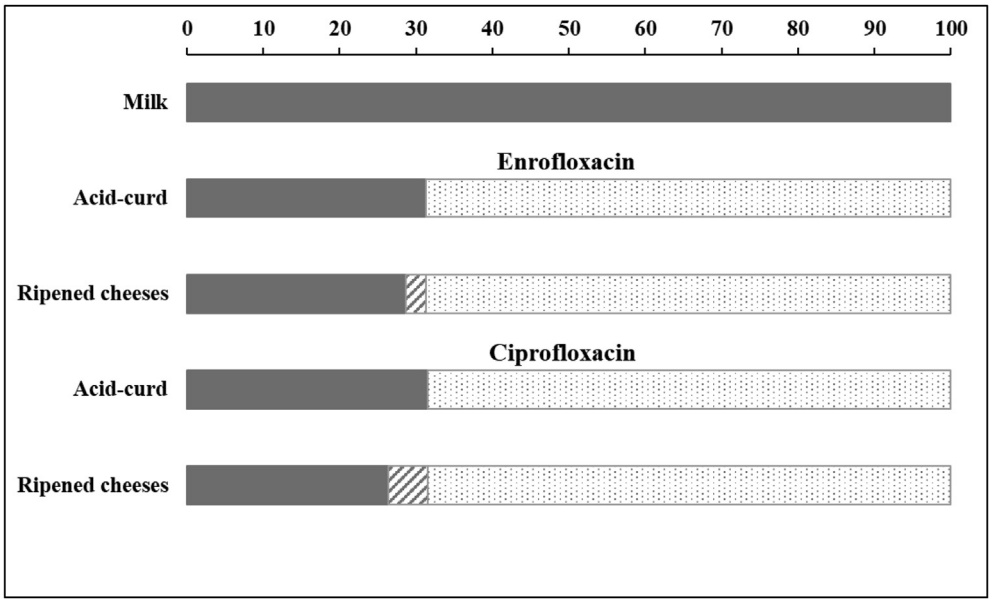

Fig. 3. Transfer (%) of enrofloxacin and ciprofloxacin during cheese-making and antibiotic persistence (%) along ripening time: ■, antibiotic retained in curd; ⬚, antibiotic degradation; □, antibiotic released into whey.

On the other hand, the highest residual amounts of quinolones in the 20-day ripened cheeses suggest low degradation rates of such substances during maturation. To assess the persistence of quinolones in the experimental cheeses, residual drug concentrations were calculated on a dry basis, observing that most of the ENR (91.6 ± 3.3%) and CPX (83.4 ± 20.3%) transferred from milk to acid-curd persist in 20-day ripened acid-curd cheeses. Thus, the high stability of quinolones along maturation and the moisture losses occurring during this period resulted in increased concentrations of antibiotics in the ripened cheeses, exceeding the EU-MRL established for such substances in milk. Beltrán, Morari-Pirlog, Quintanilla, Escriche, and Molina (2018) also reported high drug

persistence between 74.9 and 83.7%, in yoghurts from goats' milk spiked with ENR close to the EU-MRL after 28 days of refrigerated storage. In ripened rennet-cheeses, Quintanilla et al. (2019a) and Quintanilla Beltrán, Molina, & Escriche (2021) also indicate high persistence values for ENR and CPX even after 60 days of maturation (ENR: 40.4 ± 1.4% and CPX: 54 ± 8%), with residual amounts of antibiotics exceeding the drug concentration initially present in milk.

4. Conclusions

The presence of enrofloxacin and/or ciprofloxacin in goats' milk at safety levels has no technological implications for the manufacture and characteristics of acid-coagulated cheeses. However, large amounts of such residues could remain in the final products, posing a potential risk for consumer health.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgement

The authors are grateful for the financial support by the Ministerio de Ciencia e Innovación (Madrid, Spain) project AGL-2013-45147-R.

References

Beltrán, M. C., Berruga, M. I., Molina, A., Althaus, R. L., & Molina, M. P. (2015). Performance of current microbial tests for screening antibiotics in sheep and goat milk. *International Dairy Journal, 41*, 13–15.

Beltrán, M. C., Morari-Pirlog, A., Quintanilla, P., Escriche, I., & Molina, M. P. (2018). Influence of enrofloxacin on the coagulation time and the quality parameters of goat's milk yoghurt. *International Journal of Dairy Technology, 71*, 105–111.

Blokhina, S. V., Sharapova, A. V., Ol'khovich, M.,V., Volkova, T. V., & Perlovich, G. L. (2016). Solubility, lipophilicity and membrane permeability of some fluoroquinolone antimicrobials. *European Journal of Pharmaceutical Sciences, 93*, 29–37.

Cabizza, R., Rubattu, N., Salis, S., Pes, M., Comunian, R., Paba, A., et al. (2017). Transfer of oxytetracycline from ovine spiked milk to whey and cheese. *International Dairy Journal, 70*, 12–17.

Chiesa, L. M., DeCastelli, L., Nobile, M., Martucci, F., Mosconi, G., Fontana, M., et al. (2020). Analysis of antibiotic residues in raw bovine milk and their impact toward food safety and on milk starter cultures in cheese-making process. *LWT – Food Science and Technology, 131*, Article 109783.

Clark, C. R. (2013). Antimicrobial drug use in sheep and goats. In S. Giguère, J. F. Prescott, & P. M. Dowling (Eds.), *Antimicrobial therapy in veterinary medicine* (5th ed., pp. 529–539). Oxford, UK: Wiley Blackwell.

Delgado, J. V., Landi, V., Barba, C. J., Fernández, J., Gómez, M. M., Camacho, M. E., et al. (2017). Murciano-Granadina goat: A Spanish local breed ready for the challenges of the twenty-first century. In J. Simões, & C. Gutiérrez (Eds.), *Vol. 2. Sustainable goat production in adverse environments* (pp. 205–219). New York, NY, USA: Springer Science + Business Media.

EFSA. (2021). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2018-19. *EFSA Journal, 19*, Article 6490.

European Commission. (2010). Council Regulation No. 37/2010 of 22 December 2009. On pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Communities, L15*, 1–72.

Franco, I., Prieto, B., Bernardo, A., González, J., & Carvallo, J. (2003). Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). *International Dairy Journal, 13*, 221–230.

Gaborit, P., Menard, A., & Morgan, F. (2001). Impact of ripening strains on the typical flavour of goat cheeses. *International Dairy Journal, 11*, 315–325.

Gbylik-Sikorska, M., Gajda, A., Nowacka-Kozak, E., & Posyniak, A. (2021). The "force" of cloxacicillin residue will be with you in various dairy products-The last experiment evidence. *Food Control, 121*, Article 107628.

Guinee, T. P. (2016). Protein in cheese and cheese products: Structure-function relationships. In P. L. H. McSweeney, & J. O'Mahony (Eds.) (4th ed.,, *Vol. 2. Advanced dairy chemistry* (pp. 347–415). New York, NY, USA: Springer Science + Business Media.

Hakk, H., Shappell, N. W., Lupton, S. J., Shelver, W. L., Fanaselle, W., Oryang, D., et al. (2016). Distribution of animal drugs between skim milk and milk fat fractions in spiked whole milk: Understanding the potential impact on commercial milk products. *Journal of Agricultural and Food Chemistry, 64*, 326–335.

Hatzikamari, E., Litopoulou-Tzanetaki, E., & Tzanetakis, N. (1999). Microbiological characteristics of Anevato: A traditional Greek cheese. *Journal of Applied Microbiology, 87*, 465–629.

IDF. (2014). *Detecting antibiotic residues in milk. Guidance on the application of screening and confirmatory methods in integrated dairy chain management*. IDF bulletin Nº 474. Brussels, Belgium: International Dairy Federation.

ISO/IDF. (2003). *Milk and milk products. Guidelines for a standardized description of microbial inhibitor tests*. ISO 13969/IDF Standard Nº 183. Brussels, Belgium: International Dairy Federation.

Klimek, L., Aderhold, C., Sperl, A., & Trautmann, A. (2017). Allergic reactions to antibiotics – two sides of the same coin: Clearly diagnose or reliably rule out. *Allergo Journal International, 26*, 212–218.

Krajewska-Kamińska, E., Śmietana, Z., & Bohdziewicz, K. (2007). Production and characteristics of Twarog cheeses containing strains of probiotic bacteria. *Polish Journal of Food and Nutrition Science, 57*, 325–328.

Law, A. J. R., & Leaver, J. (1998). Effects of acidification and storage of milk on dissociation of bovine casein micelles. *Journal of Agricultural and Food Chemistry, 46*, 5008–5016.

Lizondo, M., Pons, M., Gallardo, M., & Estelrich, J. (1997). Physicochemical properties of enrofloxacin. *Journal of Pharmaceutical and Biomedical Analysis, 15*, 1845–1849.

López-Cadenas, C., Sierra-Vega, M., Garcia-Vieitez, J. J., Diez-Liebana, M. J., Sahagun-Prieto, A., & Fernandez-Martinez, N. (2013). Enrofloxacin: Pharmacokinetics and metabolism in domestic animal species. *Current Drug Metabolism, 14*, 1042–1058.

Lucey, J. A. (2017). Formation, structural properties, and rheology of acid-coagulated milk gels. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese: Chemistry, physics and microbiology* (4th ed., Vol. 1, pp. 179–197). Cambridge, UK: Academic Press.

Lupton, S. J., Shappell, N. W., Shelver, W. L., & Hakk, H. (2018). Distribution of spiked drugs between milk fat, skim milk, whey, curd, and milk protein fractions: Expansion of partitioning models. *Journal of Agricultural and Food Chemistry, 66*, 306–314.

Mavrogianni, V. S., Menzies, P. I., Fragkou, I. A., & Fthenakis, G. C. (2011). Principles of mastitis treatment in sheep and goats. *Veterinary Clinics: Food Animal Practice, 27*, 115–120.

McSweeney, P. L. H., Ottogalli, G., & Fox, P. F. (2017). Diversity and classification of cheese varieties: An overview. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese: Chemistry, physics and microbiology* (4th ed., Vol. 2, pp. 781–808). Cambridge, UK: Academic Press.

Morgan, F., Massouras, T., Barbosa, M., Roseiro, L., Ravasco, F., Kandarakis, I., et al. (2003). Characteristics of goat milk collected from small and medium enterprises in Greece, Portugal and France. *Small Ruminant Research, 43*, 39–49.

Pápai, K., Budai, M., Ludányi, K., Antal, I., & Klebovich, I. (2010). In vitro food–drug interaction study: Which milk component has a decreasing effect on the bioavailability of ciprofloxacin? *Journal of Pharmaceutical and Biomedical Analysis, 52*, 37–42.

Papich, M. G. (2016). *Enrofloxacin. Saunders handbook of veterinary drugs: Small and large animal* (4th ed., pp. 287–289). Amsterdam, Netherlands: Elsevier.

Pawlos, M., Znamirowska, A., Zaguła, G., & Buniowska, M. (2020). Use of calcium amino acid chelate in the production of acid-curd goat cheese. *Foods, 9*, Article 994.

Picon, A., Garde, S., Avila, M., & Nuñez, M. (2016). Microbiota dynamics and lactic acid bacteria biodiversity in raw goat milk cheeses. *International Dairy Journal, 58*, 14–22.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*, 309–318.

Quintanilla, P., Beltrán, M. C., Molina, M. P., & Escriche, I. (2021). Enrofloxacin treatment on dairy goats: Presence of antibiotic in milk and impact of residue on technological process and characteristics of mature cheese. *Food Control, 123*, Article 107762.

Quintanilla, P., Beltrán, M. C., Molina, A., Escriche, I., & Molina, M. P. (2019a). Characteristics of ripened Tronchón cheese from raw goat's milk containing legally admissible amounts of antibiotics. *Journal of Dairy Science, 102*, 2941–2953.

Quintanilla, P., Doménech, E., Escriche, I., Beltrán, M. C., & Molina, M. P. (2019b). Food safety margin assessment of antibiotics: Pasteurized goat's milk and fresh cheese. *Journal of Food Protection, 82*, 1553–1559.

Raynal-Ljutovac, K., Le Pape, M., Gaborit, P., & Barrucand, P. (2011). French goat milk cheeses: An overview on their nutritional and sensorial characteristics and their impacts on consumers' acceptance. *Small Ruminant Research, 101*, 64–72.

Redgrave, L. S., Sutton, S. B., Webber, M. A., & Piddock, L. J. V. (2014). Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology, 22*, 438–445.

Shappell, N. W., Shelver, W. L., Lupton, S. J., Fanaselle, W., Van Doren, J. M., & Hakk, H. (2017). Distribution of animal drugs among curd, whey, and milk protein fractions in spiked skim milk and whey. *Journal of Agricultural and Food Chemistry, 65*, 938–949.

Tamagnini, L. M., de Sousa, G. B., González, R. D., & Budde, C. E. (2006). Microbiological characteristics of Crottin goat cheese made in different seasons. *Small Ruminant Research, 66*, 175–180.

Trouchon, T., & Lefebvre, S. A. (2016). Review of enrofoxacin for veterinary use. *Open Journal of Veterinary Medicine, 6*, 40–58.

WHO. (2019). *Critically important antimicrobials for human medicine (6th revision 2018)*. Geneva, Switzerland: World Health Organisation. Retrived from http://www.who.int/publications/i/item/9789241515528. (Accessed 20 September 2022).

WHO. (2021). *Antimicrobial resistance*. Geneva, Switzerland: World Health Organisation. Retrieved from http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. (Accessed 25 February 2022).

Xanthopoulos, V., Polychroniadou, A., Litopoulou-Tzanetaki, E., & Tzanetakis, N. (2000). Characteristics of Anevato cheese made from raw or heat-treated goat milk inoculated with a lactic starter. *LWT – Food Science and Technology, 33*, 483, 448.