Short communication: Volatile profile of matured Tronchón cheese affected by oxytetracycline in raw goat milk

P. Quintanilla,¹ K. A. Hettinga,² M. C. Beltrán,¹ I. Escriche,³ and M. P. Molina^{1*}

¹Institute for Animal Science and Technology, Universitat Politècnica de València, Camino de Vera, s/n 46022, València, Spain

²Dairy Science and Technology Group, Chair of Food Quality and Design, Wageningen University, P.O. Box 17, 6700 AA Wageningen, the Netherlands

³Food Technology Department, Institute of Food Engineering for Development, Universitat Politècnica de València, Camino de Vera, s/n 46022, València, Spain

ABSTRACT

The presence of antibiotics in milk destined for cheese production may affect the biological processes responsible for the formation of volatile compounds, leading to alterations in the characteristic cheese flavor expected by consumers. The aim of this study was to evaluate the effect of the presence of oxytetracycline in goat milk on the volatile profile of ripened cheeses. Traditional mature Tronchón cheeses were manufactured from raw goat milk spiked with different concentrations of oxytetracycline (50, 100, and 200 µg/kg). Cheese made from antibiotic-free goat milk was used as control. We analyzed the residual amounts of the antibiotic and the volatile profile of the experimental cheeses on a fortnightly basis during maturation using liquid chromatography tandem mass spectrometry and then solid-phase microextraction followed by gas chromatography–mass spectrometry. Our results suggested that oxytetracycline was widely transferred from milk to cheese: drug concentrations in the cheeses were 3.5 to 4.3 times higher than those in raw milk. Although the residual amounts of oxytetracycline significantly decreased during maturation (88.8 to 96.5%), variable amounts of residues remained in cheese matured for 60 d (<10 to 79 µg/kg). In general, the presence of oxytetracycline in goat milk did not affect the volatile profile of Tronchón cheeses; volatile profile was significantly modified by ripening time. Still, the presence of oxytetracycline residues in cheeses ripened for 60 d could be of great concern for public health.

Key words: goat cheese, oxytetracycline, antibiotic residue, volatile profile

Short Communication

In Mediterranean and Eastern European countries, goat milk is used mainly for cheesemaking, and demand has grown in the last decade because of the particular taste, nutritional value, and great variety of traditional cheeses. Cheese flavor is one of the most important organoleptic criteria for consumer acceptance, and it is the result of a complex balance between volatile and non-volatile chemical compounds. Biochemical processes such as glycolysis, lipolysis, and proteolysis are the main pathways for producing aromatic compounds such as alcohols, aldehydes, carboxylic acids, esters, ketones, and others during ripening (McSweeney and Sousa, 2000; Delgado et al., 2010). It is generally agreed that the presence of antibiotic residues in milk—aside from their negative implications for consumer health—affect technological cheesemaking processes because they can inhibit the activity of the raw milk microflora and the starter cultures employed in the dairy industry (Katla et al., 2001). Thus, the liberation of enzymes could be altered and modify the production of aromatic compounds in matured cheese. One of the most widespread broad-spectrum antibiotics used in dairy goats is oxytetracycline, prescribed mainly to treat mastitis and urinary tract and enteric infections (Attaie et al., 2015). Oxytetracycline treatment could be the cause of antibiotic residues in milk if good farming practices are not correctly applied, especially related to the withdrawal period. In the European Union, the regulatory levels or maximum residue limits (**MRL**) for all tetracyclines in milk, including oxytetracycline, have been fixed at 100 µg/kg (European Union, 2010). In the European Union and other countries where quality-control programs have been implemented, raw milk is routinely screened for antibiotic residues. Microbial inhibitor tests are usually applied in milk quality-control laboratories; they are able to detect β-lactam antibiotics at or below MRL but cannot suitably detect other veterinary drugs such as quinolones and tetracyclines.

Received February 19, 2019.

Accepted February 1, 2020.

*Corresponding author: pmolina@dca.upv.es

at safety levels (Sierra et al., 2009; Beltrán et al., 2015). In farms and dairies, receptor-binding assays are commonly applied only to screen for β -lactam residues in goat milk, and they often do not detect other veterinary drugs such as tetracyclines. In less industrialized countries where no routine control of antibiotics in goat milk is implemented, the risk of antibiotic residues in the milk supply is increased. Studies investigating the effect of such substances in milk on cheesemaking and the organoleptic characteristics of the ripened cheese are very scarce (Cabizza et al., 2017; Quintanilla et al., 2019). The objective of this work was to evaluate the effect of different concentrations of oxytetracycline in raw goat milk on the volatile profile of Tronchón cheese during ripening.

We performed 3 trials of mature Tronchón cheese at the pilot plant of Universitat Politècnica de València (València, Spain) using Murciano-Granadina raw goat milk from the Universitat Politècnica de València experimental herd. For each cheese trial, 200 kg of milk was divided into 4 vats containing 50 kg each. Three of the vats were spiked with oxytetracycline (O4636; Sigma-Aldrich, Madrid, Spain) at different antibiotic concentrations closely related to the MRL: 50 $\mu\text{g/kg}$ (0.5 MRL), 100 $\mu\text{g/kg}$ (1 MRL), and 200 $\mu\text{g/kg}$ (2 MRL). A fourth vat was not spiked and was used as a control. We analyzed the chemical composition of the milk used for cheese production using a MilkoScan FT6000 (Foss, Hillerød, Denmark), and mean values ($\pm$ standard deviation) were as follows: $14.71 \pm 0.37\%$ total solids, $5.37 \pm 0.19\%$ fat, $3.98 \pm 0.22\%$ protein, and $4.63 \pm 0.07\%$ lactose. We made traditional semi-hard Tronchón goat cheese from the raw milk following the cheesemaking procedure reported by Quintanilla et al. (2019). Ten cheeses were obtained from each vat, and they were sampled in duplicate at 0, 15, 30, 45, and 60 d of ripening for further analysis. We quantified the oxytetracycline residues in the cheeses using liquid chromatography tandem mass spectrometry validated at the Instituto Lactológico de Lekunberri (Lekunberri, Pamplona, Spain) as previously described by Quintanilla et al. (2019). Volatile compounds analysis of the cheese samples was performed using headspace solid-phase microextraction (SPME)-GC/MS. For the SPME of volatile compounds, we followed the method developed by Hettinga et al. (2008). From each cheese, 2 samples were taken at a depth of 1 cm from the rind. For headspace extraction, 1 g of a finely grated cheese sample was weighed in 10-mL glass headspace GC vials (46×22.5 mm) and sealed with a 20-mm silicone/PTFE cap (Grace, Albany, OR). Extraction of volatile compounds by SPME was carried out using a 75- μm Carboxen-polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA) at 45°C for 40 min using an autosam-

pler. A vial filled with air was used as a blank. For GC/MS analysis, the SPME fiber was desorbed for 10 min in the GC injection port at 225°C . We performed GC/MS analysis using a Trace GC Ultra connected to a DSQ II mass spectrometer (Thermo Scientific, Austin, TX) and a Stabilwax-DA polyethylene-glycol column (30 m length, 0.32-mm internal diameter, 1- μm film thickness; Restek, Bellefonte, PA). The oven temperature was maintained at 40°C for 3 min, and then increased to 220°C at a rate of 15°C/min . When the final temperature of 220°C was reached, it was maintained for 1 min. Helium was used as a carrier gas, which was fed at a constant flow rate of 1.5 mL/min. The MS ion source was maintained at 225°C . The MS scans were collected in full scan mode, using a mass range of 33 to 250 m/z , with the electron impact mode at 70 eV. Each compound was identified using AMDIS software with the National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health database (National Institute of Standards and Technology, Gaithersburg, MD) and an in-house library (Hettinga, 2009). We used Metalign and MetalignID (<http://www.metalign.nl>, Wageningen, Netherlands) software packages for noise reduction, peak selection, peak identification, and peak integration. We used a mixed-model ANOVA to evaluate the effects of the oxytetracycline concentration in raw milk (0, 50, 100, and 200 $\mu\text{g/kg}$) and the ripening time (0, 15, 30, 45, and 60 d), as well as their respective interaction (concentration $\times$ time) on the volatile profile of the cheeses. We performed statistical analysis using Proc Mixed in SAS (version 9.2; SAS Institute Inc., Cary, NC). We used least significant difference test for multiple comparisons of the mean values. We performed multivariate analysis of the data using principal component analysis with Unscrambler X.10 software (Camo ASA, Oslo, Norway).

Cheesemaking from raw goat milk containing oxytetracycline close to MRL led to antibiotic residues in the cheeses (Figure 1). Our findings indicated that this antibiotic was widely transferred from milk to cheese, because the oxytetracycline concentration in the cheeses just before maturation (d 0) was about 4 times higher than the concentration in the raw milk used for cheese production (0.5 MRL: 3.9 ± 0.81 ; 1 MRL: 4.3 ± 0.32 ; 2 MRL: 3.5 ± 0.57). High concentration factors (3.8 to 5.7) have also been reported by Cabizza et al. (2017) and Gajda et al. (2018) when assessing the transfer of oxytetracycline from sheep and cow milk to cheese, respectively. The high fat affinity of this substance and its ability to form stable chelates with animal proteins (Giguère, 2013) could explain the high residual drug concentration in the experimental cheeses. As shown in Figure 1, the residual amounts of oxytetracycline

Figure 1. Antibiotic residues during ripening (mean $\pm$ SD) in Tronchón cheese made from goat milk spiked with different concentrations of oxytetracycline. MRL = maximum residue limit.

progressively decreased during maturation ($P < 0.001$) and was 88.8 to 96.5% lower in cheeses ripened for 60 d. Oxytetracycline is considered unstable, because its chemical structure contains 4 connected benzene rings with multiple ionizable functional groups, and under abiotic conditions it generates degradation products via epimerization, dehydration, or other pathways (Halling-Sørensen et al., 2003). The slightly acidic pH of the cheeses (5 to 5.5) and the environmental conditions of the maturation chamber (ambient temperature 10 to 12°C; relative humidity 80 to 85%) may have promoted the abiotic degradation of the oxytetracycline. Loftin et al. (2008) indicated that the degradation rate of aqueous solutions of oxytetracycline showed a positive correlation with temperature, and that oxytetracycline degrades more quickly at pH 5 than other antibiotics. In contrast, although tetracyclines have not been documented to undergo considerable microbial degradation, the highest enzymatic activity (proteolytic and lipolytic) in the cheeses during ripening could act synergistically with environmental conditions, leading to lower residual concentrations over time. Although variable amounts of oxytetracycline were present in the cheeses over the maturation period, our findings suggest that such residues were not able to significantly

affect the main biochemical pathways that produce aromatic compounds during maturation, because the volatile profile of the cheeses was unaffected by the presence of the antibiotic in the raw milk used to make the cheese ($P > 0.05$). We identified a total of 39 volatile compounds, including acids, alcohols, aldehydes, esters, ketones, and others; volatile carboxylic acids (36.3 to 66%) and ketones (61.4 to 25.8%) were the most abundant compounds in the volatile fraction of the Tronchón cheeses, similar to reports from Delgado et al. (2011) and Padilla et al. (2014) in other ripened goat milk cheeses.

Regarding the effect of the ripening time, statistical analysis showed that volatile profile values were modified throughout maturation (Table 1). In general, the total amounts of volatile organic acids, alcohols, and esters increased over the first 45 d of maturation ($P < 0.001$), whereas ketones (the group with the highest concentrations in the first 2 wk of ripening) progressively decreased ($P < 0.001$) over the same period, possibly due to a reduction of these compounds into secondary alcohols (Andiç et al., 2015). In the last 2 weeks of maturation, we observed only minor changes, likely related to the potential lower microbial activity in the cheeses at this stage, as reported by other authors

Table 1. Effect of ripening time on volatile compounds ($\text{AU} \times 10^5$, where AU = arbitrary units) in Tronchón goat cheese¹

Chemical group	Ripening time (d)					SEM
	0	15	30	45	60	
Acids						
Acetic acid	3,453 ^a	4,286 ^{cd}	4,075 ^{bc}	4,366 ^d	3,936 ^b	88.4
Propanoic acid	15 ^a	34 ^a	69 ^a	231 ^b	454 ^c	34.6
2-Methyl propanoic acid	5 ^a	19 ^b	49 ^c	67 ^d	55 ^{cd}	5.4
Butanoic acid	1,700 ^a	3,008 ^b	3,868 ^c	5,141 ^d	5,357 ^d	184.1
3-Methyl butanoic acid	10 ^a	37 ^b	87 ^c	109 ^d	116 ^d	9.3
Pentanoic acid	23 ^a	45 ^b	67 ^c	86 ^d	85 ^d	4.7
Hexanoic acid	863 ^a	1,474 ^b	2,085 ^c	2,821 ^d	2,961 ^d	179.0
Heptanoic acid	15 ^a	23 ^b	32 ^c	43 ^d	51 ^e	3.1
Octanoic acid	215 ^a	327 ^b	442 ^c	541 ^d	643 ^e	65.7
Total acids	6,299 ^a	9,253 ^b	10,774 ^c	13,405 ^d	13,661 ^d	413.1
Percentage	36.3	40.6	52.1	66.0	66.0	
Alcohols						
Ethanol	244 ^c	181 ^b	198 ^a	ND ²	ND	14.9
Butanol	ND	23 ^a	22 ^a	148 ^b	202 ^c	77.0
2-Butanol	ND	ND	120 ^a	606 ^b	330 ^a	50.3
3-Methyl 1-butanol	9 ^a	101 ^{bc}	191 ^d	124 ^c	77 ^b	13.4
Pentanol	12 ^a	31 ^c	34 ^c	23 ^b	15 ^a	2.2
2-Pentanol	24 ^a	23 ^a	223 ^b	610 ^d	443 ^c	27.0
Hexanol	20 ^a	96 ^c	54 ^b	18.6 ^a	10 ^a	10.1
1-Methoxy 2-propanol	16	25	24	19	15	3.4
Total alcohols	325 ^a	480 ^a	866 ^b	1,549 ^c	1,092 ^b	59.0
Percentage	1.9	1.9	3.7	7.8	4.4	
Aldehydes						
Hexanal	12 ^a	50 ^b	54 ^b	26 ^a	63 ^b	5.9
3-Methyl-hexanal	13 ^a	190 ^c	277 ^d	131 ^{bc}	116 ^b	25.8
Nonanal	4 ^a	59 ^b	43 ^b	46 ^b	84 ^c	6.6
Benzaldehyde	5 ^a	17 ^b	11 ^{ab}	9 ^a	9 ^a	2.1
Total aldehydes	34 ^a	316 ^{bc}	385 ^c	212 ^b	272 ^{bc}	31.0
Percentage	0.2	1.1	1.4	1.0	1.3	
Ketones						
2-Propanone (acetone)	48 ^c	67 ^d	45 ^c	13 ^a	24 ^b	5.1
2-Butanone	9 ^a	14 ^a	317 ^b	881 ^c	1,016 ^c	105.4
2,3-Butadione	1,445 ^a	2,580 ^b	2,117 ^b	1,134 ^a	1,108 ^a	203.8
2,3-Pentadione	16	18	24	22	ND	2.1
2-Pentanone	1,407 ^a	2,840 ^b	2,556 ^b	1,346 ^a	1,369 ^a	246.3
2-Hexanone	12 ^a	55 ^{bc}	50 ^b	22 ^a	67 ^c	6.4
2-Heptanone	61 ^a	1,982 ^c	1,801 ^c	683 ^{ab}	1,268 ^{bc}	301.2
3-Hydroxy 2-butanone	7,657 ^d	5,925 ^c	2,350 ^b	878 ^a	573 ^a	220.4
2-Nonanone	22 ^a	240 ^c	204 ^c	61 ^{ab}	157 ^{bc}	37.2
8-Nonen-2-one	ND	14 ^{ab}	23 ^c	9 ^a	17 ^{bc}	1.9
Total ketones	10,677 ^b	13,735 ^c	9,487 ^b	5,049 ^a	5,599 ^a	722.3
Percentage	61.4	55.9	41.7	23.5	25.8	
Esters						
Butanoic acid, ethyl ester	ND	51 ^a	95 ^b	196 ^c	217 ^c	15.7
Hexanoic acid, ethyl ester	ND	37 ^a	84 ^b	120 ^c	223 ^d	12.5
Propanoic acid, 2-methyl, propyl ester	25 ^a	45 ^b	96 ^b	52 ^{ab}	158 ^c	20.0
Total esters	25 ^a	133 ^b	275 ^c	368 ^d	598 ^e	26.3
Percentage	0.1	0.4	1.0	1.6	2.4	
Others						
2-Methyl 1,3-butadiene	10 ^a	17 ^c	15 ^c	15 ^c	13 ^b	0.9
Dimethyl disulfide	5 ^{bc}	2 ^a	4 ^b	5 ^c	4 ^{ab}	0.5
Dimethyl sulfone	10 ^c	8 ^b	8 ^b	7 ^a	6 ^a	0.4
3-Carene	3 ^a	16 ^b	3 ^a	4 ^a	ND	1.1
2,4-Dimethyl heptane	ND	ND	8 ^a	13 ^{ab}	13 ^b	1.8
Total others	28 ^a	43 ^{bc}	38 ^b	44 ^{bc}	36 ^{ab}	2.0
Percentage	0.1	0.1	0.1	0.1	0.1	

^{a-c}Means with different superscripts in the same row differ significantly ($P < 0.05$).¹Values are means of 6 determinations (2 cheeses per ripening time $\times$ 3 batch replicate). Percentage indicates the percentage of that chemical group relative to the total at a given ripening time.²ND = not detected.

typical flavor components that are perceived as a goat-like smell (Castillo et al., 2007; Delgado et al., 2011). We also detected high amounts of methyl ketones in the mature Tronchón cheese, similar to other Spanish goat cheeses such as Majorero (Castillo et al., 2007) and

Ibores (Delgado et al., 2011). The 2-pentanone, linked to a smell described as orange peel and sweet, fruity (Curioni and Bosset, 2002), was the most important ketone along the entire maturation period. We also detected large amounts of 2,3-butanedione (diacetyl) with an intensive creamy, buttery flavor (Le Bars and Yvon, 2008). We obtained no significant interactions for almost all volatile compounds (concentration $\times$ time; $P > 0.05$), suggesting that all the cheeses evolved in a similar way over time, regardless of the oxytetracycline concentration assessed. The only exception was related to some of the most quantitatively important compounds of the acid volatile fraction of the cheeses. We detected lower butanoic acid ($P < 0.05$) and hexanoic acid ($P < 0.01$) content in the cheeses from goat milk containing oxytetracycline at or above MRL, especially in the first 30 d of ripening. We also detected lower amounts of minority volatile acids such as 3-methyl butanoic acid ($P < 0.05$) and heptanoic acid ($P < 0.05$) during this period. The bacteriostatic activity of oxytetracycline, potentially able to produce an imbalance in the raw cheese microbiota involved in the biochemical changes during maturation (Cabizza et al., 2018), might have been responsible for the modifications of the volatile compounds.

We conducted principal component analysis to assess the overall effect of the concentration of oxytetracycline and ripening time on the volatile profile of the cheeses. We found 3 principal components to explain 70% of the variations in the data set, in which principal component 1 represented 43% of the variability and principal component 2 represented 20%. Figure 2 shows the score and loading plots of the principal component analysis. In general, the presence of oxytetracycline did not have an overall effect on the volatile profile. However, the different stages of maturation were very well separated along principal component 1, progressively from the left to the right quadrants (Figure 2). The circles represent the different stages of maturation (0, 15, 30, 45, and 60 d). The loading plot shows a wider distribution of volatile compounds along principal component 1 according to ripening times, with amounts of acid compounds standing out at 45 and 60 d of ripening.

The presence of oxytetracycline in goat milk close to the legal maximum concentration (0.5 to 2 MRL) did not seem to modify the volatile profile of the Tronchón cheeses ripened for 60 d. However, larger amounts of residues of this antibiotic could be present, especially when cheese is matured for a short time (2 to 4 wk). Although ripening conditions led to the degradation of residual oxytetracycline in cheese, variable amounts of this antibiotic may persist in matured cheeses, posing a risk to consumer health.

ACKNOWLEDGMENTS

This work is part of the AGL-2013-45147-R funded by the Ministry of Science and Innovation (Madrid, Spain). The authors thank the Research and Development Support Program, “Ayudas para movilidad dentro del Programa para la Formación de Personal Investigador” (2.016) of Universitat Politècnica de València (Spain), allowing Paloma Quintanilla to perform a predoctoral stay at the Food Quality and Design Group, Wageningen University and Research (Wageningen, the Netherlands). The authors state that they have no conflicts of interest.

REFERENCES

- Andić, S., Y. Tunçtürk, and G. Boran. 2015. Changes in Volatile Compounds of Cheese. V. Preedy, ed. Academic Press, Oxford, UK. <https://doi.org/10.1016/B978-0-12-404699-3.00028-7>.
- Attai, R., M. Bsharat, A. Mora-Gutierrez, and S. Woldeesenbet. 2015. Short communication: Determination of withdrawal time for oxytetracycline in different types of goats for milk consumption. *J. Dairy Sci.* 98:4370–4376. <https://doi.org/10.3168/jds.2014-8616>.
- Beltrán, M. C., R. L. Althaus, A. Molina, M. I. Berruga, and M. P. Molina. 2015. Analytical strategy for the detection of antibiotic residues in sheep and goat's milk. *Span. J. Agric. Res.* 13:e0501. <https://doi.org/10.5424/sjar/2015131-6522>.
- Cabizza, R., N. Rubattu, S. Salis, M. Pes, R. Comunian, A. Paba, M. Addis, M. C. Testa, and P. P. Urgeghe. 2017. Transfer of oxytetracycline from ovine spiked milk to whey and cheese. *Int. Dairy J.* 70:12–17. <https://doi.org/10.1016/j.idairyj.2016.12.002>.
- Cabizza, R., N. Rubattu, S. Salis, M. Pes, R. Comunian, A. Paba, E. Daga, M. Addis, M. C. Testa, and P. P. Urgeghe. 2018. Impact of a thermisation treatment on oxytetracycline spiked ovine milk: Fate of the molecule and technological implications. *Lebensm. Wiss. Technol.* 96:236–243. <https://doi.org/10.1016/j.lwt.2018.05.026>.
- Castillo, I., M. V. Calvo, L. Alonso, M. Juárez, and J. Fontecha. 2007. Changes in lipolysis and volatile fraction of a goat cheese manufactured employing a hygienized rennet paste and a defined strain starter. *Food Chem.* 100:590–598. <https://doi.org/10.1016/j.foodchem.2005.09.081>.
- Curioni, P. M. G., and J. O. Bosset. 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* 12:959–984. [https://doi.org/10.1016/S0958-6946\(02\)00124-3](https://doi.org/10.1016/S0958-6946(02)00124-3).
- Delgado, F. J., J. González-Crespo, R. Cava, J. García-Parra, and R. Ramírez. 2010. Characterisation by SPME-GC-MS of the volatile profile of a Spanish soft cheese P.D.O. Torta del Casar during ripening. *Food Chem.* 118:182–189. <https://doi.org/10.1016/j.foodchem.2009.04.081>.
- Delgado, F. J., J. González-Crespo, R. Cava, and R. Ramírez. 2011. Formation of the aroma of a raw goat milk cheese during maturation analysed by SPME-GC-MS. *Food Chem.* 129:1156–1163. <https://doi.org/10.1016/j.foodchem.2011.05.096>.
- European Union. 2010. Regulation (EU) no. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Accessed Feb. 20, 2020. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010R0037>.
- Gajda, A., E. Nowacka-Kozak, M. Gbylik-Sikorska, and A. Posyniak. 2018. Tetracycline antibiotics transfer from contaminated milk to dairy products and the effect of the skimming step and pasteurisation process on residue concentrations. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* 35:66–76. <https://doi.org/10.1080/19440049.2017.1397773>.

- Giguère, S. 2013. Antimicrobial drug action and interaction. Pages 1–10 in *Antimicrobial Therapy in Veterinary Medicine*. 5th ed. S. Giguère, J. F. Prescott, and P. M. Dowling, ed. Wiley-Blackwell, Ames, IA. <https://doi.org/https://doi.org/10.1002/9781118675014.ch1>.
- Halling-Sørensen, B., A. Lykkeberg, F. Ingerslev, P. Blackwell, and J. Tjørnelund. 2003. Characterization of the abiotic degradation pathways of oxytetracyclines in soil interstitial water using LC–MS–MS. *Chemosphere* 50:1331–1342. [https://doi.org/10.1016/s0045-6535\(02\)00766-https://doi.org/x](https://doi.org/10.1016/s0045-6535(02)00766-https://doi.org/x).
- Hettinga, K. A. 2009. Quality control of raw cows' milk by headspace analysis, a new approach to mastitis diagnosis. PhD Thesis. Wageningen University, the Netherlands.
- Hettinga, K. A., H. J. F. Van Valenberg, and A. C. M. Van Hooijdonk. 2008. Quality control of raw cows' milk by headspace analysis. *Int. Dairy J.* 18:506–513. <https://doi.org/10.1016/j.idairyj.2007.10.005>.
- Katla, A. K., H. Kruse, G. Johnsen, and H. Herikstad. 2001. Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *Int. J. Food Microbiol.* 67:147–152. [https://doi.org/10.1016/s0168-1605\(00\)00522-5](https://doi.org/10.1016/s0168-1605(00)00522-5).
- Le Bars, D., and M. Yvon. 2008. Formation of diacetyl and acetoin by *Lactococcus lactis* via aspartate catabolism. *J. Appl. Microbiol.* 104:171–177. <https://doi.org/10.1111/j.1365-2672.2007.03539.x>.
- Loftin, K. A., C. D. Adams, M. T. Meyer, and R. Surampalli. 2008. Effects of ionic strength, temperature, pH on degradation of selected antibiotics. *J. Environ. Qual.* 37:378–386. <https://doi.org/10.2134/jeq2007.0230>.
- McSweeney, P. L. H., and M. J. Sousa. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Lait* 80:293–324. <https://doi.org/10.1051/lait:2000127>.
- Padilla, B., C. Belloch, J. J. López-Díez, M. Flores, and P. Manzanares. 2014. Potential impact of dairy yeasts on the typical flavour of traditional ewes' and goats' cheeses. *Int. Dairy J.* 35:122–129. <https://doi.org/10.1016/j.idairyj.2013.11.002>.
- Quintanilla, P., M. C. Beltrán, A. Molina, I. Escriche, and M. P. Molina. 2019. Characteristics of ripened Tronchón cheese from raw goat's milk containing legally admissible amounts of antibiotics. *J. Dairy Sci.* 102:2941–2953. <https://doi.org/10.3168/jds.2018-15532>.
- Sierra, D., A. Contreras, A. Sánchez, C. Luengo, J. C. Corrales, C. T. Morales, C. De la Fe, I. Guirao, and C. Gonzalo. 2009. Short communication: Detection limits of non-β-lactam antibiotics in goat's milk by microbiological residues screening tests. *J. Dairy Sci.* 92:4200–4206. <https://doi.org/10.3168/jds.2009-2101>.
- Souza, C. F. V., T. Dalla Rosa, and M. A. Z. Ayub. 2003. Changes in the microbiological and physicochemical characteristics of Serrano cheese during manufacture and ripening. *Braz. J. Microbiol.* 34:260–266. <https://doi.org/10.1590/S1517-83822003000300016>.

ORCIDS

- P. Quintanilla <https://orcid.org/0000-0003-0422-1903>
 K. A. Hettinga <https://orcid.org/0000-0002-9017-4447>
 M. C. Beltrán <https://orcid.org/0000-0002-5335-2179>
 I. Escriche <https://orcid.org/0000-0003-0180-0360>
 M. P. Molina <https://orcid.org/0000-0002-0647-8609>