Interpretative summary

Short Communication: ***Volatile profile of matured Tronchón cheese affected by oxytetracycline in raw goat's milk***

Quintanilla

The volatile profile of Tronchón cheeses made from raw goat's milk containing different concentrations of oxytetracycline (0, 50, 100, and 200 μg/kg) were compared on a fortnightly basis during a 60-day maturation period. The volatile profile of the cheeses was unaffected by the presence of oxytetracycline in milk, being modified by the ripening time only. The antibiotic was widely transferred from milk to cheese and variable amounts of oxytetracycline (< 10 - 79 μg/kg) were detected in ripened cheese, posing a potential risk for consumer health.

Running headline: **SHORT COMMUNICATION: OXYTETRACYCLINE IN GOAT CHEESE**

Short Communication: ***Volatile profile of matured Tronchón cheese affected by oxytetracycline in raw goat's milk***

P. Quintanilla[*], K.A. Hettinga[†], M.C. Beltrán[*], I. Escriche[‡], M.P. Molina[*1],

[*]*Institute for Animal Science and Technology. Universitat Politècnica de València. Camino de Vera, s/n. 46022, Valencia, Spain.*

[†]*Dairy Science and Technology Group, Chair of Food Quality and Design, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherland.*

[‡]*Institute of Food Engineering for Development. Food Technology Department. Universitat Politècnica de València. Camino de Vera, s/n, 46022, Valencia, Spain.*

Corresponding author: Mª Pilar Molina Pons
Institute for Animal Science and Technology
Universitat Politècnica de València
Camino de Vera, s/n
46022 Valencia, Spain
Phone: + 34 963877431 Fax: +34 963877439
pmolina@dca.upv.es

[1] **Corresponding author:** pmolina@dca.upv.es

ABSTRACT

The presence of antibiotics in milk destined for cheese production may affect the biological processes responsible of the formation of volatile compounds, leading to alterations of the characteristic cheese flavor expected by consumers. The aim of this study was to evaluate the impact of the presence of oxytetracycline in goat's milk on the volatile profile of ripened cheeses. Traditional mature Tronchón cheeses were manufactured from raw goat's milk spiked with different concentrations of oxytetracycline (50, 100, and 200 μg/kg). Cheese from antibiotic-free goat's milk was used as control. Residual amounts of the antibiotic and the volatile profile of the experimental cheeses were analyzed on a fortnightly basis during maturation by LC/MS-MS and SPME-GC/MS methods, respectively. Results herein suggest that oxytetracycline was widely transferred from milk to cheese; the drug concentration in the cheeses being 3.5 - 4.3 times higher than the drug concentration in raw milk. Although the residual amounts of oxytetracycline significantly decreased during maturation (88.8 - 96.5%), variable amounts of residues remained in 60-days matured cheeses (< 10 - 79 μg/kg). In general, the presence of oxytetracycline in goat's milk did not affect, the volatile profile of Tronchón cheeses which was significantly modified by the ripening time. In any case, the presence of oxytetracycline residues in the 60-day ripened cheeses could be of great concern for public health.

Key Words: Goat cheese, oxytetracycline, antibiotic residue, volatile profile.

Short Communication

In the Mediterranean and eastern European countries, goat's milk is mainly used for cheese-making, with a growing demand in the last decade due to its particular taste, nutritional value and the great variety of traditional cheeses. Cheese flavor is one of the most important organoleptic criteria for consumer acceptance, being 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 to produce aromatic compounds like alcohols, aldehydes, carboxylic acids, esters, ketones, among others, during ripening (McSweeney and Sousa, 2000; Delgado et al., 2010). It is generally agreed that the presence of antibiotic residues in milk, besides the negative implications on consumer health, affects technological cheese-making processes as they could inhibit the activity of the raw milk microflora and/or of the starter cultures usually employed in the dairy industry (Katla et al., 2001). Thus, the liberation of enzymes could potentially be altered and, consequently, modify the production of aromatic compounds in matured cheese. One of the most widespread broad-spectrum antibiotics used in dairy goats is oxytetracycline mainly prescribed to treat mastitis, urinary tract and enteric infections (Attaie et al., 2015). Oxytetracycline treatments 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 (**EU**), the regulatory levels or Maximum Residue Limits (**MRL**) for all tetracyclines, included oxytetracycline, in milk have been fixed at 100 μg/kg by legislation (European Union, 2010). In EU and other countries, where quality control programs are well implemented, raw milk is routinely screened for antibiotic residues. Microbial inhibitor tests are usually applied in milk quality control laboratories, being able to detect β-lactam antibiotics at or below MRLs but cannot suitably detect other veterinary drugs such as quinolones and tetracyclines, at safety levels

(Sierra et al., 2009; Beltrán et al, 2015). In farms and dairies, receptor-binding assays are commonly applied only for screening β-lactam residues in goat milk often not detecting other veterinary drugs such as tetracyclines. On the other hand, in less industrialized countries where no routinely control of antibiotics in goat milk are implemented, the risk of antibiotic residues in the milk supply increases. Studies available on the effect of such substances in milk on the cheese-making and the organoleptic characteristics of the ripened cheese are very scarce (Cabizza et al., 2017; Quintanilla et al., 2019). Therefore, the objective of this work was to evaluate the impact of different concentrations of oxytetracycline in raw goat's milk on the volatile profile of Tronchón cheese during ripening.

Three trials of mature Tronchón cheese were performed at the pilot plant of Universitat Politècnica de València (UPV, Valencia, Spain) using Murciano-Granadina raw goat's milk from the UPV experimental herd. For each cheese trial, 200 kg of milk were divided into four 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 μg/kg (0.5 MRL), 100 μg/kg (1 MRL) and 200 μg/kg (2 MRL), and the last one was not spiked to be used as reference. The chemical composition of the milk used for cheeses production were analyzed using MilkoScan FT6000 (Foss, Hillerød, Denmark), the mean values (mean ± standard deviation) being: 14.71 ± 0.37% total solids, 5.37 ± 0.19% fat, 3.98 ± 0.22% protein and 4.63 ± 0.07% lactose. Traditional semi-hard Tronchón goat cheese was made from raw milk following the cheese-making procedure reported by Quintanilla et al. (2019). Ten cheeses were obtained from each vat, which were sampled in duplicate at 0, 15, 30, 45 and 60 days of ripening for further analysis. Oxytetracycline residues in the cheeses were quantified using liquid chromatography tandem-mass spectrometry (LC/MS-MS) method validated at the

Instituto Lactológico de Lekunberri (Lekunberri, Pamplona, Spain) previously described by Quintanilla et al. (2019). The volatile compounds analysis of the cheese samples was performed by the headspace SPME-GC/MS method. For the solid phase micro-extraction (SPME) of volatile compounds, the method developed by Hettinga et al. (2008) was followed. From each cheese, two samples were taken at a depth of 1 cm from the rind. For headspace extraction, one gram of a finely grated cheese sample was weighed in a 10 mL glass headspace GC vials (46 x 22.5 mm) and sealed with a 20 mm silicone/PTFE cap (Grace, Albany, OR, USA). Extraction of volatile compounds by SPME was carried out with a 75 µm Carboxen™–PDMS SPME fiber (Supelco, Bellefonte, PA, USA) at 45°C for 40 min using an auto-sampler. A vial filled with air was used as blank. For GC/MS analysis, the SPME fiber was desorbed for 10 min in the GC injection port at 225°C. GC/MS analysis was performed using a Trace GC Ultra connected to a DSQ II mass spectrometer (Thermo Scientific, Austin, TX, USA). The Stabilwax®-DA polyethylene-glycol column with 30 m length, 0.32 mm internal diameter, and 1 µm film thickness (Restek, Bellefonte, PA, USA) was used. The oven temperature was maintained at 40°C for 3 min, 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 carrier gas, which was fed at a constant flow rate of 1.5 mL/min. The MS ion source was maintained at 225°C. MS scans were collected in full scan mode, using a mass range of 33-250 m/z with electron impact mode at 70 eV. Each compound was identified with AMDIS software using the NIST/EPA/NIH database (NIST, Gaithersburg, MD, USA) and an in-house library (Hettinga, 2009). Metalign and MetalignID (http://www.metalign.nl, Wageningen, Netherlands) software packages were used for noise reduction, peak selection, peak identification and peak integration. A mixed model analysis of variance was employed to evaluate the effects of the oxytetracycline concentration in raw milk (C: 0, 50, 100, and 200 µg/kg) and the ripening time (t: 0, 15, 30, 45, and 60 days), as well as their respective interaction (C x t) on the volatile profile of the cheeses. Statistical analysis was performed using the Proc Mixed of the SAS software (SAS Version 9.2, SAS Institute Inc., Cary, NC, USA). The Least Significant Difference (LSD) test was employed for multiple comparisons of the mean values. Multivariate analysis of the data was done by Principal Component Analysis (PCA) using the Unscrambler X.10 software (Camo ASA, Oslo, Norway).

Cheese-making from raw goat's milk containing oxytetracycline close to MRL led to antibiotic residues in the cheeses (Figure 1). Results herein indicate that this antibiotic was widely transferred from milk to cheese, as the oxytetracycline concentration in the cheeses just before maturation (day 0) was about 4 times higher than the drug concentration in 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 - 5.7) were reported also 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 quelates 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 progressively decreased during maturation ($p < 0.001$), being on average 88.8 - 96.5% lower in 60-day ripened cheeses. It is known that oxytetracycline is considered to be instable as its chemical structure contains four connected benzene rings with multiple ionizable functional groups, and under abiotic conditions generate degradation products via epimerization, dehydration or other pathways (Halling-Sørensen et al., 2003). The slightly acidic pH of the cheeses (5 - 5.5) and the environmental conditions of the maturation chamber (T^a = 10 - 12ºC, RH = 80 - 85%) may promote the abiotic degradation of the oxytetracycline. Loftin et al. (2008)

indicated that the degradation rate of the aqueous solutions of oxytetracycline showed a positive correlation with temperature and, also, that oxytetracycline degrades more quickly at pH 5 than other antibiotics. On the other hand, although tetracyclines are not documented to undergo considerable microbial degradation, the highest enzymatic activity (proteolytic and lipolytic) in the cheeses during this period could act synergistically with environmental conditions leading to lower residual concentrations along time. Although variable amounts of oxytetracycline were present in the cheeses during the entire maturation period, results herein suggest that such residues were not able to affect significantly the main biochemical pathways producing aromatic compounds during maturation as the volatile profile of the cheeses was unaffected by the presence of this antibiotic in the raw milk used for cheese manufacture ($p > 0.05$). A total of thirty-nine volatile compounds including acids, alcohols, aldehydes, esters, ketones and others were identified; volatile carboxylic acids (36.3 - 66%) and ketones (61.4 - 25.8%) were the most abundant compounds in the volatile fraction of the Tronchón cheeses, as similarly reported by Delgado et al. (2011) and Padilla et al. (2014) in other ripened goat's milk cheeses.

Regarding the effect of the ripening time, statistical analysis showed that the volatile profile values were modified throughout maturation (Table 1). In general, the total amounts of volatile organic acids, alcohols, and esters increased during the first 45 days of maturation ($p < 0.001$), whereas ketones, the group with the highest concentrations in the two first weeks of ripening, progressively decreased ($p < 0.001$) in this period, possibly due to a reduction of these compounds into secondary alcohols (Andiç et al., 2015). In the last two weeks of maturation only minor changes were observed, likely to be related to the potential lower microbial activity in the cheeses at this stage, as reported by other authors in goat cheeses with similar maturation times (Souza et al., 2003;

Delgado et al. 2011). In 60-day ripened Tronchón cheeses, acetic, butanoic, and hexanoic acids were the most abundant volatile compounds, more than 50%, being typical flavor components perceived as a goat-like smell (Castillo et al., 2007; Delgado et al., 2011). High amounts of methyl ketones were also detected in the mature Tronchón cheese, as occurred in 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 one along the entire maturation period. Large amounts of 2,3 butadione (diacetyl) with an intensive creamy, buttery flavor (Le Bars and Yvon, 2008) were also detected. No significant interactions were obtained for almost all volatile compounds (C x t; $p > 0.05$), 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. Thus, lower content of butanoic acid ($p < 0.05$) and hexanoic acid ($p < 0.01$) were detected in the cheeses from goat milk containing oxytetracycline at or above MRL, especially in the first 30 days of ripening. Similarly, lower amounts of minority volatile acids like 3-methyl butanoic acid ($p < 0.05$), and heptanoic acid ($p < 0.05$) were detected in 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 be responsible for the modifications of the volatile compounds.

A PCA was conducted to assess the overall effect of the concentration of oxytetracycline and the ripening time on the volatile profile of the cheeses. Three principal components were found to explain 70% of the variations in the data set in which PC1 represents 43% of the variability and PC2 20%. Figure 2 shows the score (A) and loading (B) plots of the PCA performed. 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 PC1, progressively from the left to the right quadrants (Figure 2). The circles represent the different stages of maturation (0, 15, 30, 45, and 60 days). The loading plot shows a wider distribution of the volatile compounds along PC1 according to the ripening times, with amounts of acid compounds standing out at 45 and 60 days of ripening.

The presence of oxytetracycline in goat's milk close to the legal maximum concentration (0.5 - 2 MRL) does not seem to modify the volatile profile of the Tronchón cheeses ripened for a 60-day period. However, larger amounts of residues of this antibiotic could be present especially when cheese is matured for a short time (2 - 4 weeks). Although ripening conditions lead 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 Food Quality and Design Group, Wageningen University & Research (Wageningen, Netherlands).

REFERENCES

Andiç, S., Y. Tunçtürk, and G. Boran. 2015. Changes in Volatile Compounds of Cheese. V. Preedy, ed. Academic Press, Oxford, UK. http://doi.org/10.1016/B978-0-12-404699-3.00028-7.

Attaie, R., M. Bsharat, A. Mora-Gutierrez, and S. Woldesenbet. 2015. Short communication: Determination of withdrawal time for oxytetracycline in different types of goats for milk consumption. J. Dairy Sci. 98:4370-4376. http://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. Spanish J. Agric. Res. 13. http://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. LWT - Food Sci. 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. http://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. http://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.

http://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. http://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. Off. J. L 15:1–72.

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. http://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, Iowa. 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-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, 128 pp.

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.

http://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.
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. http://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. 2009b. 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. D. Rosa, and M. A. Z. Ayub. 2003. Changes in the microbiological

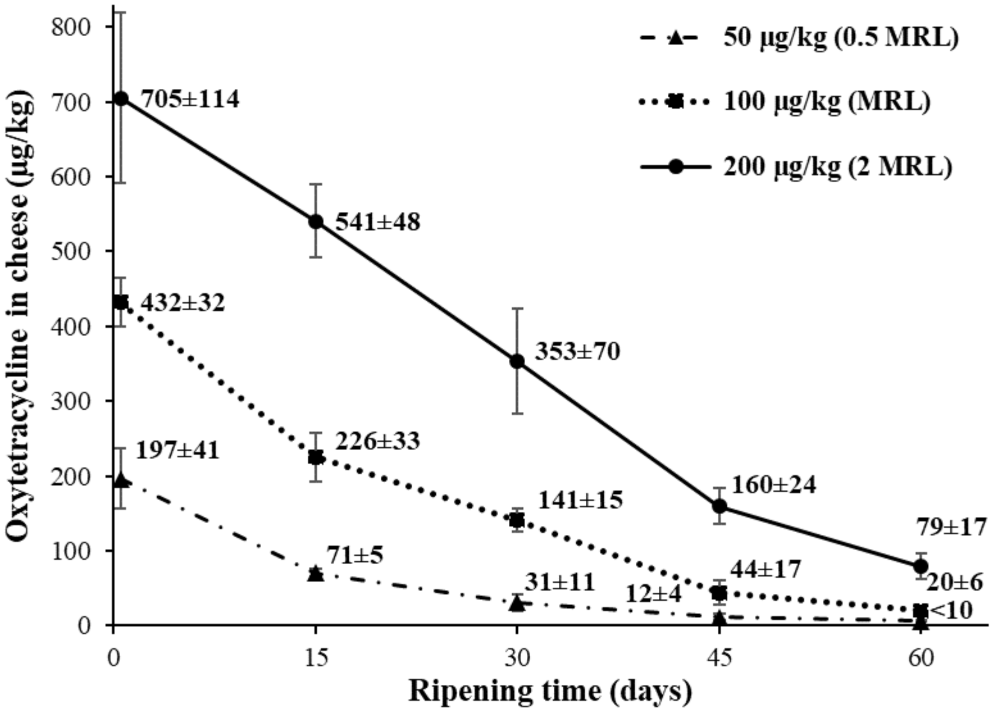
and physicochemical characteristics of Serrano cheese during manufacture and ripening. Braz. J. Microbiol., 34:260-266.

Table 1. Effect of the ripening time on the volatile compounds (AU x 10^5) of Tronchón goat cheese.

Chemical group	Ripening time (days)					
	0	15	30	45	60	SEM
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	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**	

Chemical group	Ripening time (days)					
	0	15	30	45	60	SEM
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**	

Values are means of six determinations (two cheeses per ripening time x three batch replicate); SEM: standard error; $^{a, b, c, d, e}$: Superscript letters in the same row indicate significant differences ($p < 0.05$), nd = not detected.



Oxytetracycline in cheese (µg/kg)
800
700
600
500
400
300
200
100
0
0
15
30
45
60
Ripening time (days)
50 µg/kg (0.5 MRL)
100 µg/kg (MRL)
200 µg/kg (2 MRL)
705±114
541±48
353±70
160±24
79±17
432±32
226±33
141±15
44±17
20±6
197±41
71±5
31±11
12±4
<10

Figure 1.

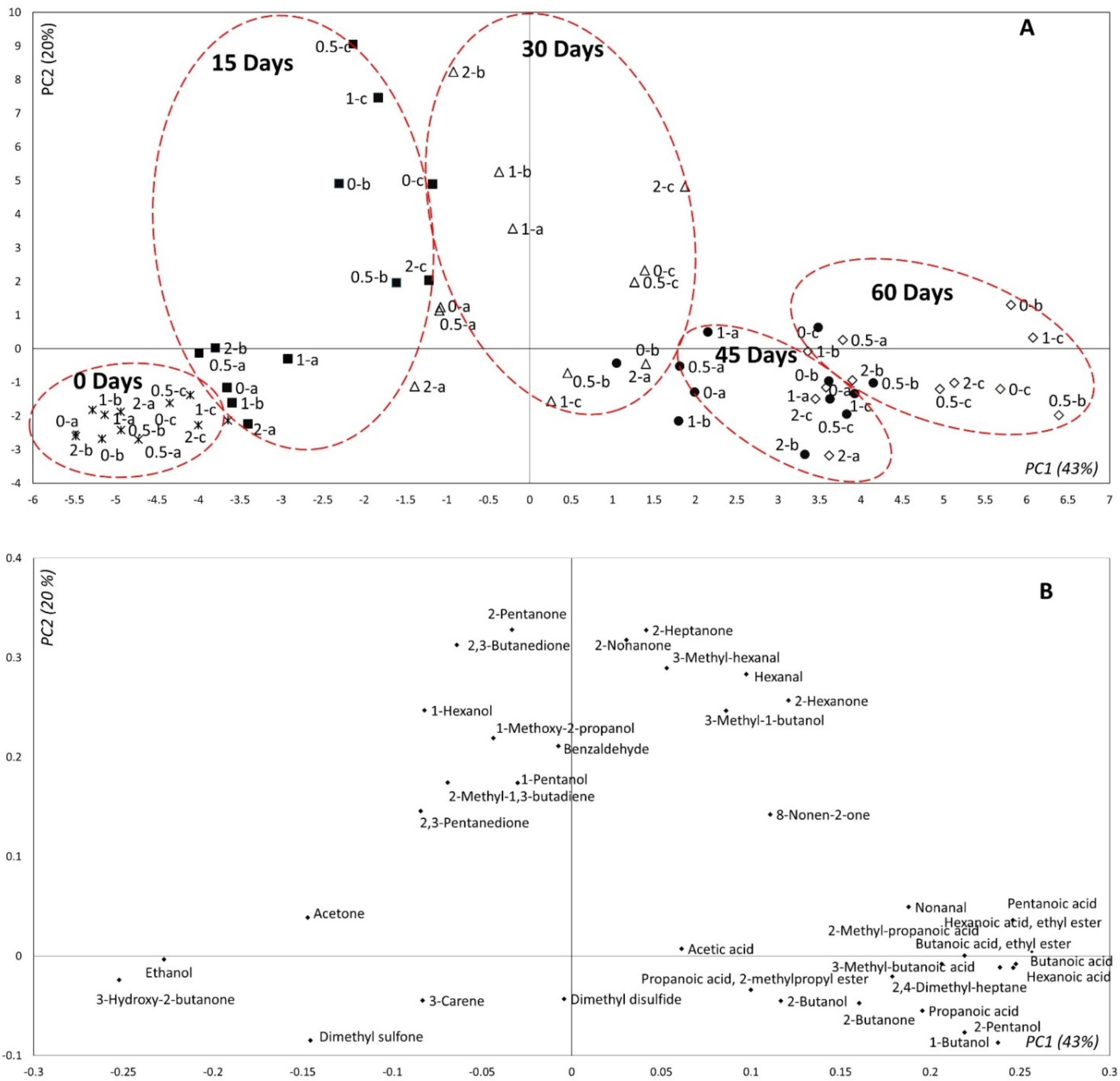

A
15 Days
30 Days
0 Days
45 Days
60 Days
PC2 (20%)
PC1 (43%)
B
PC2 (20 %)
PC1 (43%)
2-Pentanone
2,3-Butanedione
2-Heptanone
2-Nonanone
3-Methyl-hexanal
Hexanal
2-Hexanone
3-Methyl-1-butanol
1-Hexanol
1-Methoxy-2-propanol
Benzaldehyde
1-Pentanol
2-Methyl-1,3-butadiene
2,3-Pentanedione
8-Nonen-2-one
Acetone
Nonanal
Pentanoic acid
2-Methyl-propanoic acid
Hexanoic acid, ethyl ester
Butanoic acid, ethyl ester
Acetic acid
Ethanol
3-Methyl-butanoic acid
Butanoic acid
Propanoic acid, 2-methylpropyl ester
Hexanoic acid
2,4-Dimethyl-heptane
3-Hydroxy-2-butanone
3-Carene
Dimethyl disulfide
2-Butanol
2-Butanone
Propanoic acid
2-Pentanol
Dimethyl sulfone
1-Butanol

Figure 2.

Figure captions

Figure 1. Antibiotic residues during ripening (Mean ± SD) in Tronchón cheese made from goat's milk spiked with different concentrations of oxytetracycline.

Figure 2. Score (A) and loading (B) plots of the two first principal components (PC_1 and PC_2) for the volatile profile of Tronchón cheese. The explained variance of PC_1 and PC_2 was 43% and 20%, respectively. Codes in the score plots refer to the oxytetracycline concentrations in goat's milk (0, 0.5 MRL, 1 MRL, and 2 MRL), ripening time (0: *; 15: ■; 30: Δ, 45:● and 60 days: ◊) and the triplicate of cheese manufactured for each concentration (a, b, and c).