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Differential microbiological groups affecting the clotting properties of sheep milk

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Abstract. Milk quality criteria are established in many countries according to a variety of requirements, in order to answer the need of milk processors and consumers. Moreover, sheep's milk, unlike cows' or goats' milk, is used almost exclusively for the production of cheese so its quality is based not only on its nutrient content but also on its renneting ability, a key factor in cheese yield. However the technological properties of many Spanish breeds have not been fully studied. On the other hand, the differential microflora of milk has varied effects on the coagulation process of milk. Information of factors affecting the technological characteristics of sheep's milk is scarce and research on the evolution of lactodynamographic parameters in relationship with the microbiological aspects of milk has been limited. The aim of this work was to study the relationship between differential microbiological quality and technological parameters. The differential microbiological analysis of milk gives a clear idea about milk quality depending of the group. When the differential counts are classified attending to r parameter, it is observed that the samples with higher counts have lower renneting times. On the other hand, the value of A_{30} parameter is affected by the counts of SPC, THERMO, PSYCHRO, PSEUDO, COLIT, LACT and STREP, as well as, the pH value. In view of the results it shows that it is necessary to continue studying the influence of the microbial load of sheep's milk cheese, as the main product obtained from processing.

Keywords. Differential microbiological quality – Clotting properties – Sheep milk.

Groupes microbiologiques différentiels affectant les propriétés de coagulation du lait de brebis

Résumé. *Les critères de qualité du lait sont établis dans de nombreux pays selon une variété de besoins, afin de répondre à la nécessité des transformateurs de lait et des consommateurs. En outre, le lait de brebis, à la différence du lait de vache ou de chèvre, est utilisé presque exclusivement pour la production de fromage, de sorte que sa qualité est basée non seulement sur sa teneur en éléments nutritifs, mais aussi sur sa capacité d'emprésurage, un facteur clé du rendement en fromage. Toutefois, les propriétés technologiques de nombreuses races espagnoles n'ont pas été entièrement étudiées. D'autre part, la microflore différentielle du lait a des effets variés sur le processus de coagulation du lait. Les informations sur les facteurs qui influent sur les caractéristiques technologiques du lait de brebis sont rares et la recherche sur l'évolution des paramètres de coagulation en relation avec les aspects microbiologiques du lait a été limitée. Le but de ce travail était d'étudier la relation entre la qualité microbiologique différentielle et les paramètres technologiques. L'analyse microbiologique différentielle du lait donne une idée claire de la qualité du lait en fonction du groupe. Lorsque les dénombrements différentiels sont classés en fonction du paramètre r, il est observé que les échantillons avec des dénombrements plus élevés ont des temps d'emprésurage inférieurs. D'autre part, la valeur du paramètre A_{30} est affectée par les dénombrements de CPS, THERMO, PSYCHRO, PSEUDO, COLIT, LACT et STREP, ainsi que la valeur du pH. Compte tenu des résultats, il est montré qu'il est nécessaire de continuer à étudier l'influence de la charge microbienne du fromage de lait de brebis, comme principal produit obtenu à partir de la transformation.*

Mots-clés. *Qualité microbiologique différentielle – Propriétés de coagulation – Lait de brebis.*

I – Introduction

Milk quality criteria are established in many countries according to hygienic, sanitary, physico-chemical, technological and sensorial requirements, in order to answer the need of milk processors and consumers. Therefore, milk protein, fat contents, bacteriology, somatic cell count (SCC), immunoglobulin (IgG), inhibitors, freezing point and, in an optional way, lipolysis (like in France) are some criteria included in control quality systems. This list and the thresholds depend on the countries and bonuses or penalties are applied on the price of the milk according to the thresholds (Raynal-Ljutovac *et al*., 2005).

Sheep's milk, unlike cows' or goats' milk, is used almost exclusively for the production of cheese, so its quality is based not only on its nutrient content but also on its renneting ability, a key factor in cheese yield. Some research has contributed to the understanding of the renneting properties of dairy ewe breeds from other Mediterranean countries (Martini *et al.,* 2008; Sitzia *et al.,* 2015); however the technological properties of many Spanish breeds have not been fully studied.

On the other hand, the microflora of cheese may be divided into two groups: starter lactic acid bacteria and secondary microorganisms. Starter lactic acid bacteria are involved in acid production during manufacture and contribute to the ripening process. Secondary microorganisms do not contribute to acid production during manufacture, but generally play a significant role during ripening (Beresford *et al*., 2001).

Information of factors affecting the technological characteristics of sheep's milk are scarce and research on the evolution of lactodynamographic parameters in relationship with the microbiological aspects of milk has been limited (Beresford and Williams 2004; Elmoslemany *et al*., 2009).

Castilla-La Mancha region, with around 137,687 thousands of litters (MAGRAMA, 2013), is the second sheep milk productive region in Spain and a place of origin of Manchego Cheese. So that, our research group has developed some research works with the final objective of studying the global quality of sheep milk used in cheese-making. Some results about the differential microbiological quality of bulk tank milk in relationship with the technological aspects are shown in this work.

II – Material and methods

Between October 2012 and November 2013, a total of 302 bulk-tank milk samples were collected from 79 sheep farms distributed in the region of Castilla-La Mancha (Spain). The farms were selected by different factors: to be a member of Regulatory Council of Manchego Cheese, the geographical distribution and size of farms and to be a member of National Association of Manchega Breeders (AGRAMA).

Milk sampling was performed before homogenization, transported to the Dairy Laboratory of CERSYRA under refrigerated conditions (below 5ºC) and analysed within 24 h for the differential counts of micro-organisms. In addition, two samples more were taken in parallel: one for the study of the technological features [through Formagraph Foss-Electric (Hillerød, Denmark) at the Dairy Small Ruminant Laboratory of University of Cordoba (Spain)], and a second one for the study of somatic cell count (SCC) [with Fossomatic FC (Hillerød, Denmark) at the Interprofessional Dairy Laboratory of Castilla-La Mancha (Spain)].

1. Microbiological analysis

Samples (1 ml) were homogenized in 9 ml of sterile 0.1% peptone-water solution (w/v). Appropriated serial decimal dilutions were made and inoculated on several specific media. A fixed quantity of 0.1 ml of the corresponding dilution was plated for different microbiological analyses by surface plating.

The total bacterial count or Standard plate count (SPC), Thermodurics (THERMO), and Psychrotrophics (PSYCRO) were plated in the plate count agar (PCA) (Panreac, Barcelona, Spain). SPC and Thermodurics (after milk pasteurization, 62.8ºC for 30 min) were incubated in aerobic conditions at 30ºC for 72 h (ISO 4833:2003). Psychrotrophics were incubated at 6.5º for 10 days (ISO 6730/IDF 101:2005). The evaluation of *Pseudomonas* spp. (PSEUDO) was made incubating at 35ºC for 24-48 h in a Centrimida medium. The determination of *Escherichia coli* (ECOLI) and Total Coliforms (COLIT) was achieved with CromoIDTM Coli (bioMérieux, Madrid, Spain) and plates were incubated 37ºC for 24 h. Moreover, the use of plates incubated at 37ºC for 24 h with Agar Baird Parker + RPF (bioMérieux, Madrid, Spain) allowed the differentiation of Baird Parker + RPF coagulase positive (CP) and Baird Parker + RPF coagulase negative (CN). Lactic Acid Bacteria (LACT) strains were incubated in MRS medium (Panreac, Barcelona, España), acidified to pH = 5.7 under anaerobic conditions (ISO 15214: 1998), incubating at 30ºC for 48-72 h. Finally, *Streptococcus* spp. (STREP) were plated in Edwards modified medium supplemented with colistin sulfate (5 mg/L) and oxolinic acid (2.5 mg/L) (Oxoid, Cambridge, UK), incubating at 35ºC for 48 h.

2. Technological characteristics

Lacto-dynamography is based on recording the movement of a small loop pendulum immersed in a linearly oscillating sample of coagulating milk, with the degree of movement taken to represent curd firmness (CF). Three single-point measures (McMahon and Brown, 1982) were considered to be useful for assessing milk coagulation properties: (1) Renneting time (**r**, min), which is the interval between the addition of rennet to the time at which the baseline begins to widen due to milk gelation; (2) The time interval between r and a measured amplitude of oscillation of 20 mm on the cream (K_{20}, min), which represent the curd-firming rate; and (3) the amplitude of oscillation (representing the final CF) recorded 30 and 60 min after rennet addition (A_{30} and A_{60}, mm).

The percentage ratio between the curd weight and the milk weight or Cheese Yield (CY) and pH (pH-meter Crison Basic 20), have been evaluated too at the Dairy Small Ruminant Laboratory of University of Cordoba (Spain).

The results given in this work concern only the variables Renneting time (r, min) and Curd Firming recorded 30 min (A_{30} , mm).

3. Statistical analysis

Data analysis was performed using R version 3.1.2. (R Core Team 2014). Significance level was established at $P < 0.05$. Prior to data analysis, the assumption of normality was checked and results of microbiological counts were log-transformed (log10). In a first experiment, we examined how microbiological counts and pH value affected renneting time (r). For that, four groups based on r value were established: Group 1 (n = 24): 0< r<15 ; Group 2 (n = 110): 15 ≤r <30; Group 3 (n = 124): 30 ≤ r < 45; and Group 4 (n = 44): 45 ≤ r<60. Groups have been established taking into account the distribution of the renneting times of the samples and according to the referential times marked by Specifications of Manchego Cheese (Clotting time: 30-60 min) (ORDEN APA/3273/2007). Then, in a second experiment, we performed regression analysis to study how microbiological counts and pH value affected the A_{30} parameter. For that, a subset of samples that had coagulated at time 30 min were used (133 from 302).

III – Results and discussion

The relationship among microbiological counts and pH with renneting time (r) is presented in Table 1. The differential microbiological analysis of milk gives a clear idea about milk quality depending of the group. There are different groups of microorganisms that inform about the hygienic conditions

of the productive system of farms: Standard plate count, Thermodurics (those microorganisms that survive the pasteurization conditions), Psychrotrophics (those microorganisms that develop at refrigeration conditions), *Escherichia coli* and Total Coliforms. On the other hand, there are some microorganisms that indicate the incidence of intramammary infections (IMI): CP, CN and some of Streptococcus group. In milk from IMI glands, there is a decrease in the level of lactose and an increase in whey proteins which have negative effects on the suitability of milk for cheese-making (Marti-de Olives *et al*., 2011; Giadinis *et al*., 2012). Finally Lactic acid bacteria, that are the principal organisms involved in fermentation and subsequent processing of the milk to produce cheese. A positive correlation between LACT count and the ability to acidify milk was shown by Tosi *et al*. (2008).

In Table 1, SPC, THERMO, PSYCHRO and STREP for Group 1 (0< r <15) had significantly higher values than other groups. These mean that in samples with lower renneting time, the microbiological counts are higher. Beresford *et al*. (2001) suggested that secondary microorganisms do not contribute to acid production during manufacture of the cheese, but generally play a significant role during ripening. The general trend is that the lower count of microorganisms, the higher renneting time of milk. Significantly positive values were also observed at pH values, with a general trend of more acidic pH values with lower renneting times. For counts of PSEUDO, LACT and COLIT, there are not significant differences between Group 1 (0< r<15) and Group 2 (15 ≤r <30), but a significant difference is observed between these groups and Groups 3 (30≤r<45) and 4 (45 ≤r<60), with lower counts in general. However, there are not significant differences for specific microorganisms groups like ECOLI, CP and CN.

The result of the regression analysis of A_{30} is shown in Tables 2 and 3.

Means and standard error for microbial counts according to A_{30} established for the samples that had coagulated are shown in Table 2. The mean values for SPC and THERMO was 5.86 and 3.36 respectively. These results were higher that those indicated by the study of De Garnica *et al*. (2013) in a study about the relationship among specific bacterial counts and total bacterial and somatic cell counts and factors influencing their variation in ovine bulk tank milk. The mean values for PSYCHRO, PSEUDO and LACT were 5.51, 3.33, and 4.95. The groups that are in relationship with the hygienic conditions of the farm, ECOLI and COLIT had mean values of 1.77 and 3.32 respectively. Finally, the mean values for the microbiological counts related to herd health, STREP, CP and CN was 4.37, 2.38 and 4.33 respectively.

Finally, the relations between the differential microbiology and the value of A_{30} parameter are shown in Table 3. A significant and positive relationship with SPC, THERMO, PSYCHRO, PSEUDO, COLIT, LACT and STREP was observed. This mean that each unit that increase the count of the different microorganisms groups (measured in cfu/ml) or pH (measured in pH units), the value of A_{30} will increase the respective estimated value indicated (measured in mm). With the result obtained, it is observed that higher microbial count is in relationship with a greater average curd-firming rate at 30 min in the case of milk with coagulating capacity. Not correlation appeared with other groups as ECOLI, CP and CN.

IV – Conclusions

The differential microbiological analysis of milk gives a clear idea about technological quality depending of the group. When the differential counts are classified attending to r parameter, it is observed that the samples with higher counts have lower renneting times. On the other hand, the value of A_{30} parameter is affected by the counts of SPC, THERMO, PSYCHRO, PSEUDO, COLIT, LACT and STREP, as well as, the pH value. In view of the results, it is necessary to continue studying the influence of the microbial load of sheep's milk cheese, as the main product obtained from processing.

Table 1. Means and standard error for microbiological counts (log cfu ml^{-1}) according groups established by the clotting time in raw ewe's milk

r Categories	SPC	THERMO	PSYCHRO	PSEUDO	LACT	STREP	ECOLI	COLIT	CP	CN	pH
0 < r < 15	6.43 ± 0.22^{a}	3.87 ± 0.28^{a}	6.26 ± 0.32^{a}	3.67 ± 0.16^{a}	5.23 ± 0.09^{a}	4.75 ± 0.19^{a}	1.71 ± 0.27	3.64^{a} ± 0.18	2.43 ± 0.29	4.44 ± 0.13	6.41 ± 0.05^{a}
15 ≤ r < 30	5.78 ± 0.09^{b}	3.26 ± 0.09^{b}	5.34 ± 0.11^{b}	3.31 ± 0.09^{ab}	4.97 ± 0.08^{ab}	4.34 ± 0.08^{b}	1.82 ± 0.12	3.28^{ab} ± 0.09	2.43 ± 0.14	4.36 ± 0.04	6.63 ± 0.01^{bc}
30 ≤ r < 45	5.57 ± 0.06^{b}	3.16 ± 0.07^{b}	5.03 ± 0.10^{b}	3.13 ± 0.08^{b}	4.86 ± 0.07^{b}	4.07 ± 0.07^{bc}	1.62 ± 0.12	3.09^{bc} ± 0.09	2.60 ± 0.13	4.31 ± 0.06	6.59 ± 0.01^{b}
45 ≤ r < 60	5.56 ± 0.11^{b}	3.29 ± 0.13^{b}	5.09 ± 0.18^{b}	2.60 ± 0.17^{c}	4.75 ± 0.11^{b}	3.85 ± 0.13^{c}	1.26 ± 0.21	2.74^{c} ± 0.23	2.53 ± 0.24	4.31 ± 0.08	6.71 ± 0.02^{c}

Different superscripts $^{a, b, c}$: p<0.05.

Table 2. Means and standard error for microbiological counts (log cfu ml^{-1}) according to A_{30} established for the samples that had coagulated

	A_{30}	SPC	THERMO	PSYCHRO	PSEUDO	LACT	STREP	ECOLI	COLIT	CP	CN	pH
MEAN	27.91	5.86	3.36	5.51	3.33	4.95	4.37	1.77	3.32	2.38	4.33	6.60
SE	1.28	0.08	0.08	0.11	0.07	0.06	0.07	0.09	0.08	0.12	0.04	0.01

Table 3. Relations between differential microbiology and A_{30} established for samples that had coagulated

	SPC	THERMO	PSYCHRO	PSEUDO	LACT	STREP	ECOLI	COLIT	CP	CN	pH
ESTIMATE ± SE	7.61 ± 1.56	5.59 ± 1.15	5.23 ± 0.96	6.45 ± 1.80	4.99 ± 1.62	8.48 ± 1.38	0.45 ± 1.87	6.68 ± 1.41	-0.45 ± 2.16	-1.50 ± 2.49	-14.78 ± 6.08
CI 95 %	[5.33; 9.90]	[3.31; 7.88]	[3.33; 7.13]	[2.89; 10.01]	[1.79; 8.19]	[5.76; 11.21]	[-3.27; 4.16]	[3.89; 9.46]	[18.44; 43.92]	[-6.42; 3.43]	[-26.82; -2.75]
P-VALUE	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	0.812	<0.001	0.835	0.549	<0.01

***: p<0.001; **: p<0.01; *: p<0.05; ^{NS}: p>0.05.

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References

Beresford T.P., Fitzsimons N.A., Brennan N.L. and Cogan T.M., 2001. Recent advances in cheese microbiology. *Int. Dairy J.,* 11, p. 259-274.

Beresford T. and Williams A., 2004. The Microbiology of Cheese Ripening. In: P.F. Fox, P.L.H. McSweeney, T.M. Cogan and T.P. Guinee (eds), *Cheese: Chemistry, Physics and Microbiology*, Volume 1, p. 287-317.

De Garnica M.L., Linage B., Carriedo J.A., De la Fuente L.F., García-Jimeno M.C., Santos J.A. and Gonzalo G., 2013. Relationship among specific bacterial counts and total bacterial and somatic cell counts and factors influencing their variation in ovine bulk tank milk. *J. Dairy Sci.*, 96, p. 1021-1029.

Elmoslemany A.M., Keefe G.P., Dohoo I.R. and Jayarao B.M., 2009. Microbial quality of bulk tank milk in Prince Edward Island dairy herds. *J. Dairy Sci.*, 92, p. 4239-4248.

Giadinis N.D., Arsenos G., Tsakos P., Psychas V., Dovas C.I., Papadopoulos E., Karatzias H. and Fthenakis G.C., 2012. Milk-drop syndrome of ewes: investigation of the causes in dairy sheep in Greece. *Small Rumin. Res.*, 106, p. 33-35.

MAGRAMA, 2013. Consulted 17th May 2015. http://www.magrama.gob.es/es/ganaderia/temas/produccion-y-mercados ganaderos/CARACTERIZACI %C3%93N_DEL_SECTOR_OVINO_Y_CAPRINO_EN_ESPA%C3%91A_2013_tcm7-271704.pdf.

Marti-De Olives A., Le Roux Y., Rubert-Aleman J., Peris C. and Molina M.P., 2011. Effect of subclinical mastitis on proteolysis in ovine milk. *J. Dairy Sci.*, 94, p. 5369-5374.

Martini M., Scolozzi C., Cecchi F., Mele M. and Salari F., 2008. Relationship between morphometric characteristics of milk fat globules and the cheese making aptitude of sheep's milk. *Small Rumin. Res.*, 74, p. 194-201.

McMahon D.J. and Brown R.J., 1982. Evaluation of Formagraph for comparing rennet solutions. *J. Dairy Sci.*, 65, p. 1639-1642.

ORDEN APA/3273/2007, de 25 de octubre, por la que se publica el pliego de condiciones de la Denominación de Origen Protegida "Queso Manchego" (BOE nº 272 de 13-11-07).

Raynal-Ljutovac K., Gaborit P. and Lauret A., 2005. The relationship between quality criteria of goat milk, its technological properties and the quality of the final products. *Small Rumin. Res.*, 60, p. 167-177.

Sitzia M., Bonanno A., Todaro M., Cannas A., Atzori A.S., Francesconi A.H.D. and Trabalza-Marinucci M., 2015. Feeding and management techniques to favour summer sheep milk and cheese production in the Mediterranean environment. *Small Rumin. Res.*, 126, p. 43-58.

Tosi F., Sandri S., Tedeschi G., Malacarne M. and Fossa E., 2008. Variazioni di composizione e proprietà fisico-chimiche del Parmigiano-Reggiano durante la maturazione e in differenti zone della forma. *Sci. Tecn. Latt. Cas.,* 59, p. 507-528.