Microbiological changes during manufacture and ripening of a naturally ripened blue cheese (Valdeón, Spain)

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1. Introduction

Valdeón cheese is a blue-veined variety made nowadays during spring and summer, in farmhouses in Valdeón Valley (Picos de Europa, province of León). Raw goat's milk (occasionally mixed with cow's milk) is coagulated (20-30°C for 1-2 h) with animal rennet (goat kid) or commercial rennet. The whey is removed with a saucepan and suitable cylindrical moulds (tinplates) are filled with the curd. For 2 d drainage proceeds and during this time coarse salt is sprinkled over the surfaces. During the first 10 to 15 d of ripening the cheeses are kept in a warm room (17-23°C and 70-90 % relative humidity, RH). For the next stage in maturing, the cheeses are moved to natural mountain caves (700-1,500 m altitude; 7-12°C and 95-99 % RH) and turned at intervals throughout the remainder of the ripening period of several months (1).

Although Valdeón cheese has a low production (1,500-2,000 kg/year), it is a unique variety (a natural ripened blue cheese made with goat's milk) and is regarded as a high quality cheese (2). However, no reports on its microbiology have been published.

In this report the microbiological changes during manufacture and ripening were studied as part of a project which aims to commercialize the production of this traditional variety.

2. Materials and methods

2.1 Samples

Four lots of cheese made with goat's milk were made by 2 different farms (A and C) in 2 different villages in the same valley. Cheese sampling was performed according to the ICMSF (3). The following samples were taken during the manufacture of each lot: 1) from milk (M); 2) from the curd before moulding (Cd); 3) from the cheeses after salting (C1); and 4) during the ripening period, on days 10-15 (after drying, C2), day 30 (C3) and day 60 (finished product, C4).

2.2 Microbiological analysis

Serial ten-fold dilutions of milk samples (10 ml) were made using sterile peptone water (0.1 %). Representative 25 g samples of curd or cheese taken from the interior at all stages and in the final product, and 10 g samples taken from the surface (5 mm deep) from drying onwards, were homogenised in a Stomacher Lab-blender for 90 s, with 225 ml and 90 ml of sterile warm (45°C) 2% aqueous solution of sodium citrate, respectively. Decimal dilutions were prepared in sterile peptone water (0.1%).

The microbiological groups studied in milk, curd and interior cheese, the media and conditions of incubation used were as follows:

a) Mesophilic aerobes, Plate Count Agar (PCA, Oxoid), 30°C/48 h (4).
b) Enterococci, Kanamycin Aesculin Azide Agar (KAA, Oxoid), 35°C/16-24 h (5).
c) Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBGA, Oxoid), 37°C/24 h (4).
d) Lactic acid bacteria, Man, Rogosa & Sharpe Agar (MRS, Oxoid), 30°C/48 h (6) for lots from farm A and Rogosa Agar (Oxoid), 30°C/2-5 d (7), MSE Agar (Biokar), 22°C/4 d (8), and M17 Agar (Biokar), 30°C/18-24 h (9), for detection and enumeration of *Lactobacillus, Leuconostoc* and lactic streptococci in samples of lots from farm C, respectively.
e) Gram positive, catalase positive cocci (micrococcaceae), Mannitol Salt Agar (MSA, Oxoid), 30°C/2 d (10).
f) Moulds and yeasts, Oxytetracycline Glucose Yeast Extract Agar (OGYEA, Oxoid), 25°C/5 d (11).

In samples taken from the surface of the cheese, only mesophilic aerobes, micrococci and moulds and yeasts were studied, using the media and conditions listed above.

2.3 Chemical/physical analysis

The following measurements were made during the manufacture: pH at all stages, electrometrically (12), NaCl content at all stages except milk (13) and a_w from curd onwards (14).

3. Results and discussion

Lactic acid bacteria were the dominant group throughout the whole process (Fig. 1). Mean log counts of colony forming units (CFU)/g or ml ($x \pm S.D.$) of this group reached their maximum level (9.2 ± 0.1) during salting. Moulds and yeasts were also prominent during ripening, reaching the maximum count (8.2 ± 0.6) in dried cheese. In the initial stages yeasts were dominant, their counts being higher than moulds up to ripening in caves (Table 1). From then on, both groups reached high levels and at the end of the process had similar log counts (7.7 ± 0.2, for moulds and 7.15 ± 0.6, for yeasts). Micrococci and enterococci reached significant levels during ripening (7.6 ± 0.5 and 6.5 ± 0.3, respectively, in the finished product). Finally, enterobacteria were present in lower numbers throughout the whole process, although at the end the level was significant (3.9 ± 0.05).

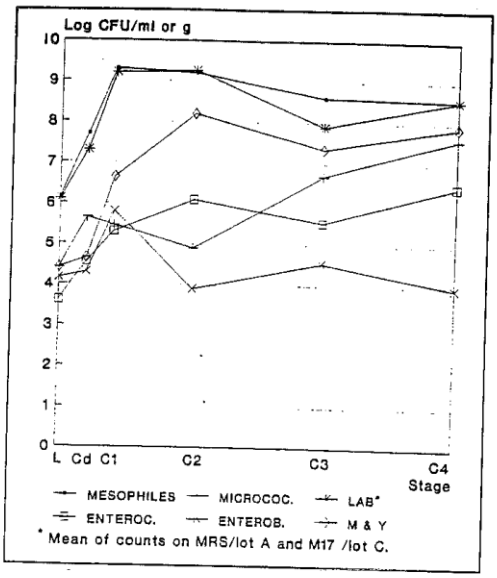

Fig. 1: Changes in counts of mesophiles, micrococci, lactic acid bacteria (LAB), enterococci, enterobacteria and moulds and yeasts. Mean values of lots from farms A and C (average variation, 0.6 log CFU/ml or g)

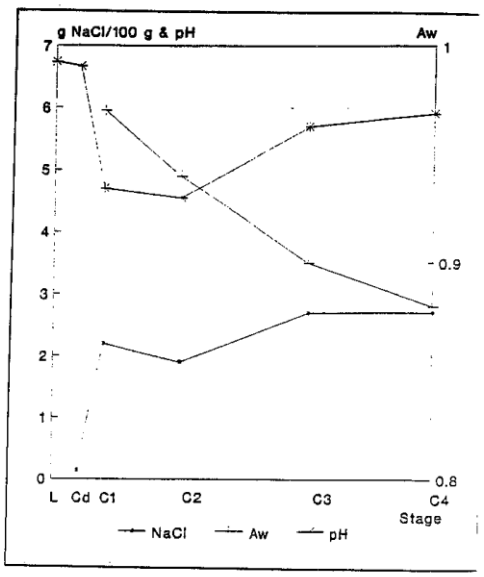

Fig. 2: Changes in salt content, a_w and pH. Mean of lots from farms A and C (average variation, 0.15).

Table 1: Counts of moulds and yeasts during manufacture (x ± S.D. log CFU/ml or g)

	M[1]	Cd	Cl	C2	C3	C4
Moulds	2.2±0.3	2.6±0.7	3.4±1.5	1.9±0.0	7.0±0.6	7.7 ±0.2
Yeasts	3.0±0.4	3.8±0.8	4.9±1.5	7.3±0.9	8.3±0.1	7.15±0.6

[1]Stages as explained in materials and methods

The evolution of the different groups can be better understood by the division of the process into stages and the study of the physico-chemical changes (Fig. 2). In the period up to salting (L-C1), the log counts of lactic acid bacteria increased markedly in numbers, from 6.1±0.4 in milk to 9.2±0.1 (maximum level) in curd after salting. During this stage, the remaining microbial groups also multiplied, with moulds and yeasts being the most active, increasing more than 2 log units/g (Fig. 1). Within this group, yeasts were dominant over moulds, the difference being up to 1.5 log units/g in salted cheese (Table 1). The pH fell from 6.7±0.03 to 4.7±0.02 (Fig. 2), presumably due to lactic acid production by lactic acid bacteria (15), and the salt content reached 2.2±0.4 g NaCl per 100 g of curd after salting. A satisfactory acidification in blue cheeses is specially important. It must be slow but intense. If it is too rapid, the curd loses whey very quickly and the cheese has a close texture that does not allow the development of fungi. The optimum pH is 5 after 48 h (16), and in Valdeón cheese this condition was present.

During drying, when cheeses were kept at high temperature (around 20°C) and low RH, there was variable behaviour of the different groups. Lactic acid bacteria remained at similar levels and enterococci and micrococci varied slightly, the first decreasing by 0.5 log units and the second increasing by 0.8 log units. In contrast, moulds and particularly yeasts multiplied actively (more than 10-fold) (Table1) and enterobacteria decreased markedly (100 times) (Fig. 1). During this period, the water activity decreased from 0.97±0.01 in salted cheese (C1) to 0.94±0.0 in dried cheese (C2), due to the low relative humidity of the atmosphere (70-90 %) and the presence of salt. In addition, the pH and salt content remained nearly constant (Fig. 2). Under these conditions, only some microorganisms including fungi were able to multiply, with groups like enterobacteria being affected in the opposite way.

Finally, during ripening in caves, when cheeses were kept at low temperature (around 10°C) and high RH (close to saturation), the pH rose finally to 5.9±0.7, probably as a result of the utilization of lactic acid by moulds and yeasts. The neutralization of the curd is one of the most interesting roles played by these organisms during ripening of cheese (17, 18, 19). Throughout this period, salting and other factors reduced a_w from 0.94±0.0 to 0.88±0.0 at the end of the ripening, a value lower than those found in other blue cheeses (20). Finally, the salt content increased to a final concentration of 2.7±0.05 g NaCl/100 g of cheese. Factors such as low temperature, low a_w and high salt content prevented further growth of most microorganisms, with the levels found at the end of manufacture being very similar to those found at the beginning of the ripening. Only

two groups changed markedly and those were moulds and micrococci. The filamentous fungi increased notably during the first 30–45 d of the period in the caves (Table 1). This could be related to natural contamination of the cheese in the caves and to the physiology of this group of microorganisms. It is well known that many genera and species of fungi have the ability to grow under what would be limiting conditions for other microorganisms (low temperature, low a_w, low pH) (21, 22). Micrococci increased more than 2 log units/g at this stage. This fact could be related to their halotolerance and ability to grow at low temperatures (15).

In the samples taken from the surface of the cheese during this phase the development of all groups studied was parallel to that observed in the samples from the interior (Table 2). Counts of mesophiles gave high numbers (8.3 – 8.5) and counts of moulds and yeasts were slightly higher than those found in the interior samples. Changes of micrococci counts were similar to those observed in the interior samples.

Table 2: Numbers of microorganisms in samples taken from the surface (x ± S.D. log CFU/g)

	C2[1]	C3	C4
Mesophiles	8.35±0.3	8.3±0.4	8.55±0.05
Micrococci	5.5 ±2.0	7.2±0.7	8.4 ±0.3
Moulds and yeasts	7.6 ±0.6	8.2±0.7	8.35±0.4

[1]Stages as explained in materials and methods

The counts of lactic acid bacteria obtained on different media in the lots from farm C are shown in Table 3. The numbers of the three lactic acid bacteria groups, streptococci, *Leuconostoc* and lactobacilli, were very similar throughout the whole process although streptococci were always slightly higher. This group gave counts which were closest to the numbers found on MRS, used for lactic acid bacteria in samples from farm A.

Table 3: Changes in lactic acid bacteria in lots from farms A (counts on MRS) and C (counts on Rogosa, M17 and MSE) (x ± SD log CFU/ml or g)

		M[1]	Cd	Cl	C2	C3	C4
A	LAB[2]	6.6±0.5	7.8±0.5	9.3±0.1	9.4±0.06	7.2	8.6
C	LB	4.7±0.4	5.5±0.7	8.3±0.03	7.7±0.1	7.8±0.3	6.9±0.2
	SC	5.5±0.2	6.8±0.3	9.2±0.03	9.1±0.2	8.7±0.2	8.5±0.1
	LC	5.4±1.1	6.0±0.5	8.5±0.02	8.1.±0.1	8.0±0.1	8.0±0.05

[1] Stages as explained in materials and methods. [2] LAB, lactic acid bacteria (counts on MRS;); LB, lactobacilli (counts on Rogosa Agar); SC, streptococci (counts on M17 agar); LC, leuconostoc (counts on MSE)

The results of microbial changes found in our study coincide with the findings of other authors on similar varieties of cheese (*i.e.*, blue cheese made with raw milk). DEVOYOD *et al.* (23), found a similar pattern of development and changes in levels of mesophiles, yeasts and micrococci in both surface and interior samples in Roquefort cheese. Enterobacteria were not surveyed by these authors, but they found decreasing counts of coliforms through the process and very low final levels (close to 10 CFU/g). In Cabrales cheese (24), a variety produced in a very similar way to Valdeon cheese, although in a different area and with a mixture of sheep, goat and cow milk, the findings were similar in the first stages, but from salting and drying onwards there were significant differences. Thus, a striking decrease in the numbers of all groups, including moulds and yeasts, was observed, reaching even lower levels than those found in our study. This fact could be related to the length of the ripening period, which was longer in Cabrales cheese (120 d). In addition, the counts of *Leuconostoc* in this cheese were always lower (3-4 log units/g) than ours.

In conclusion, the microbial changes during manufacture of Valdeón cheese found in our study were similar to other blue cheeses and characterised by the dominance of lactic acid bacteria and moulds and yeasts. The quantitative prominence of yeasts during the whole process suggests that it would be of particular interest to carry out the identification of members of this group, apart from lactic acid bacteria and moulds, as the next step in this project.

Acknowledgements

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6. Summary

LOPEZ-DIAZ, T.M., ALONSO, C., SANTOS, J., GARCIA, M.L., MORENO, B.: **Microbiological changes during manufacture and ripening of a naturall ripened blue cheese (Valdeón, Spain).** Milchwisser schaft **50** (7) 381–384 (1995).

56 Blue cheese (microbiology)

The microbiological changes during manufacture of Spanish blue-cheese made with raw goat's milk wer studied. Throughout the whole process, lactic aci bacteria were the dominant group (8-9 log units/g, fror salting onwards), with moulds and yeasts also bein prominent during ripening (7-8 log units/g). Micrococc and enterococci reached high levels during ripening an at the end of the process (7 and 6 log units/g, respec tively). Enterobacteria decreased from salting and re mained constant during ripening, giving final levels of log units/g. Changes of mesophiles, micrococci an moulds and yeasts in surface samples were similar. Ac cording to the evolution of the microflora throughout th process, several stages could be observed. These an the relation with the physico-chemical parameters were discussed.

LOPEZ-DIAZ, T.M., ALONSO, C., SANTOS, J., GARCIA, M.L., MORENO, B.: **Microbiologische Veränderungen während der Herstellung und Reifung eines natürlich reifenden „Blue cheese" (Valdeón. Spanien).** Milchwissenschaft **50** (7) 381–384 (1995).

56 Blue cheese (Mikrobiologie)

Es wurden die mikrobiologischen Veränderungen während der Herstellung eines spanischen „Blue cheese" aus Ziegenrohmilch untersucht. Während des gesamten Verfahrens waren Milchsäurebakterien die vorherrschende Gruppe (8–9 log Einh./g, beginnend mit dem Salzen). Auch Pilze und Hefen waren während der Reifung ausgeprägt vorhanden (7–8 log Einh./g). Mikrokokken und Enterokokken erreichten während der Reifung und am Ende des Verfahrens hohe Werte (7 bzw. 6 log Einh./g). Enterobakterien nahmen nach dem Salzen ab und blieben während der Reifung mit Endwerten von 4 log Einh./g konstant. Die Veränderungen der mesophilen Keime, Mikrokokken, Pilze und Hefen in Oberflächenproben waren ähnlich. Entsprechend der Entwicklung der Mikroflora während der Reifung konnten mehrere Stadien beobachtet werden. Diese und das Verhältnis zu den physikochemischen Parametern wurden diskutiert.

Heat denaturation of bovine, caprine and ovine whey proteins

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1. Introduction

Denaturation of bovine whey proteins occurs when milk is heated above 60 °C, and involves an initial reversible step in which the intramolecular disulphide bonds are broken and the proteins lose their globular configuration (1, 2). On more severe heating, the whey proteins undergo irreversible denaturation, and become associated with the caseins through hydrophobic interaction and disulphide linkage (3, 4, 5, 6). On subsequent acidification to pH 4.6, the denatured whey proteins precipitate together with the caseins (7), and the decrease in concentration of the individual whey proteins in the acid filtrate can be used to determine the extent of their irreversible denaturation. The order of ease of irre-