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Effect of an autochthonous starter culture, including lactococci and *Geotrichum candidum* strains, on the ripening of a semi-hard goat's milk cheese

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Four batches of Armada semi-hard goat cheese were elaborated from pasteurized milk inoculated with a natural starter culture, constituted of two lactococci strains, combined with a *Geotrichum candidum* strain. The four *G. candidum* strains tested as co-starter were selected for their proteolytic and lipolytic activities. The effects of an autochthonous starter on physico-chemical, microbiological and sensorial characteristics during the ripening of this cheese were evaluated. The depth of proteolysis was very low, which confirms the presence of low-level aminopeptidase activity. Fungal population was involved in lipolysis. The cheeses elaborated with *G. candidum* strains developed a desirable flavour characteristic of goat cheese and a creamy texture. Cheeses from batch I (that included the strain of *G. candidum* with high lipolytic activity and low proteolytic activity) presented a hardness profile that differed from the others, as it was the batch with the highest scores at thirty days of ripening and was even the best evaluated at the end of ripening because of their odour, fresh balanced pleasant taste and creamy smooth texture.

Key words: Cheese, starter culture, *Geotrichum candidum*, lactic acid bacteria.

INTRODUCTION

The autochthonous microbiota of traditionally made cheeses elaborated from raw milk gives them their particular characteristics, and thus constitute an excellent source of new strains of microbes with phenotypic and genotypic diversity, which could be of technological interest (Rademaker et al., 2007). This microbial diversity is responsible for the production of compounds giving rise to flavour during ripening (Coolbear et al., 2008). The activity of the starter which is made up of lactic acid bacteria and could include strains producing aroma, could be reinforced by adding a secondary microbiota that will contribute to the ripening process of the cheeses. Non-starter yeast and molds (NSYM) population in cheese is very diverse and its role in the ripening is often

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underestimated (Lavoie et al., 2012).

The importance of using the filamentous yeast-like fungus, *Geotrichum candidum* as a co-starter in dairy products is well known because of its various properties related to the development of flavour and texture in semi-hard cheeses (Gaborit et al., 2001). In fact, in recent years, there has been increasing interest in the use of this microorganism, especially in the manufacturing of cheeses from pasteurized milk in order to reproduce the characteristics of cheese made from raw milk (Boutrou and Guéguen, 2005). *G. candidum* was the dominant yeast species in the first and second week of Armada ripening (Fresno et al., 1996; Tornadijo et al., 1998). Then, it could have important contributions to the flavour and texture of this one or similar cheeses. Nonetheless, *G. candidum* strains differ in their biochemical capacity to produce aromatic compounds in dairy products (Spinnler et al., 2001), which require a selection process on the basis of their technological suitability. In an earlier study, several properties of technological relevance were studied in *G. candidum* and the strains with greater technological capacities were characterized at a molecular level (Sacristán et al., 2012, 2013).

The aim of this study was to investigate the contribution of a natural starter composed of strains of *Lactococcus* and *G. candidum* as co-starter on the chemical, microbiological and sensorial characteristics of a goat's milk cheese. The *G. candidum* strain that provides the best sensorial properties was selected to be included as co-starter in the Armada cheese manufacture.

MATERIALS AND METHODS

Cheese manufacture and sampling

All the strains used in the cheese manufacturing were isolated from a traditional Armada cheese elaborated by the artisanal cheese-makers themselves (Tornadijo et al., 1995). Lactic acid bacteria were characterized from a technological point of view including acidifying activity and proteolytic and lipolytic activities and selected in order to obtain a starter (Herreros et al., 2003; 2007). *Lactococcus lactis* subsp. *lactis lactis* (TAUL 1292) was selected because of its acidifying capacity and its proteolytic activity, and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (TAUL 12) for its high dipeptidase activity and its capacity to produce aroma compounds. The *G. candidum* strains were selected for their proteolytic and lipolytic activities (Sacristán et al., 2012).

Four batches of Armada cheese were manufactured by duplicate (8 batches in total) using the traditional method (Tornadijo et al., 1995). The goat's milk used for the manufacture of a total of 32 cheeses (4 cheeses per batch) was pasteurized and inoculated with an autochthonous starter culture composed of *Lactococcus lactis* subsp. *lactis* (TAUL 1292) and *L. lactis* subsp. *lactis* biovar. *diacetylactis* (TAUL 12) at a level of 0.5% for each, and *G. candidum* as co-starter, inoculated at 1%. Batch I included the *G. candidum* Ge-1886 strain with high lipolytic activity and low proteolytic activity. Ge-1903 strain (with low lipolytic activity and high proteolytic activity) was added in batch II. Batch III incorporated the Ge-1889 strain with both lipolytic and proteolytic activity at a high level. Ge-1893 strain (with both lipolytic and proteolytic activities at an intermediate level) was used in batch IV.

Table 1. Autochthonous starter cultures used in the manufacture of the Armada cheeses.

Batches[a]	Autochthonous starter culture strains[b]
Control batch	TAUL 12 + TAUL 1292
Batch I	TAUL 12 + TAUL 1292 + Ge-1886
Batch II	TAUL 12 + TAUL 1292 + Ge-1903
Batch III	TAUL 12 + TAUL 1292 + Ge-1889
Batch IV	TAUL 12 + TAUL 1292 + Ge-1893

[a]All the batches were manufactured from pasteurized goat's milk; [b]All the strains were isolated from the artisanal Armada cheese; *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* TAUL 12 was selected because of its acidifying capacity and its proteolytic activity; *L. lactis* subsp. *lactis* TAUL 1292 was selected for its high dipeptidase activity and its capacity to produce aroma compounds; *Geotrichum candidum* Ge-1886 was selected because of its high lipolytic activity and low proteolytic activity; *G. candidum* Ge-1903 was selected for its low lipolytic activity and high proteolytic activity; *G. candidum* Ge-1889 was selected because of its both high lipolytic and proteolytic activities; *G. candidum* Ge-1893 was selected for its both intermediate lipolytic and proteolytic activities.

The control batch was composed of the Armada cheese elaborated with the LAB strains (Herreros, 2010) (Table 1).

After thirty minutes of the inoculation of the starter cultures, 10 mL of commercial calf rennet (1:10000 strength) were added to every batch (100 L of milk), which was left to coagulate for about 2 h at room temperature (20 to 25°C). The curd was then cut and transferred to cheese-cloths where the whey was drained off over a period of 48 h. The curd was then kneaded in a very rigorous manual operation called "Sobado". Salting was carried out by adding dry salt (1.68%; w/w) to the curd during the second kneading process, and finally the curd was hand-moulded to produce its characteristic square shape. Subsequently, the cheeses were placed on a plate for four days at 10°C and 90% relative humidity. The ripening process took place over 60 days at 11°C and 86% relative humidity.

Milk as well as 2-, 15-, 30- and 60-day-old cheese samples were taken from each batch. Each sample was made up of one cheese and was analysed as specified as follows.

Microbiological analysis

Samples for microbiological analysis of milk and cheese were prepared according to the International Dairy Federation (IDF) standard 122B (IDF, 1992).

Aerobic mesophilic bacteria were enumerated on standard plate count agar (PCAm) (Oxoid, Unipath Ltd., Basingstoke, U.K.) following the APHA method (APHA, 1978) after incubation at 30°C for 48 h. General population of lactic acid bacteria (LAB) were determined on MRS agar (De Man et al., 1960), after incubation at 30°C for 72 h; lactococci were counted on the M17 agar (Biokar, Beauvais, France) incubated at 30°C for 18 to 24 h (Terzagui and Sandine, 1975) and lactobacilli were counted on ROGOSA agar (Oxoid, Unipath Ltd., Basingstoke, U.K.) after incubation at 30°C for 5 days (Rogosa et al., 1951). Yeasts and moulds were counted on oxytetracycline glucose yeast extract agar (OGYEA) (Oxoid, Unipath Ltd., Basingstoke, U.K.) after incubation at 22°C for 5 days (Mossel et al., 1970). Enterobacteriaceae were enumerated on violet red bile glucose agar (VRBGA) (Oxoid, Unipath Ltd., Basingstoke, U.K.) after incubation at 37°C for 18 to 24 h (Mossel et al., 1962).

Table 2. Physico-chemical parameters (average values ± standard deviation) throughout ripening of Armada cheeses elaborated with an autochthonous starter culture.

	Ripening time (days)				Batch	Time
	2	15	30	60		
Moisture [a]	64.60 ± 2.86	50.00 ± 2.43	40.46 ± 3.01	27.55 ± 2.23	**	***
a_w	0.995 ± 0.002	0.972 ± 0.003	0.959 ± 0.005	0.929 ± 0.010	NS	***
Salt [b]	0.54 ± 0.09	3.08 ± 0.29	2.99 ± 0.16	3.28 ± 0.48	**	***
S/M [c]	0.30 ± 0.05	3.08 ± 0.23	4.42 ± 0.46	8.65 ± 1.21	*	***
pH	4.94 ± 0.20	4.61 ± 0.29	4.51 ± 0.30	4.56 ± 0.09	NS	***
TA [d]	2.22 ± 0.24	1.56 ± 0.18	1.12 ± 0.16	0.90 ± 0.14	NS	***
Lactose [b]	5.23 ± 0.98	2.94 ± 0.76	2.21 ± 0.63	1.57 ± 0.48	**	***
FAI [e]	1.96 ± 0.48	5.86 ± 2.73	1.93 ± 0.65	1.74 ± 0.54	NS	***
Fat [b]	56.68 ± 5.47	59.10 ± 3.06	60.64 ± 2.36	59.71 ± 1.63	NS	NS
Protein [b]	32.57 ± 2.48	28.85 ± 0.65	28.98 ± 1.11	28.81 ± 0.86	NS	***

The last two columns are referred to the significant differences between batches and between the ripening time. NS: no significant differences; *: significant differences ($p < 0.05$); **: significant differences ($p < 0.01$); ***: significant differences ($p < 0.001$); [a]Expressed as g 100 g^{-1} of cheese; [b]Expressed as g 100 g^{-1} of dry matter; [c]expressed as g salt 100 g^{-1} of moisture; [d]expressed as g lactic acid 100 g^{-1} of dry matter; [e]expressed as mg KOH g^{-1} of fat.

Cheese physico-chemical analysis

The contents of dry matter (DM), fat, protein, lactose and salt were determined in cheese according to standard methods (IDF, 2004, 2008, 2001, 1967; AOAC, 1990a).

The pH was determined potentiometrically from samples homogenized with 100 mL of distilled water warmed at 45-50°C so as to disperse the fat and then cooled down to 20°C ± 2°C. Water activity (a_w) was measured using an Aqua Lab CX-2 water activity meter (Decagon, WA, USA). The titratable acidity of the cheese and the fat acidity index (FAI) were determined according to standard methods (AOAC, 1990b; IDF, 1969).

Extraction and quantification of pH 4.4 soluble nitrogen (pH4.4-SN), trichloroacetic acid soluble nitrogen (TCA12%-SN) and phosphotungstic acid soluble nitrogen (PTA5%-SN) fractions were carried out using the method described by Bütikofer et al. (1993). All analyses were carried out in duplicate.

Sensory analysis

Cheeses were analysed after 15, 30 and 60 days of ripening by a panel of 20 trained tasters following the standard recommendations (ISO, 2005; 2012). Several parameters, related to appearance (mouldy rind, yellowish rind, white paste, mouldy spotted paste and cracked paste), taste (bitter taste, sweet taste, acid taste, salty taste, metallic taste, spicy taste, astringency, aftertaste and persistence), odour (fresh milky odour, mouldy odour, rennet odour, buttery odour and farmyard odour) and texture (hardness, buttery texture, grainy texture, crumbly texture and sticky texture) were evaluated on a 7-point intensity scale from 1 (dislike extremely) to 7 (like extremely), with 4 being an “acceptable” value. Finally, the cheeses were scored from 1 to 10 on the the basis of overall sensory impression.

Statistical analysis

In order to investigate possible significant differences among batches, the ANOVA/MANOVA analysis using Fisher's least significant difference (LSD) test (Statistica 8.0 computer program: Statsoft, Tulsa, Oklahoma, U.S.A.) was carried out with the confidence intervals set at 95% level and other higher levels (99 and 99.9%). The correlation between changes in the physico-chemical parameters and the log counts of the major microbial groups was also studied by the Pearson’s correlation coefficient, so as to discover what influence the ripening process had on microbial development.

RESULTS AND DISCUSSION

Changes in physico-chemical parameters

Changes in chemical and physico-chemical parameters during the ripening of the different batches of Armada cheese elaborated with an autochthonous starter culture and various selected strains of *G. candidum* as a co-starter are shown in Table 2. The changes throughout ripening time of the physico-chemical parameters which showed significant differences between batches are also shown in Figure 1.

Over the ripening process, there was a notable decrease in moisture and water activity. The final values for moisture even fell below 30%, with significant differences ($p < 0.01$) between batches on different sampling days.

The values for the relationship between salt and moisture (S/M) after two days of ripening were practically identical for all the batches, rising over the whole ripening period, in particular up to fifteen days and after sixty days of ripening. With regard to the influence from the specific batch, significant differences ($p < 0.05$) were noted, especially in samples taken after sixty days. In all cases, the results obtained for S/M ratio at the end of the ripening process were similar to those observed by Herreros (2010).

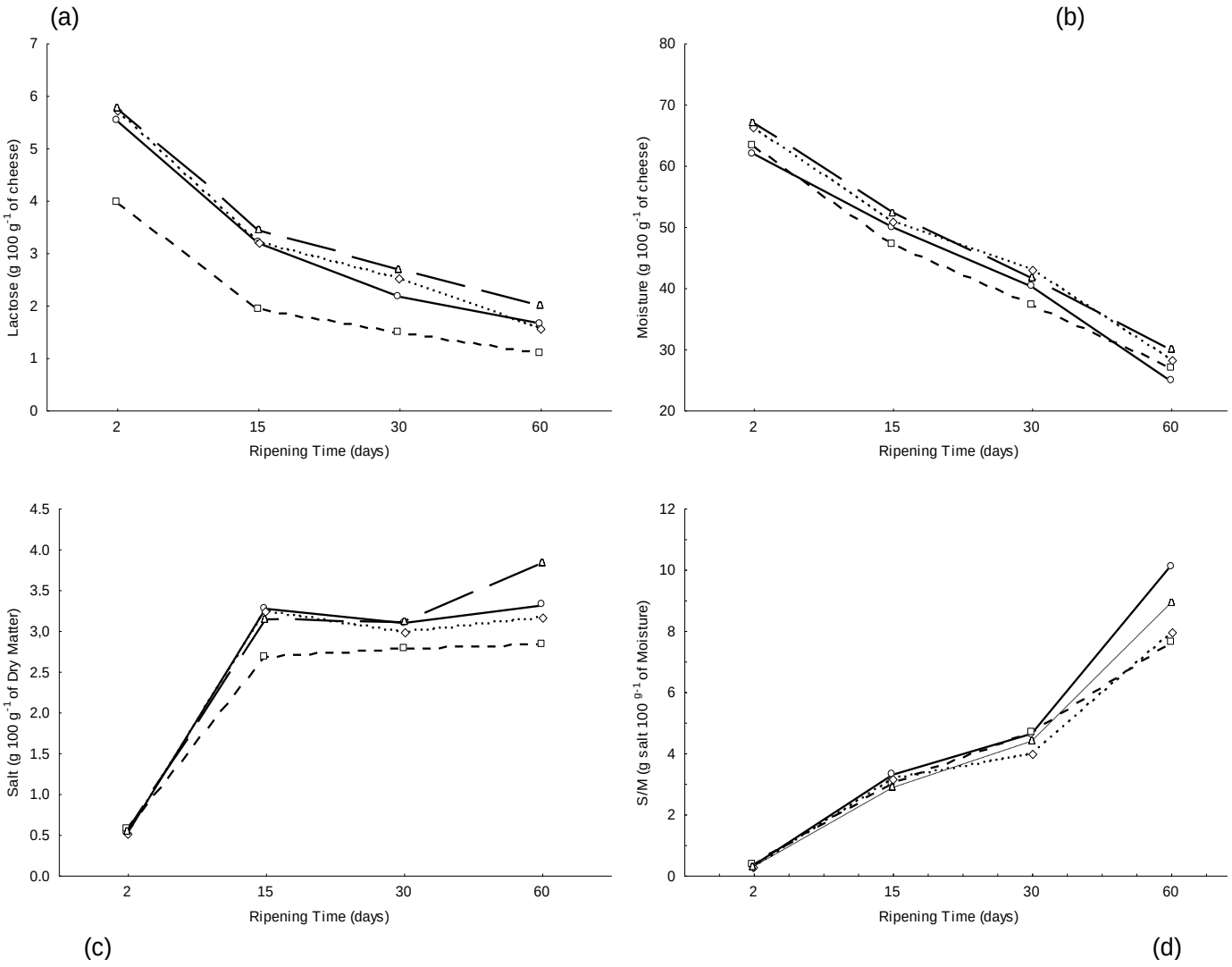

Figure 1. Changes in the physico-chemical parameters which showed significant differences between batches throughout the ripening time of Armada cheeses manufactured using the autochthonous starter cultures. Batches of cheeses were made with: batch I (-○-), Ge-1886; batch II (-□-), Ge-1903; bath III (-◊-), Ge-1889; batch IV (-Δ-), Ge-1893.

The use of *Lactococcus lactis* subsp. *lactis* (TAUL 1292) strain selected for its acidifying and proteolytic activity (Herreros et al., 2003), caused a very marked fall in pH at early stages. The slight increase in pH observed after sixty days of ripening could have been due to the capacity of *G. candidum* to metabolize lactic acid, which would favour the implantation of another type of microbiota (Cosentino et al., 2001; Fadda et al., 2004). Titratable acidity (TA), expressed as g of lactic acid 100 g^{-1} of DM, decreased during the ripening ($p < 0.001$), with no significant differences between batches. With regard to changes in lactose, significant differences ($p < 0.01$) were noted between the different batches. The lactose content in the curds and at the start of the ripening was similar to the content observed in Armada cheeses manufactured from pasteurized milk and the LAB strains (Herreros, 2010). The lactose content dropped sharply up to the fifteenth day of ripening. This decline is attributed to the rapid development of the lactic microbiota included in the starter, favoured by the low salt to moisture ratio (S/M) in two-day-old cheese. These results agree with those obtained by Herreros (2010) in batches made form pasteurized goat's milk and the LAB strains.

The FAI showed no significant differences between batches. However, there were significant differences ($p < 0.001$) as a function of ripening time, with much higher values after fifteen days. The presence of cracks in the cheeses, especially after fifteen days of ripening, favoured the growth of fungi and hence that of lipolysis. After the fifteenth day of ripening, a fall in the FAI was observed until the end of ripening. The lipoprotein lipase in milk is deactivated by pasteurization and the addition

Table 3. Nitrogen fractions content (average values ± standard deviation)[a] throughout ripening of Armada cheeses elaborated with an autochthonous starter culture.

Nitrogen fractions	Ripening time (days)				Batch	Time
	2	15	30	60		
pH4.4-SN	12.18 ± 2.23	12.69 ± 1.85	12.47 ± 1.30	12.29 ± 1.49	NS	NS
TCA12%-SN	2.38 ± 0.64	3.35 ± 0.93	3.43 ± 0.66	3.41 ± 0.48	*	**
PTA5%-SN	0.13 ± 0.15	0.40 ± 0.54	0.68 ± 0.49	0.98 ± 0.55	***	***
Polypeptide N	9.80 ± 2.60	9.34 ± 2.33	9.04 ± 1.70	8.88 ± 1.84	NS	NS
Peptide N	2.25 ± 0.57	2.95 ± 0.71	2.76 ± 0.81	2.42 ± 0.74	NS	NS

[a]Values expressed as g 100 g^{-1} of total nitrogen; pH4.4-SN: pH 4.4 soluble nitrogen; TCA12%-SN: trichloroacetic acid soluble nitrogen; PTA5%-SN: phosphotungstic acid soluble nitrogen; Polypeptide N: Polypeptide nitrogen; Peptide N: peptide nitrogen. The last two columns are referred to the significant differences between batches and between the ripening time. NS: no significant differences; *: significant differences ($p < 0.05$); **: significant differences ($p < 0.01$); ***: significant differences ($p < 0.001$).

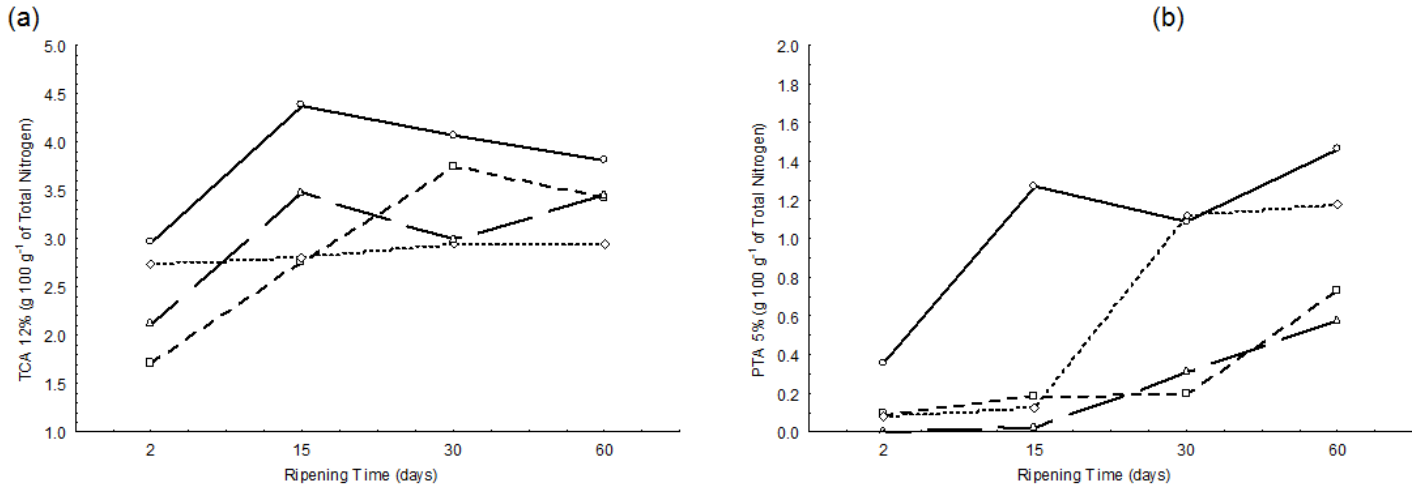

Figure 2. Changes in the nitrogen fractions content which showed significant differences between batches throughout the ripening time of Armada cheeses manufactured using the autochthonous starter cultures. Batches of cheeses were made with: batch I (-○-), Ge-1886; batch II (-□-), Ge-1903; bath III (-◊-), Ge-1889; batch IV (-Δ-), Ge-1893.

of a starter culture triggers off a marked decrease in pH at the beginning of ripening, which could negatively affect the lipases present.

The protein content decreased slightly during the first two weeks of ripening, and then remained stable until the end of the process. Significant differences were then observed with regard to the ripening time ($p < 0.01$), but in contrast there were no significant differences between batches (Table 2). In order to determine the degree of proteolysis in cheese, the changes in different nitrogen fractions were evaluated (Table 3). The nitrogen fractions which showed significant differences between batches were also illustrated in Figure 2.

The formation of pH 4.4 soluble nitrogen during the ripening of the cheese is mainly due to the rennet action on caseins and, to a lesser extent, to the plasmin and acid protease action in milk. No significant differences were found as a function of the batch or ripening time in the batches of Armada cheese elaborated using the various *G. candidum* strains. The pH 4.4-SN values remained stable at about 12.4%, which indicates that only slight proteolysis took place. These results were very similar to those reported by Herreros (2010) for Armada cheeses elaborated from pasteurized milk and the LAB strains. With regard to the trichloroacetic acid soluble nitrogen, significant differences were noted during ripening ($p < 0.01$). The TCA12%-SN increased slightly up to thirty days, but then stabilized. Significant differences ($p < 0.05$) were also observed between the various batches. The TCA12%-SN, measures the depth of proteolysis and it is nevertheless an indicator of proteolytic activity due to the microbiota present in cheese. In the case of phosphotungstic acid soluble nitrogen (PTA5%-SN), significant differences ($p < 0.001$) were found with regard to both ripening time and the various manufactured batches. In all cases, the average

Table 4. Microbial counts (average values ± standard deviation)[a] throughout manufacture and ripening of Armada cheeses made with an autochthonous starter culture.

	Milk	Cheese (days of ripening)				Batch	Time
		2	15	30	60		
PCAm (aerobic mesophilic bacteria)	7.04 ± 0.05	9.91 ± 0.56	9.27 ± 0.29	8.33 ± 0.39	6.11 ± 1.67	NS	***
MRS (lactic acid bacteria)	7.05 ± 0.07	9.76 ± 0.19	9.19 ± 0.19	8.37 ± 0.48	6.21 ± 1.78	*	***
M17 (Lactococci)	6.99 ± 0.27	9.82 ± 0.38	9.38 ± 0.34	8.37 ± 0.33	6.07 ± 0.94	*	***
ROGOSA (Lactobacilli)	0.11 ± 0.30	0.63 ± 0.95	3.37 ± 1.59	5.15 ± 1.17	5.42 ± 0.98	*	***
VRBGA (Enterobacteriaceae)	0.81 ±1.01	1.00 ±1.51	0.17 ± 0.24	0.74 ± 1.40	0.00 ± 0.00	**	**
OGYEA (yeasts and moulds)	2.52 ± 0.30	3.30 ± 0.30	5.88 ± 0.63	4.01 ± 1.91	3.60 ± 0.81	NS	***

[a]Microbial counts are expressed as log CFU g^{-1}; the last two columns are referred to the significant differences between batches and between the ripening time. NS: no significant differences; *: significant differences ($p < 0.05$); **: significant differences ($p < 0.01$); ***: significant differences ($p < 0.001$).

values detected at each sampling point were very low, which confirms the presence of low-level aminopeptidase activity. These results were lower than those found by Herreros (2010) in the same type of cheese elaborated without the addition of the relevant *G. candidum* strains. Polypeptide nitrogen (Polypeptide N), calculated from the difference between pH4.4-SN and TCA12%-SN, decreased over the ripening process, but without any significant differences being noted either in ripening time or in batches. As for peptide nitrogen (peptide N), calculated from the difference between TCA12%-SN and PTA5%-SN, no significant differences were seen either with regard to ripening time or between the batches (Table 3).

Changes in the main microbial group counts

Changes in the microbial counts during the ripening process of the various batches of Armada cheese are shown in Table 4. The microbial counts which showed significant differences between batches are also illustrated in Figure 3.

Most of the microbial groups had an increase of approximately two logarithmic units in two-day-old cheese due to the physical retention of microorganisms in curds, their multiplication during coagulation and drainage and the delay of salting process in this cheese. The highest counts of mesophilic aerobic microorganisms (PCAm) were recorded after two days of ripening, but thereafter there was a gradual decline in counts until at the end of ripening. The concentration of NaCl dissolved in the moisture in cheese, which increased during ripening, has an inhibitory effect on microorganisms (Beresford et al., 2001). All the batches showed a very similar evolution and no significant differences were observed with regard to the batch in the counts of mesophilic aerobic bacteria. Nevertheless, there were significant differences with regard to ripening time ($p < 0.001$). The counts recorded were similar to those in Armada cheese elaborated from pasteurized goat's milk using the same lactic acid bacteria strains as starter culture (Herreros, 2010).

The counts observed on MRS and M17 agar followed a pattern practically identical to that observed on PCAm. On both culture media, significant differences were observed with regard to both ripening time ($p < 0.001$) and batch ($p < 0.05$) up to sixty days of ripening. Counts were also similar to those reported for other goat and cow's cheeses (Arenas et al., 2004; González et al., 2003; Herreros et al., 2007). Counts on M17 agar reached their highest values between two and fifteen days of ripening because *Lactococcus* break down lactose and their counts increase rapidly (Williams et al., 2000). Then, slowly dropped until the end of ripening at which point significant differences were noticeable between batches. A significant positive correlation ($p < 0.01$) was found in the *Lactococcus* on M17 counts with regard to the a_w levels ($r = 0.88$) and moisture ($r = 0.86$) (Table 5).

Lactobacilli count on ROGOSA agar increased progressively as ripening proceeded ($p < 0.001$), with significant differences ($p < 0.05$) being observed between batches. The slower lactobacilli metabolism and its greater capacity to adapt to adverse conditions (acidity, low values for a_w or high NaCl concentrations) related to other LAB could contribute to its increasing predominance as the number of days of ripening increases. In fact, there was a significant negative correlation ($p < 0.01$) between the developments in counts on ROGOSA agar and titratable acidity ($r = -0.52$), a_w ($r = -0.45$) and moisture ($r = -0.45$). On the other hand, a positive correlation ($p < 0.05$) was found between the ROGOSA agar counts and the S/M ratio ($r = 0.39$) (Table 5).

Significant differences were detected in VRBGA medium with regard to the batch ($p < 0.01$) and ripening time ($p < 0.01$). After fifteen days of ripening, the counts for Enterobacteriaceae decreased up to undetectable count, and eventually they completely disappeared owing to the unfavourable growth conditions which gradually

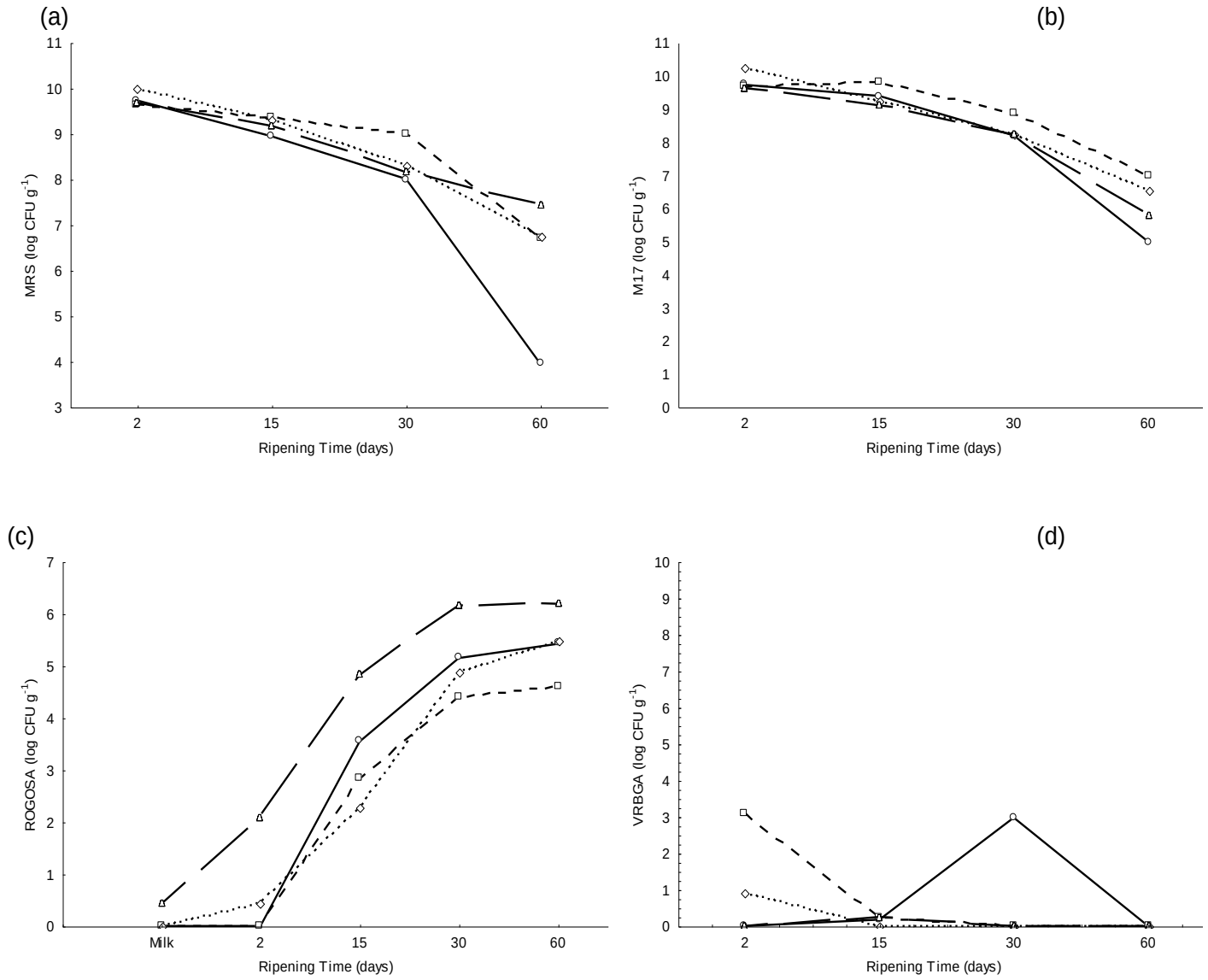

Figure 3. Changes in the microbial counts which showed significant differences between batches throughout the ripening time of Armada cheeses manufactured using the autochthonous starter cultures. Batches of cheeses were made with: batch I (-○-), Ge-1886; batch II (-□-), Ge-1903; bath III (-◊-), Ge-1889; batch IV (-Δ-) and Ge-1893.

prevailed in the cheese as this process develops (Buffa et al., 2001). The detection of Enterobacteriaceae in the cheeses of one of the batches after thirty days of ripening may have been due to later contamination.

Counts for moulds and yeasts on OGYEA medium increased up to fifteen days of ripening, when they reached their highest levels. They then decreased until the end of ripening. The evolution of this microbial group was similar in all batches manufactured. However, there were significant differences ($p < 0.001$) with regard to ripening time. *G. candidum* was the predominant fungal species during the manufacturing and ripening of the cheeses elaborated from the lactic starter culture and the *G. candidum* co-culture. Thus, OGYEA counts constitute an indicator of changes undergone by *G. candidum* during the ripening. The highest counts were reached after fifteen days of ripening, without apparent inhibition of growth. Interactions of *G. candidum* and starter culture were reported by other authors (Šípková et al., 2015). From here on, the counts fell more or less steeply depending on the batch, until after sixty days when they reached values similar to those at the start of the ripening.

Lipolytic activity by LAB is generally very limited. Their lipases act primarily on mono- and di-glycerides previously formed by indigenous lipases of the milk, and their capacity to act on triglycerides is very slight (El Soda et al., 1986). Positive correlation between the values for

Table 5. Correlations between the microbiological and physico-chemical parameters in the manufacture of Armada goat's cheeses.

	Culture medium				
	M17	**MRS**	**ROGOSA**	**OGYEA**	**PCAm**
Moisture	0.86[a]	0.80[a]	-0.45[a]	0.01	0.82[a]
a_w	0.88[a]	0.80[a]	-0.45[a]	0.07	0.83[a]
S/M	-0.92[a]	-0.84[a]	0.39[a]	-0.07	-0.85[a]
TA	0.77[a]	0.73[a]	-0.52[a]	-0.11	0.73[a]
Lactose	0.63[a]	0.58[a]	-0.48[a]	-0.16	0.63[a]
FAI	0.37[a]	0.29	-0.08	0.64[a]	0.31

[a]Marked correlations are significant at $p < 0.05$.

Table 6. Overall sensory impression[a] throughout ripening of the Armada cheeses elaborated with an autochthonous starter culture.

Batches[b]	**Ripening time (days)**		
	15	**30**	**60**
I	6.8 ± 0.22	6.9 ± 0.29	7.3 ± 0.90
II	6.2 ± 0.33	6.1 ± 0.14	6.0 ± 0.40
III	6.2 ± 0.48	6.2 ± 0.39	6.5 ± 0.02
IV	6.5 ± 0.35	7.6 ± 0.20	6.5 ± 0.73

[a] Average values evaluated on a scale running from 1 to 10 by a panel of 20 tasters; [b] The batches were elaborated with four starter cultures: Starter batch I: *Lactococcus lactis subsp. lactis* (TAUL 1292), *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (TAUL 12) and *G. candidum* Ge-1886 strain. Starter batch II: TAUL 1292, TAUL 12 and *G. candidum* Ge-1903 strain. Starter batch III: TAUL 1292, TAUL 12 and *G. candidum* Ge-1889 strain. Starter batch IV: TAUL 1292, TAUL 12 and *G. candidum* Ge-1893 strain.

the FAI and the counts obtained for OGYEA shows the involvement of fungal population in lipolysis (Table 5).

Sensory evaluation

The overall sensory evaluation of the Armada cheeses elaborated by adding an autochthonous starter culture and co-starter culture of *G. candidum* is shown in Table 6. These cheeses developed a notable flavour characteristic of goat cheese, which gives individuality and quality to this type of cheese (Gaborit et al., 2001). In fact, flavour is one of the main sensory attributes of cheese's quality (Zabaleta et al., 2015). The evaluation of the texture and taste parameters in cheeses after sixty days of ripening is shown in Figure 4.

With regard to the overall sensory evaluation of the manufactured cheeses, no significant differences could be seen throughout the ripening period, but there were significant differences ($p < 0.05$) with regard to batches. Batches I and IV received the best scores after sixty and thirty days of ripening, respectively.

Because of the particular techniques used in the manufacturing of these cheeses, in which salting is carried out after two days, kneading stage was required, and a dry grainy texture is a constant in cheeses after fifteen and thirty days of ripening. This was also seen in the cheeses elaborated with pasteurized milk using commercial and autochthonous starter culture in the test carried out by Herreros (2010). Generally, as the ripening process went on, hardness increased ($p < 0.001$) and soft buttery texture decreased ($p < 0.001$), as did the stickiness of the cheese paste in the mouth ($p < 0.05$). Batch I presented a hardness profile that differed from the others, as it was the batch with the softest texture after fifteen and thirty days as well as had the greatest increase in hardness. Stickiness decreased in all the batches, although somewhat irregularly, depending on the batch.

The fresh milky odour characteristic of these cheeses declined slightly during the ripening period ($p < 0.01$), with significant differences observed among batches ($p < 0.05$). Another pleasant attribute of these cheeses was a buttery odour, which increased slightly over the ripening period in all the cheeses. A rennet odour and a farmyard odour were two negative attributes of some of these

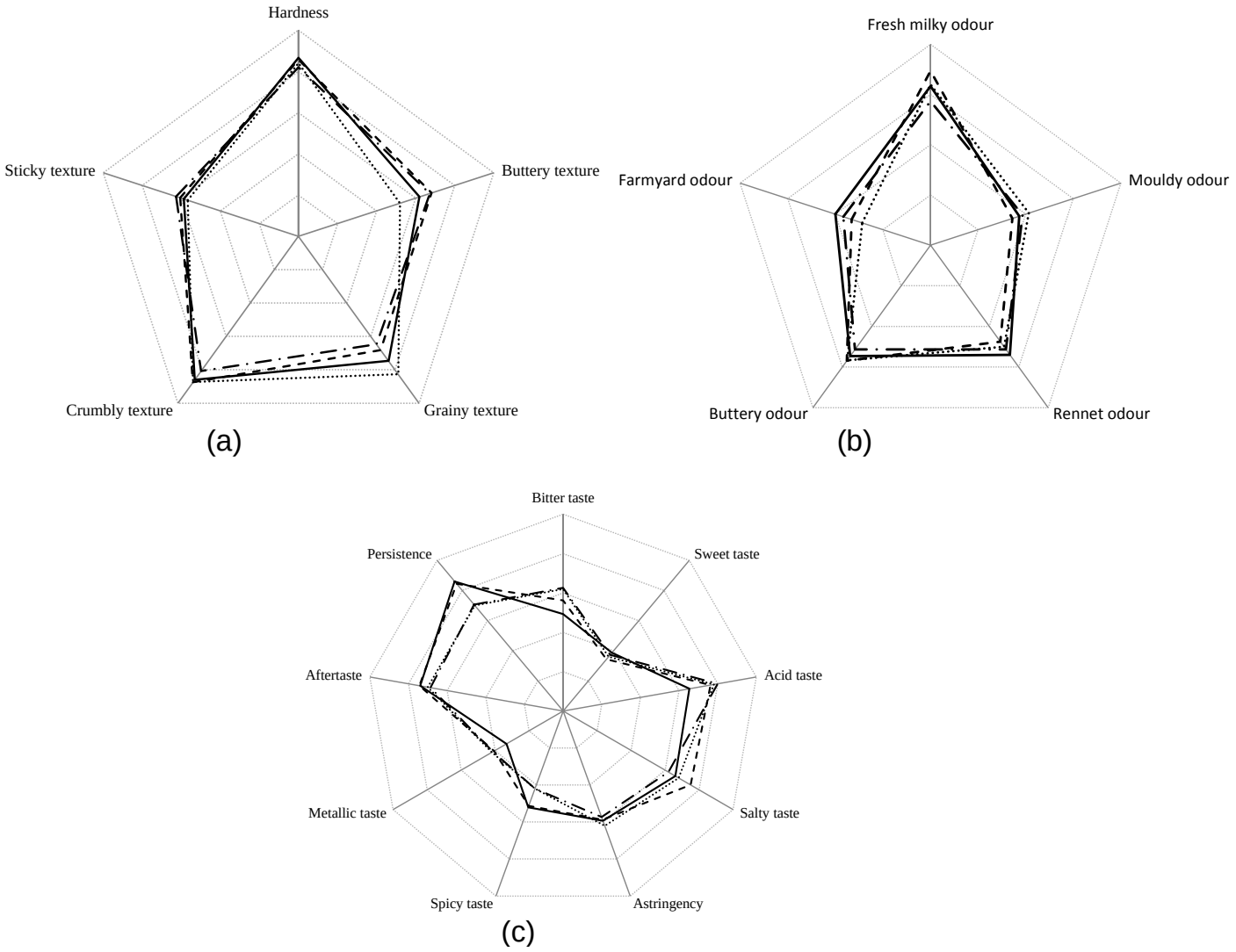

Figure 4. Sensorial evaluation of texture (a), odour (b) and taste (c) parameters of Armada cheese at 60 days of ripening. Parameters evaluated on a 7-point intensity scale. (**-**) Batch I, (**⋯**) Batch II, (**-·-**) Batch III, (**---**) Batch IV.

cheeses, with significant differences ($p < 0.05$) arising as ripening progressed. In general, the two unpleasant parameters were observed more in thirty-day-old cheeses, then decreased after sixty days of ripening.

The cheeses were characterized by a fresh acid taste which decreased somewhat during the ripening period ($p < 0.05$). The desirable odour and aftertaste of goat which developed was a parameter for quality in these cheeses, associated with *G. candidum*. In fact, these cheeses received higher scores than those elaborated from pasteurized milk without the addition of *G. candidum* culture. Astringency was also affected by ripening time ($p < 0.01$), increasing from the fifteenth to the sixtieth day. The spicy taste characteristic of these cheeses also increased during ripening ($p < 0.05$). A further parameter that was affected was the persistency on the palate, which notably increased after sixty days of ripening period ($p < 0.001$), which indicates a greater potency of the mixture of tastes and odours at the end of the ripening process.

With regard to the assessment of batches as a function of ripening time, at fifteen days of ripening the taste was equal, milky and fresh in all batches, if not very intense. In some cheeses it was possible to note a certain mouldy taste, and in those from batch I a typical goat odour. In general, after thirty days the cheeses in batches II and III showed a drier and grainier texture and an unequal and metallic taste, with an unpleasant aftertaste and marked bitterness. In some cases, odour defects were observed, such as a mouldy or rennet odour. At sixty days of ripening, cheeses of the batch III presented a more acceptable texture (stronger buttery texture and less grainy) and a strong buttery odour, but it did not reach high overall scores. Although, cheeses in batch IV

achieved the best scores at thirty days of ripening, then they worsened in texture, becoming grainier and crumblier, as well as having a taste that was excessively acid and astringent. With regard to cheeses of the batch I, they obtained high scores at thirty days of ripening and were even the best evaluated at the end of ripening because of their odour, fresh balanced pleasant taste and creamy smooth texture.

Conclusions

The use of an autochthonous starter culture constituted lactic acid bacteria strains and *G. candidum* as co-culture affected the evolution of chemical and physico-chemical parameters in the case of Armada cheese, in particular lactose, pH and titratable acidity. The impact that this starter culture had on the sensorial characteristics of the Armada cheese was observed from the fifteenth day of ripening. Cheeses elaborated with the autochthonous lactic starter culture and *G. candidum* co-culture had a marked odour characteristic of goat cheese and a stronger buttery and creamy texture, as they did not undergo excessive drying-out and did not develop any residual rancid taste. Cheeses in batch I, elaborated with the Ge-1886 strain (with high lipolytic activity and low proteolytic activity) were given the best assessments for presenting a wider and more intense range of pleasant odours, and a soft buttery and creamy texture.

Conflict of Interests

The authors have not declared any conflict of interests.

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