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ORIGINAL ARTICLE

Influence of the starter culture on the microbiological and sensory characteristics of ewe's cheese

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Changes which take place in the sensory characteristics of cheeses during ripening are influenced by different factors, involving rennet, starter culture and adventitious contamination of the cheese by non-starter lactic acid bacteria. The objective of this work was to study the influence of the starter on sensory and microbiological ewe's cheese properties during ripening time. Four batches (two with starter added and two without) were manufactured. Milk and cheeses at different stages of ripening were analysed. Cheeses manufactured without adding starter showed a significantly higher level of mesophilic aerobic microflora, lactobacilli, facultatively heterofermentative lactobacilli and enterococci (indigenous microflora) than cheeses manufactured with starter. This study has also shown that adding or not adding starter affects the flavour profile of the cheese. Cheeses with starter added showed greater intensity of the following attributes: refreshing, astringent, sweet; and received lower scores on bitterness. With respect to texture, the said cheeses develop a more homogenous texture and greater elasticity throughout ripening.

Introduction

Changes which take place in the sensory properties of cheeses during ripening are complex and little information exists, mainly in ewe's cheeses. By application of multi-variate statistics, the key changes in flavour and texture have been identified (Muir et al. 1995) and the effects of the experimental treatments examined in detail (Muir et al. 1996).

Cheese ripening is influenced by factors, involving rennet (Foltman 1993), starter culture (Crow et al. 1994) and adventitious contamination of the cheese by non-starter lactic acid bacteria (McSweeney et al. 1993).

Until now, the influence of native flora on the sensory properties of raw milk cheeses has not been exactly established (Bachmann et al. 1996). However, evidence has been provided to show that commercial starter affects ripening rate and can promote substantial differences in the flavour profile in cow's cheeses (Muir et al. 1996). It is also worth noting that the role of proteases and peptidases on ripening rate has been clearly demonstrated in studies of accelerated ripening (Law 1984, Fernández-García and López-Fandiño 1994).

Lactic acid bacteria (LAB) added as the starter culture or present as non-starter lactic acid bacteria (NSLAB) are able to transform lactic acid, citrate, lactate, proteins and fat into volatile compounds which, together with amino

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acids and other products produced by casein hydrolysis, play a critical role in the development of cheese flavour (Steele and Ünlü 1993, Martley and Crow 1993).

NSLAB and mainly facultative heterofermentative lactobacilli reach high cell densities in both cow's and ewe's hard cheese varieties that require a long ripening time (Jimeno et al. 1995, Arizcun et al. 1997a). Many authors have pointed out the proteolytic and lipolytic activities of these bacteria in cheese (for example Broome et al. 1990). They can also participate directly in the production of some major aroma compounds, such as acetic, formic acids and gas (CO_2) (Demarigny et al. 1996). These bacteria are able to metabolize citrate to acetate, formate, carbonate and succinic acid. Small amounts of diacetyl are also produced during citrate fermentation (Hungenholz 1993, Jimeno et al. 1995).

Counts of enterococci tend to be particularly high (i.e. 10^5–10^8 cfu g^{-1}) in ewe's milk cheeses (Arizcun et al. 1997b). These levels observed make it likely that their enzymatic activities are important for aroma formation (González de Llano et al. 1992).

The objective of this work was to study the influence of the starter on sensory and microbiological ewe's cheese properties during ripening time. Given the similarities with respect to raw material and technology, this paper constitutes a scientific contribution to improved knowledge of two ewe's cheeses with the Spanish Appellations d'Origin: Idiazábal (Ministerio de Agricultura, Pesca y Alimentación 1993) and Roncal (Ministerio de Agricultura, Pesca y Alimentación 1991).

Materials and Methods

Samples

Two controlled batches of cheese elaborated with raw ewe's milk were studied. The only factor modified between the two batches was the addition or not of starter cultures to the original milk. In the first case, a freeze-dried starter (Ezal; Texel, Dangé Saint Romain, France) was used at a rate of 1 U 100^{-1}. It contained a combination of *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris*. Both batches were elaborated on the same day and the experiment was repeated two days later. The batch report is shown in Table 1.

Samples from the milk stored for up to 6 h at 4°C, from the time it was collected and the manufacturing process was started (original milk), were analysed. Analyses were also run on the milk from the four vats when the heating temperature reached 30°C (in two of the vats starters had been added at 24°C). Coagulation of the milk was recorded at 36·7°C (Table 1). For each batch, two cheese samples were taken on day 1 (cheese just removed from the brine), 15, 30, 60, 120 and day 180. Additionally, 270-day cheeses were sampled to see if changes in the sensory analyses were observed. In all, 56 cheeses were analysed and two replications of all analyses were performed. The milk samples were cold-transported with azidiol preservative (Zangerl et al. 1992) to the Instituto Lactológico in Lekunberri (Interprofessional Dairy Laboratory, Navarra, Spain) where the main physicochemical parameters were analysed and the total bacteria and somatic cells were counted in 24 h or less from the time of sampling. Samples of the said milk and the cheeses elaborated with it were transported to the Universidad Pública de Navarr, Pamplona, Spain, and were submitted for microbiological analysis on the same day. The sensory analysis was carried out at the Universidad del País Vasco, Vitoria, Spain, in less than 1 week from the time the samples were taken. The samples were taken in accordance with the International Commission for Microbiological Specifications for Foodstuffs. (ICMSF 1982).

Physiochemical analyses

The milk was automatically analysed three times by the Milko-Scan-255 aparatus (Foss Electric® España S.A., Madrid, Spain). This instrument is based on the infrared spectroscopy technique used for calculating fat, protein, lactose and non-fat dry matter. The pH was determined by the Berdague and Grappin method (Berdague and Grappin 1987).

Table 1. Batch report for cheese made without the addition (C) and with the addition (F) of starter cultures

		C	F
Milk	pH of the original milk[a]	6·75	6·75
	pH of the milk before adding the rennet	6·53	6·45
Additives	Mixed: 3 g natural/4 cc industrial		
	Rennet temperature: (for 24 l of milk)	31·2°C	31·2°C
	Cultures—lyophilized	No	Yes[b]
	—temperature		24°C
Vat	Ripening time of the milk (min)	40	40
	Coagulation temperature of the milk	36·7°C	36·7°C
	Reheating temperature	35·8°C	35·8°C
	pH of the whey	6·40	6·34
Press	Pressing temperature	18°C	18°C
	Pressing time	4 h	4 h
	pH of the cheeses leaving the press	6·17	5·09
Brine	Brine temperature	9–11°C	9–11°C
	Brine density (°B)	saturation	saturation
	pH of the brine	6·15	6·15
	Time in brine	14 h	14 h
Airing	Relative humidity	70–80%	70–80%
	Temperature	15°C	15°C
	Days	4–5	4–5
Ripening	Temperature	12–14°C	12–14°C
	Relative humidity	85–89%	85–89%
	Months (at least)	2	2
Observations:	Performance = 5·14 l $kg^{-1.}$		

[a] Milk stored for up to 6 h at 4°C, from the time it was collected and the manufacturing process was started.
[b] A freeze-dried starter was used at a rate of 1 U 100^{-1}. It contained a combination of *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris*.

Microbiological analyses

The total bacteria and somatic cells were counted in milk samples with the Bacto Scan-8000 (Foss Electric® España) and Fossomatic-250 apparati (Foss Electric® España) respectively. Likewise, the following microbiological analyses were run on the aforementioned milk and cheese samples: aerobic mesophilic flora on PCA agar (Difco Laboratories, Detroit, Michigan, USA) at a temperature of 32°C over 48 h, lactobacilli on MRS agar (Difco) at 32°C over 48–72 h under conditions of anaerobiosis (5% CO_2), and facultatively heterofermentative lactobacilli (*L. casei, L. rhamnosus* and *L. plantarum*) in FH medium, a specific and selective medium for these micro-organisms, at 32°C over 72 h. This medium contains vancomycin and restricts the growth of other lactobacilli (Isolini et al. 1990). Enterococci were counted on KF Streptococcus agar (Difco) at 37°C over 48 h.

Sensory Analyses

Cheese samples were evaluated by a sensory panel comprised of 12 judges who were all members of the A.O.P. Sensory Quality Control Committee, initially screened to establish their ability to recognise and rank the primary taste stimuli. The judges were highly experienced and formally trained over a period of 1 year in descriptive sensory assessment of cheeses following the guidelines used in this study.

A discriminatory test (duo-trio) was performed in order to establish if the assessors were able to globally distinguish between the samples manufactured with and without addition of a starter culture (UNE 87-010 1993). The panel evaluated the cheeses throughout ripening (2, 4, 6 and 8 months) twice each time using some of the descriptive sensory terms published by Berodier et al. (1997) for odour and flavour characteristics and by Lavanchy et al. (1993) for texture attributes adapted to ewe's cheeses. These modifications have been developed within two different European research programs: COST 95 (improvement of the quality of the production of raw milk cheeses) and AIR3-CT94-2039 (the influence of native flora on the characteristics of cheeses with 'Appellation d'Origin Protegée' made from raw milk), and are in the course of preparation to be published in the open scientific literature by the Sensory Analysis Working Group within the above mentioned projects. Four samples were evaluated during a single session and presented in such a design as to minimise order and carry-over effects (Muir and Hunter 1991).

Statistical analyses

The data were analysed with the SPSS statistics package, Version 6.1 for Macintosh (SPSS Inc., Chicago, Illinois, USA). A two-factor analysis of variance was applied (temperature of the milk and addition of cultures) to determine the existence of significant differences among the different variables studied in the milk. Likewise, a two-factor analysis of variance was also carried out for each of the microbial groups studied in cheese in order to detect the existence of significant differences in terms of ripening time and the addition of cultures.

Tables published by the International Standards Organisation (ISO-10.399, 1991) were followed for the interpretation of the discriminitive sensory tests. Mean sensory profiles across assessors and duplicates were calculated. The resulting data matrix was analysed by principle component analysis (PCA) (Piggott 1988). The solution was rotated by the varimax procedure.

Results and Discussion

Physicochemical and microbiological analyses of milk

Significant differences were observed in terms of the ferment factor for fat, lactose, non-fat dry matter and enterococci (Table 2). The addition of starter significantly reduced the fat, lactose and non-fat dry matter content and favoured the growth of enterococci.

Differences observed in the amount of lactose between original milk and milk at

Table 2. Mean values and their standard deviations for the parameters measured in the milk. L 4°C; milk at 4°C; LC 30°C, milk without starter added, at 30°C; LF:30°C milk, with starter added, at 30°C

	L 4°C	LC 30°C	LF 30°C	ET	EF	Int
Fat (%)	6·80 ± 0·05	6·84 ± 0·10	6·76 ± 0·02	NS	**	NS
Protein	5·31 ± 0·04	5·31 ± 0·04	5·25 ± 0·03	NS	NS	NS
Lactose	5·29 ± 0·03	5·21 ± 0·05	5·23 ± 0·04	**	NS	NS
Non-fat dry matter (%)	11·5 ± 0·04	11·5 ± 0·03	11·4 ± 0·03	NS	NS	NS
Somatic cells (10^3)	303 ± 21	322 ± 39	329 ± 41	NS	NS	NS
Total count[a] (BactoScan)	5 ± 0·04	4·98 ± 0·05	6·05 ± 0·04	***	**	**
Total count[a] (PCA)	5·21 ± 0·05	5·51 ± 0·30	6·63 ± 0·32	***	***	***
Lactobacilli[a] (MRS)	5·09 ± 0·41	5·14 ± 0·36	4·98 ± 0·53	NS	NS	NS
Lactobacilli[a] (FHL)	1·84 ± 0·04	1·92 ± 0·30	2·13 ± 0·56	NS	NS	NS
Enterococci[a] (KF)	3·46 ± 0·42	3·85 ± 0·31	4·02 ± 0·39	*	NS	NS

*$P \leq 0{\cdot}05$; ** $P \leq 0{\cdot}01$; *** $P \leq 0{\cdot}001$.
ET, Effect of temperature; EF, effect of starter; Int, temperature–starter interaction; NS, not significant.
[a] Data expressed in log ufc ml^{-1}.

30°C (with and without starter added), are justified because during the heating period acidifying mesophilic flora were able to use this sugar in their metabolism. The differences found in the amount of fat can be attributed to the greater reduction of pH observed in milk to which starter had been added; the Milko-Scan shows differences in readings for different degrees of milk acidification (Grappin et al. 1987).

The starter and temperature factors have shown a significant interaction for milk at 30°C for the total bacteria counts (measured in Bactoscan and in PCA). The interaction effect found between the temperature of the milk and the starter culture on the total bacteria count is easily explained since the micro-organisms are favoured by the temperature. Therefore, the combined effect of both factors determines the existence of a total number of bacteria, which is higher in ewe's milk with added culture.

Microbiological analysis of the cheeses

As observed in Table 3, the evolution of the aerobic mesophilic flora thoughout ripening is influenced by the addition or not of starters to the original milk. The amounts are significantly higher ($P \leq 0{\cdot}001$) in the cheeses made without starter cultures. This fact can be attributed to the inhibition of these bacteria as a consequence of the drastic reduction of the pH, due to the production of lactic acid by the bacteria that make up the starter culture (observe the pH of the cheeses as they come out of the press in Table 1) (Farkye and Fox 1990). On day 15 of ripening, the pH of cheeses made without starters reached the normal value, nearly 5·2.

Both batches evolve similarly throughout ripening. During the first 15 days of ripening, the value increases significantly ($P \leq 0{\cdot}001$). Later, and up to 60 days, a reduction of approximately one logarithm unit is observed. The value then increases and after 120 days, the level of mesophiles decreases significantly ($P \leq 0{\cdot}001$), which correlates with the evolution of the lactobacilli and is justified because these micro-organisms constitute a quantitatively important fraction of the aerobic mesophilic flora (Poullet 1991).

With respect to the lactobacilli (Table 3) in general, the cheeses made without the addition of starters register a significantly higher value ($P \leq 0{\cdot}001$) throughout ripening. This is fundamentally due to the fact that, in this type of cheese, the starter culture only contains mesophilic lactococci strains and, in cheeses made with raw milk, proliferation of the lactobacilli can be greater since less competition exists with the lactococci. A significant increase in the amount of lactobacilli is observed during the first 15 days. From day 30 to day 60, the counts decrease significantly in all the cheeses, although it is worth highlighting that the reduction is greater in the cheeses with starters. The values even out by the day 180.

Table 3 shows the significant increase ($P \leq 0{\cdot}001$) of more than 4 logarithmic units of the facultative heterofermentative lactobacilli, grown in FH medium during the first 13 days of ripening. Until day 60, the value increases progressively and then remains almost stable (except from day 120 to day 180 in cheeses without starter added). Other authors (Demarigny et al. 1996) have described similar evolution curves. Significantly higher counts can be seen in the cheeses made without the addition of starter cultures, which corresponds to the above comments about the lactobacilli. Such high values confirm that this group of micro-organisms constitutes one of the predominant microbial groups throughout the ripening period (Jimeno et al. 1995), and that their influence can be relevant to the development of organoleptic characteristics of cheese, as some authors (see Broome et al. 1990) have pointed out.

Table 3 shows that the levels of enterococci found in cheeses made with starters are significantly lower ($P \leq 0{\cdot}001$) than those detected in the cheeses made without the addition of starter. They always remained at a level 10- to 100-fold lower than in raw milk cheeses. The differences detected in the enterococci counts could be explained by the greater resistance of these micro-organisms (Arizcun et al. 1997b) and phenomena of microbiological antagonism of the bacteria that make up the starter culture

Table 3. Evolution (mean ± s.d.) of the mesophiles, Lactobacilli grown on MRS agar (Lactobacilli), facultative heterofermentative lactobacilli grown on FH (heterofermentative lactobacilli) and enterococci throughout the ripening period of the 4 batches. Cheese made with raw milk (QC) and cheese made with the addition of starter (QF). Units expressed in log ufc g^{-1}

	Mesophiles			Lactobacilli			Heterofermentative lactobacilli			Enterococci		
Ripening (days)	QC	QF	*P*	QC	QF	*P*	QC	QF	*P*	QC	QF	*P*
1	$8{\cdot}71 \pm 0{\cdot}12^{a}$	$8{\cdot}39 \pm 0{\cdot}07^{a}$	***	$7{\cdot}27 \pm 0{\cdot}64^{a}$	$6{\cdot}97 \pm 0{\cdot}75^{a}$	NS	$2{\cdot}60 \pm 0{\cdot}16^{a}$	$2{\cdot}49 \pm 0{\cdot}23^{a}$	NS	$6{\cdot}38 \pm 1{\cdot}14^{a}$	$4{\cdot}65 \pm 0{\cdot}10^{a}$	***
15	$9{\cdot}07 \pm 0{\cdot}20^{b}$	$8{\cdot}55 \pm 0{\cdot}10^{b}$	***	$8{\cdot}83 \pm 0{\cdot}25^{b}$	$8{\cdot}66 \pm 0{\cdot}27^{b}$	$6{\cdot}66 \pm 0{\cdot}42^{b}$	**	$6{\cdot}87 \pm 0{\cdot}21^{b}$	$5{\cdot}46 \pm 0{\cdot}18^{be}$	***		
30	$8{\cdot}49 \pm 0{\cdot}13^{c}$	$7{\cdot}84 \pm 0{\cdot}10^{c}$	***	$8{\cdot}76 \pm 0{\cdot}34^{b}$	$8{\cdot}42 \pm 0{\cdot}33^{b}$	*	$7{\cdot}76 \pm 0{\cdot}13^{c}$	$7{\cdot}14 \pm 0{\cdot}11^{c}$	***	$7{\cdot}52 \pm 0{\cdot}34^{c}$	$6{\cdot}65 \pm 0{\cdot}11^{c}$	***
60	$8{\cdot}09 \pm 0{\cdot}15^{d}$	$7{\cdot}36 \pm 0{\cdot}17^{d.}$	***	$8{\cdot}05 \pm 0{\cdot}12^{c}$	$6{\cdot}9 \pm 0{\cdot}30^{a}$	***	$8{\cdot}10 \pm 0{\cdot}11^{d}$	$7{\cdot}34 \pm 0{\cdot}49^{cd}$	**	$7{\cdot}40 \pm 0{\cdot}29^{c}$	$5{\cdot}54 \pm 0{\cdot}5^{b}$	***
120	$8{\cdot}33 \pm 0{\cdot}16^{e}$	$7{\cdot}53 \pm 0{\cdot}27^{e}$	***	$8{\cdot}29 \pm 0{\cdot}11^{c}$	$7{\cdot}66 \pm 0{\cdot}19^{c}$	***	$8{\cdot}08 \pm 0{\cdot}13^{d}$	$7{\cdot}55 \pm 0{\cdot}20^{de}$	***	$7{\cdot}54 \pm 0{\cdot}09^{c}$	$5{\cdot}76 \pm 0{\cdot}12^{d}$	***
180	$7{\cdot}48 \pm 0{\cdot}18^{f}$	$6{\cdot}74 \pm 0{\cdot}10^{f}$	***	$7{\cdot}57 \pm 0{\cdot}12^{d}$	$7{\cdot}58 \pm 0{\cdot}06^{c}$	NS	$7{\cdot}59 \pm 0{\cdot}15^{bc}$	$7{\cdot}33 \pm 0{\cdot}14^{ce}$	**	$7{\cdot}06 \pm 0{\cdot}14^{b}$	$5{\cdot}28 \pm 0{\cdot}10^{e}$	***

NS, $P > 0{\cdot}05$; * $P \leq 0{\cdot}05$; ** $P \leq 0{\cdot}01$; *** $P \leq 0{\cdot}001$.
Means with the same superscript lowercase letters in a column are not significantly different ($P > 0{\cdot}05$).

with other bacteria genera (Gaya et al. 1986), which permits a greater development of the enterococci.

The fact that the counts are between 5·54 and 7·52 (in log ufc g^{-1}) after 30 days of ripening in all cheeses could lead one to consider that enterococci can play an important role in the ripening of the cheese, as has already been indicated by some authors (Litopoulou-Tzanetaki et al. 1993). Very high levels of enterococci produce the appearance of bitter (Wessels et al. 1990) and hot (Prato and Messina 1990) tastes as well as defects in the external appearance of the cheese (Hernández et al. 1989). This is reflected in lower scores when evaluated by a panel of tasters, as has been attested in sensory analysis of the cheeses made with raw milk without the addition of a starter.

Cheese sensory analysis

Results obtained from duo-trio discriminatory tests at each step of the ageing period reported the existence of overall sensory differences between the samples manufactured with and without the addition of a starter culture. This fact indicates the necessity of describing these main characteristics.

Cheese flavour/odour

Main changes experimented by odour/flavour attributes through ageing and the principal differences among cheeses manufactured with and without the addition of a starter culture were examined by principal component analysis (PCA).

The variance accounted for by the first three principal dimensions was 41·0, 29·1 and 14·8% respectively (85·0% in total). The solution was selected according to two criteria suggested by Jolliffe (1986): examination of the breakpoint of the screen plot and components accounting for 80–90% of the total amount of variance.

The vector loadings examination facilitated the interpretation of dimensions of the PCA solution. The principal components matrix is shown in Table 4. The first flavour/odour dimension was high in flavour intensity, acid taste, metallic, aftertaste and odour intensity, all showing positive values. The second component was mainly attributable to refreshing, astringent and sweet (positive values) and bitter and persistent (negative values). The third dimension was associated with the terms hot and salty.

Table 4. Principal component analysis: interpretation of odour/flavour space of cheeses. Vector loadings rotated and sorted on first three components

Odour/Flavour attribute	Factor 1	Factor 2	Factor 3
Flavour intensity	0·90	−0·17	−0·33
Acid	0·90	0·16	0·34
Metallic	0·89	−0·02	0·38
Aftertaste	0·69	−0·08	0·67
Odour intensity	0·64	0·59	−0·29
Refreshing	0·04	0·90	0·08
Bitter	0·29	−0·79	0·39
Persistent	0·40	−0·76	0·13
Astringent	0·51	0·74	0·26
Sweet	0·46	0·67	0·13
Hot	−0·10	−0·24	0·84
Salty	0·30	0·41	0·74
% Variance explained	41·00	29·10	14·80

Changes in the flavour/odour character of the individual samples in the main sensory dimensions are shown in Fig. 1; the first dimension seems to be the most representative of the cheeses, independently of the addition of any starter culture. The movement of cheese samples along this axis to positive values during aging is mainly due to an increase in the intensity of the flavour and odour attributes, aftertaste and acid, and the trigeminal sensation called metallic. It has been stated that most of the sensory attributes measured in Cheddar cheese increase to some extent during ripening (Roberts and Vickers 1994), although no published research has been found on the evolution of the metallic attibute.

Muir et al. (1996) noted progressive increases in acid, bitter and salty flavours during ripening of Cheddar cheese, however, in this study this effect is only observed for acid taste.

The second dimension seems to be the axis that separates the samples according to the addition of the starter. There can be seen a slight reduction in the sample scores along this dimension during ripening for both types of

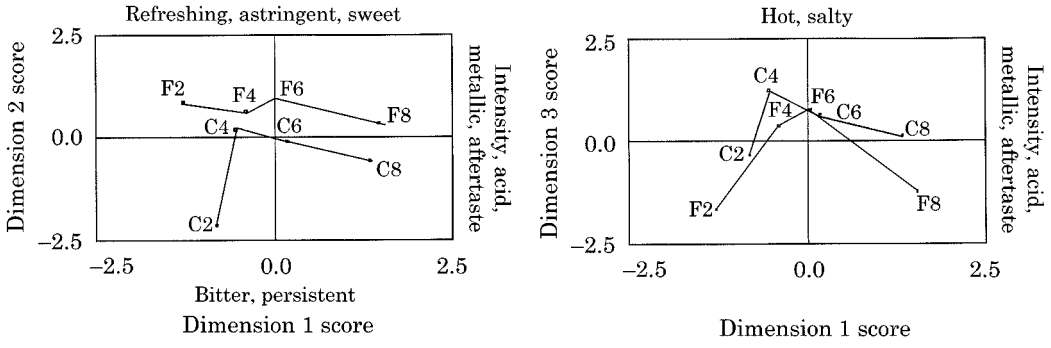

Figure 1. Sensory space maps for odour/flavour attributes of cheese constructed on scores on first, second and third dimensions of the configuration derived from varimax rotation after principal component analysis. Samples are coded C (without the addition of any starter) and F (with a starter culture added); ripening time numbers correspond to 2, 4, 6 and 8 months respectively.

cheeses (with and without a starter added) in a similar way. It is interesting to note the extreme values were observed for the samples not manufactured with a starter and 2 months of ripening. Thus, it can be deduced that the effect of the starter culture added during the manufacture of these cheeses is more appreciable at the beginning of the ripening period. Taking into account the composition of this culture (*Lactococcus* spp.), several authors (such as Chapman and Sharpe 1990) have reported that its amount decreases through ripening, with greater activity during the earliest stages. Table 3 shows the higher quantity of *Lactobacilli* present in the samples without starter added; presumably due to the lack of competition with *Lactococci* (Urbach 1995).

These score values in the second dimension show an important bitter and persistent character, which are opposed to the refreshing, sweet and astringent notes present in the samples with a starter. Lemieux and Simard (1994) reported that bitterness and astringency in dairy products were frequently correlated. However, in this study, these two attributes appear opposite in the principal component map, and could be due to difficulty in the measurement of the sensory description called astringency (Lemieux and Simard 1994).

Bitterness in cheese has been studied by several authors, most of them stressing the great importance of this sensory attribute on consumer acceptance of dairy products (Bouton et al. 1996, Habibinajafi and Lee 1996). Thus, in this study, it can be clearly seen that the addition of a starter culture during the manufacture of cheese avoids the large sensory scores obtained for this negative descriptor by the samples produced with native flora, with no addition of a starter micro-organism.

While several authors have seen that young cheeses had a sweet character (Barlow et al. 1989), in this study, no difference in this parameter due to ripening time has been noticed. This fact could be affected by the high level of bitterness present in the samples manufactured without the addition of a starter, making the perception of sweetness more difficult. This indicates that the addition of a starter culture has more influence on matured bitter and persistence characteristics than does the length of maturation. It is also worth noting that the addition of a starter affected not only the ripening rate but also the flavour profile.

The cheese samples do not show a clear tendency to group around the third factor, neither according to the addition of the starter nor the stage of ripening. These results suggest that the first two components would have been sufficient to explain the main effects of the starter culture on ewe's cheese ageing.

Cheese texture

Changes in cheese texture during ripening were examined in the same manner as flavour/odour. The three components retained in the

chosen solution condensed variance percentages of 45·2, 30·7 and 10·2% respectively (86·1% in total).

As shown in the loading matrix for texture attributes in the first three components (Table 5), in the first one firmness, rugosity and adhesiveness appear opposite solubility. The second one appears mainly characterized by the term humidity, on the surface and in the mouth, and with negative values, friability. Elasticity seems to be the representative of the third dimension characteristics.

As for odour/flavour characteristics, the first dimension is mainly associated with the changes observed during the ageing of the cheese. In Fig. 2, it can be seen that cheese samples obtain higher scores through ripening time due to the larger ratings for firmness, rugosity and adhesiveness in aged cheeses, opposed to the higher ratings for solubility for the 2-month cheeses. In samples with a starter added, the evolution of the sensory texture is homogeneous through ripening. However, in samples without a starter, an important difference exists from the second to the fourth month of ripening, with a slight evolution until the eighth month. Starter culture seems to highlight the differentiation of the samples in terms of ageing time more accurately.

Dikeman (1988) and Piggott and Mowat (1991) did not observe any changes in texture that could be attributed to ageing of Cheddar cheese, while Barlow et al. (1989) described cheeses with a more firm, brittle and crumbly character in the last stages of ripening.

Table 5. Principal component analysis: interpretation of texture space of cheese. Vector loadings rotated and sorted on first three components

	Factor 1	Factor 2	Factor 3
Firmness	0·95	−0·16	0·06
Solubility	−0·83	0·22	0·44
Rugosity	0·72	0·07	−0·60
Adhesiveness	0·63	0·49	−0·31
Humidity	−0·23	0·94	0·17
Friability	0·01	−0·73	−0·17
Surface humidity	0·67	0·68	−0·03
Elasticity	−0·11	0·29	0·92
% Variance explained	45·20	30·70	10·20

Samples with 4 and 6 months of ripening in the case of the addition of a starter, and with 4 months of ageing without a starter, clearly moved away from the rest of the samples in the second dimension. These cheeses are more friable and have less marked humidity, although this increases in advanced stages of ripening.

The third dimension, mainly defined by the term elasticity, emphasises a positive development on this attribute with ageing until intermediate stages, especially in the case of the samples with a starter added. It is worth discussing the important decrease of this component for the samples without a starter from the

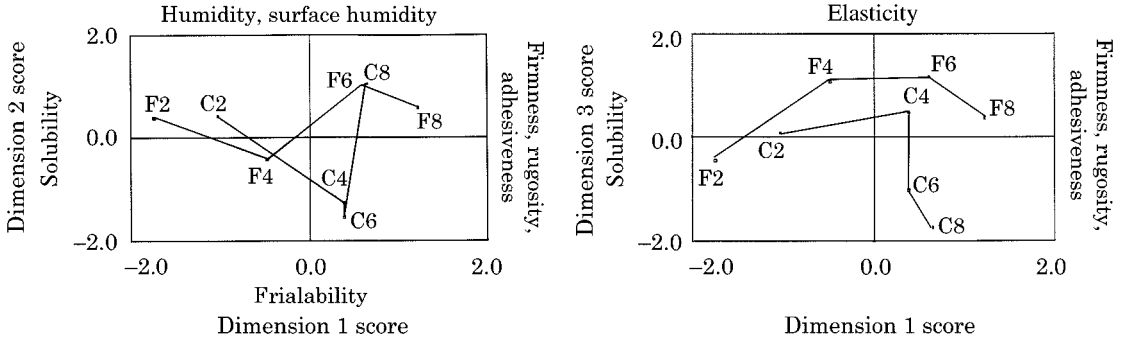

Figure 2. Sensory space maps for texture attributes of cheese constructed on scores on first, second and third dimensions of the configuration derived from varimax rotation after principal component analysis. Samples are coded C (without the addition of any starter) and F (with a starter culture added); ripening time numbers correspond to 2, 4, 6 and 8 months respectively.

fourth month. This fact indicates that the selected starter produces more elastic cheeses that develop their sensory characteristics properly during ageing.

Conclusions

The addition of a starter culture in the manufacture of ewe's cheese is a process which affects the quality of these cheese varieties. There is evidence that cheeses made without adding starter cultures present a significantly higher level ($P \leq 0{\cdot}001$) of aerobic mesophilic flora, lactobacilli, heterofermentative lactobacilli and enterococci (micro-organisms present in their autochthonous flora) than cheeses made with the addition of a starter. This particular study has demonstrated the importance of starter in determining the sensory properties of ewe's cheeses. However, the direct and interactive effects of cheese composition cannot be discounted. Further progress will depend on a better understanding of the influence of cheese composition on the ecology and activity of both starter and non-starter bacteria.

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