

Original article

**Effect of milk pasteurization  
on lipolysis during ripening of ovine cheese  
manufactured at different times of the year**

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**Abstract** — Lipolysis was studied in cheeses manufactured with pasteurized and raw ovine milk with a starter culture added in winter, spring and summer, up to 180 d of ripening. Pasteurized milk cheeses had significantly lower levels of lipolysis than raw milk cheeses in winter after 180 d of ripening and in spring both after 90 and 180 d of ripening. The relative amounts of individual FFA after 180 d of ripening changed from winter to summer, both in pasteurized and in raw milk cheeses. In pasteurized milk cheeses made in winter the predominant FFA were C18:1 ( $2152 \pm 386 \mu\text{mol}\cdot\text{kg}^{-1}$ ), C4 ( $1954 \pm 354 \mu\text{mol}\cdot\text{kg}^{-1}$ ), C16 ( $1541 \pm 406 \mu\text{mol}\cdot\text{kg}^{-1}$ ) and C10 ( $1452 \pm 188 \mu\text{mol}\cdot\text{kg}^{-1}$ ). In contrast, in pasteurized milk cheeses made in summer C16 ( $2860 \pm 1305 \mu\text{mol}\cdot\text{kg}^{-1}$ ) and C18:1 ( $2677 \pm 973 \mu\text{mol}\cdot\text{kg}^{-1}$ ) were the major FFA. The percent FFA composition of both types of cheeses changed during ripening: short-chain (C4 to C10) FFA increased from approximately 25% to approximately 40 to 45%, whereas long chain ( $\geq$  C16:0) FFA decreased from approximately 55% to approximately 40 to 45% in winter and spring. However, in both types of cheese made in summer long chain FFA represented 52% and volatile FFA represented approximately 28 to 32% of the total after 180 ripening days. Milk pasteurization reduced the levels of acetic acid by 99% after 90 ripening days at the three times of the year studied.

**ovine milk cheese / pasteurized milk cheese / season / cheese ripening / lipolysis**

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**Résumé — Effet de la pasteurisation du lait sur la lipolyse au cours de l'affinage du fromage de brebis fabriqué à différentes saisons de l'année.** Le travail a été consacré à l'étude de la lipolyse dans des fromages fabriqués à partir de lait de brebis pasteurisé et cru, additionné d'un levain lactique, en hiver, au printemps et en été, jusqu'à 180 j d'affinage. La lipolyse est significativement plus faible dans les fromages pasteurisés que dans ceux élaborés avec du lait cru en hiver après 180 j d'affinage et au printemps après 90 et 180 j d'affinage. Le passage de l'hiver à l'été modifie la teneur relative des différents acides gras libres (AGL) après 180 j d'affinage dans les fromages pasteurisés et dans ceux élaborés avec du lait cru. Dans les fromages pasteurisés fabriqués pendant l'hiver, les acides gras les plus abondants sont C18:1 ( $2\,152 \pm 386 \mu\text{mol}\cdot\text{kg}^{-1}$ ), C4 ( $1\,954 \pm 354 \mu\text{mol}\cdot\text{kg}^{-1}$ ), C16 ( $1\,541 \pm 406 \mu\text{mol}\cdot\text{kg}^{-1}$ ) et C10 ( $1\,452 \pm 188 \mu\text{mol}\cdot\text{kg}^{-1}$ ). En revanche, dans les fromages pasteurisés fabriqués pendant l'été les plus abondants sont C16 ( $2\,860 \pm 1\,305 \mu\text{mol}\cdot\text{kg}^{-1}$ ) et C18:1 ( $2\,677 \pm 973 \mu\text{mol}\cdot\text{kg}^{-1}$ ). Les pourcentages d'acides gras libres sont modifiés dans les deux sortes de fromage au cours de l'affinage, tandis que la teneur en acides gras à chaîne courte (C4 à C10) augmente de 25 % à 40–45 %, la teneur en acides gras à chaîne longue (= C16) diminue de 55 % à 40–45 % en hiver comme au printemps. Par contre, dans les deux types de fromage fabriqués pendant l'été, les acides gras à chaîne longue représentent 52 % et les acides gras volatils 28–32 % du total après 180 j d'affinage. La pasteurisation du lait diminue les niveaux d'acide acétique de 99 % après 90 j d'affinage dans les trois saisons de l'année étudiées.

**fromage au lait de brebis / fromage au lait pasteurisé / saison / affinage / lipolyse**

## 1. INTRODUCTION

Pasteurization introduces several changes in the milk which can certainly modify the characteristics of the cheese made with it. In addition to the well-known reduction of mesophilic aerobic organisms, it inactivates or decreases the activity of milk lipoprotein lipase and other milk enzymes [2, 12, 19] while activating plasmin [15]. Many whey proteins are denatured favouring their interaction with  $\kappa$ -casein [24] which, in turn, does not readily interact with chymosin [25], thus increasing renneting time. Milk pasteurization also increases cheese yield [36]. Because most cheeses are manufactured with either pasteurized or raw milk, comparisons between pasteurized and raw milk cheeses of the same type are not frequently reported. Furthermore, the influence of milk pasteurization on lipolysis has not been well characterized. Milk pasteurization affects both proteolytic [23] and lipolytic processes [17, 29, 38] in different manners which apparently do not depend on the origin of the milk used. Reduced levels of lipolysis have been reported in both a bovine cheese such as Cheddar [29] and in an ovine cheese

like Manchego [17] when compared to the corresponding cheeses manufactured with raw milk. In contrast, in the Portuguese Serra cheese manufactured with ovine milk and plant coagulant, pasteurization increased the levels of lipolysis [38]. In any case, as a result of milk pasteurization, flavour is also affected, with that of cheeses made from pasteurized milk being less intense than the flavour of their raw milk counterparts [18].

Idiazabal cheese is a typical product of the Basque Country region of Northern Spain, made from raw milk of the 'latxa' breed of sheep according to the traditional method approved by its Denomination of Origin [4]. It is presently protected by the European Union [14]. Although its Denomination of Origin does not actually accept milk pasteurization, the present study was undertaken in an attempt to determine its possible authenticity through a scientific knowledge of the characteristics of this cheese made with pasteurized milk and relate sensory and analytical parameters. In the present paper we report the changes introduced by milk pasteurization on lipolysis.

## 2. MATERIALS AND METHODS

### 2.1. Cheese manufacture

Cheeses were made in the pilot plant of Queserías Araia (Araia, Alava, Spain). The industrial ewe's milk was from several local, commercial flocks of *latxa* sheep. The lactation period for an individual flock extends approximately 5 months between November and July, depending on the altitude where the flock is located, with flocks on lower elevations starting lactation earlier. Industrial cheesemaking (using mixed milk from different flocks) extends from December until the end of June. Thus, industrial milk in January contains a large percentage of milk from flocks in their early lactation stage, while industrial milk in June corresponds to the late lactation period of most flocks. In April milk could be a mixture (of unknown composition) of milk from late, mid- and early lactation period flocks. Milk had been refrigerated at 4 to 6 °C for up to 48 h prior to cheesemaking. Cheeses were made according to the traditional method for the industrial production of Idiazabal cheese approved by its Denomination of Origin [4], using commercial calf rennet (Ch. Hansen, Madrid, Spain), in 200-L vats. A starter culture (90% citrate-positive *Lactococcus lactis* and 10% *Lactobacillus casei* ssp. *casei*), isolated from Idiazabal [34] was used both for raw milk and pasteurized milk cheeses. Milk was pasteurized at 72 °C for 15 s in the factory upon arrival. Cheeses of both types (1.0 to 1.2 kg and 13 cm diameter) were made in three consecutive weeks in winter (last week in January and first two weeks in February), spring (last three weeks in April) and summer (last three weeks in June) (total of 9 batches with 2 vats in each batch: raw and pasteurized milk). From each vat, two different cheeses were taken for analysis after 1, 90 and 180 d of ripening, with the entire cheeses used for analysis on each sampling day. In addition to these sampling days, cheeses from each experimental unit of batches 2 (first week in February),

5 (third week in April) and 8 (third week in June) were also taken after 15, 30, 60 and 120 d of ripening. Cheese samples were wrapped in plastic film and aluminum foil and frozen at -80 °C until analyzed.

### 2.2. Free fatty acid analysis

Gas-liquid chromatographic analysis of each cheese sample was made in duplicate. FFA were analyzed underivatized by gas-liquid chromatography as described previously [11]. Briefly, cheese (1.0 g) was ground and extracted from an acidic medium with diethyl ether:heptane (1:1, v/v) after addition of an internal standard mixture consisting of pentanoic, nonanoic and heptadecanoic acids (1.0 mg each acid per mL diethyl ether:heptane). Triacylglycerols were separated from the FFA on aminopropyl-bonded phase columns (500 mg, Waters Corporation, Milford, MA, USA; previously equilibrated with heptane) by elution with chloroform:isopropanol (2:1, v/v). FFA were eluted with 5.0 mL diethyl ether containing 20 mL formic acid per L. This fraction containing underivatized FFA was injected directly into the gas chromatograph (Hewlett Packard, model 5890, series II, equipped with a flame ionization detector) and FFA were separated on a fused silica capillary column (25 m × 0.32 mm) coated with free fatty acid phase (cross-linked polyethylene glycol, 0.52 µm layer thickness). The carrier gas (helium) flow rate was 2 mL·min<sup>-1</sup>, and the temperature was raised from 65 °C to 240 °C at 10 °C·min<sup>-1</sup>, then held at 240 °C for 20 min. All solvents (Merck, Darmstadt, Germany) were of the highest grade available and were not redistilled before use.

### 2.3. Microbiological analyses

During cheese ripening total log counts of microorganisms were determined in duplicate after 90 and 180 d. Mesophiles were determined as described [1]. The method of Broome et al. [9] was used to determine the

total counts of lactic acid bacteria. Yeasts were determined as described by Caballero et al. [10]. Pseudomonas and micrococci were determined with the methods of Blazevic et al. [6] and García et al. [16], respectively. The method of Isenberg et al. [22] was used to determine the total counts of enterococci.

#### 2.4. Statistical analysis

The BMPD statistical package [7] was used for statistical treatment of the results. Three-way split-plot analysis of variance (ANOVA) was done to establish the presence or absence of significant difference in the FFA contents of the cheese, considering time of the year and pasteurization as factors and ripening time as sub-plot factor [20, 28]. Eta-square is referred to as size effect. Student's t-tests were done to determine the presence of significant differences in FFA contents on different days of ripening (1, 90 and 180) between the two types of cheeses (raw and pasteurized ones) made at different times of the year (winter, spring and summer). Simple linear regression analysis was done to fit the total FFA content with the ripening time (for batches 2, 5 and 8).

### 3. RESULTS AND DISCUSSION

The influence of the factors 'milk pasteurization', 'season of the year' and 'ripening time' on the concentration of individual FFA, short-, medium- and long-chain FFA and total lipolysis was analyzed by a three-way ANOVA (Tab. I). Milk pasteurization significantly affected ( $P = 0.01$ ) the levels of short-chain (C4-C10) FFA as well as their total amount, whereas season of the year and ripening time significantly affected all variables studied. The size effect of 'ripening time' was largest for all variables except for C2 which was primarily affected (largest size effect value) by 'milk pasteur-

ization'. The size of the effect 'ripening time' was particularly large for short-chain FFA and the long-chain FFA stearic, oleic and linoleic acids. There was a significant interaction ( $P = 0.001$ ) between 'milk pasteurization' and 'season of the year' which affected the concentration of all variables studied except that of C4, indicating that the effect of pasteurization was not the same throughout the cheesemaking season. The interaction between 'season of the year' and 'ripening time' significantly affected ( $P = 0.05$ ) the concentrations of all variables studied. In contrast, the interaction between 'milk pasteurization' and 'ripening time' was significant primarily for the concentration of short-chain FFA and total lipolysis. Short-chain FFA, both individually (except C4) and collectively, were the only variables significantly affected by the three factors simultaneously.

As observed in Table I, lipolysis (as total FFA) was primarily affected by 'milk pasteurization' and 'season of the year'. The value obtained for total lipolysis in Idiazabal cheese manufactured with pasteurized milk was approximately 40% lower in cheeses manufactured in spring than in those manufactured in winter, and between 50 and 60% lower than those manufactured in summer after 90 and 180 ripening days (Tab. II). In cheeses manufactured with raw milk, lipolysis in spring was between 30 and 40% lower than in winter and summer. The levels of lipolysis in pasteurized milk cheeses were significantly lower than those in raw milk cheeses after 180 ripening days in winter (about 30% lower) and 90 and 180 ripening days in spring (about 20% lower; Tab. II). In summer, although the mean lipolysis values on each ripening day were higher for pasteurized milk cheeses than for raw milk cheeses, these differences were not statistically significant due, most likely, to the large standard deviations obtained for pasteurized milk cheeses.

The physico-chemical composition of the cheeses made with raw milk was not

**Table I.** Significance levels and size effects of the three-way analysis of variance for the effects “milk pasteurization” (P), “season of the year” (S) and “ripening time” (T).

**Tableau I.** Les niveaux de signification de l’analyse de variance à trois facteurs pour les effets « pasteurisation du lait » (P), « saison » (S) et « durée d’affinage » (T).

	FFA		P S		T		PS		PT		ST		PST	
	sig	size effect												
C2	***	0.604	***	0.080	***	0.475	**	0.062	***	0.160	*	0.062	***	0.091
C4	***	0.062	***	0.075	***	0.612	NS	0.016	NS	0.026	*	0.056	NS	0.037
C6	***	0.058	***	0.362	***	0.849	***	0.132	**	0.055	***	0.253	**	0.068
C8	**	0.045	***	0.485	***	0.851	***	0.122	NS	0.024	***	0.296	**	0.069
C10	***	0.061	***	0.361	***	0.683	***	0.125	**	0.057	***	0.279	***	0.110
C12	NS	0.000	***	0.095	***	0.508	***	0.080	NS	0.002	**	0.071	NS	0.036
C14	NS	0.011	***	0.347	***	0.562	***	0.138	NS	0.007	***	0.180	NS	0.047
C16	NS	0.011	***	0.448	***	0.627	***	0.151	NS	0.005	***	0.205	NS	0.041
C16:1	*	0.027	***	0.304	***	0.559	***	0.142	NS	0.017	***	0.127	NS	0.049
C18	*	0.026	***	0.600	***	0.782	***	0.127	NS	0.012	***	0.273	NS	0.021
C18:1	*	0.034	***	0.521	***	0.808	***	0.098	NS	0.018	***	0.244	NS	0.017
C18:2	NS	0.010	***	0.535	***	0.785	***	0.107	NS	0.004	***	0.260	NS	0.024
C18:3	NS	0.003	***	0.343	***	0.626	***	0.099	NS	0.004	***	0.142	NS	0.022
Short (C4–C10)	***	0.100	***	0.382	***	0.810	***	0.091	**	0.056	***	0.278	***	0.101
Medium (C12–C14)	NS	0.001	***	0.269	***	0.580	***	0.128	NS	0.004	***	0.145	NS	0.046
Long (> C16)	NS	0.006	***	0.477	***	0.736	***	0.130	NS	0.003	***	0.215	NS	0.029
Total	***	0.376	***	0.196	***	0.790	***	0.081	**	0.063	**	0.087	NS	0.046

\*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; NS: not significant.

significantly different from that of cheeses made with pasteurized milk after 90 and 180 ripening days. The average value of the pH for both types of cheeses in all seasons studied was  $5.1 \pm 0.1$ , water activity was  $0.955 \pm 0.005$ , the percent dry matter was  $66.6 \pm 0.5$  and the percent total nitrogen per dry matter was  $5.7 \pm 0.1$  [27]. McSweeney et al. [29] did not observe changes in the physico-chemical composition of raw and pasteurized milk Cheddar cheeses. In contrast to the physico-chemical composition, pasteurized milk cheeses made in winter and spring had (averages on days 90 and 180 of ripening) between 15 and 20% lower total counts of mesophiles ( $7.20 \pm 0.19$  cfu·g<sup>-1</sup> cheese), lactic acid bacteria ( $7.18 \pm 0.14$  cfu·g<sup>-1</sup>) and enterococci ( $6.60 \pm 0.92$  cfu·g<sup>-1</sup>) than cheeses made with raw milk, whereas in summer the reduction in these groups of microorganisms was only between 5 and 10%. In pasteurized milk cheeses yeasts were reduced by about 70% ( $0.69 \pm 0.36$  cfu·g<sup>-1</sup>), micrococci by 55% (winter and spring:  $1.84 \pm 0.39$  cfu·g<sup>-1</sup>) or 45% (summer:  $1.97 \pm 1.13$  cfu·g<sup>-1</sup>) and pseudomonas by 30% ( $2.05 \pm 1.73$  cfu·g<sup>-1</sup>). Milk pasteurization in summer appeared to be less effective in reducing the total counts of the above-mentioned microorganisms than in winter and spring. Beuvier et al. [5] reported reductions in the total counts of enterococci, mesophiles, micrococci and yeasts in Swiss-type cheese made with pasteurized milk with respect to the counts observed in cheese made with raw milk. Likewise, in their review article, Martley and coworkers [26] found lower total counts of mesophiles and lactic acid bacteria in various types of cheeses made with pasteurized milk than in the same cheeses made with raw milk.

The effect of milk pasteurization on lipolysis levels appears to vary widely with the type of cheese. Both McSweeney et al. [29] and Gaya et al. [17] report decreases in the levels of lipolysis between 38 and 50% for Cheddar and Manchego cheeses, respectively, when compared to their corresponding raw

**Table II.** Total amounts of FFA ( $\mu\text{mol}\cdot\text{kg}^{-1}$  cheese) accumulated during ripening of cheeses manufactured in winter, spring and summer with raw and pasteurized milk.

**Tableau II.** Quantités totales d'acides gras ( $\mu\text{mol}\cdot\text{kg}^{-1}$ ) accumulés pendant l'affinage des fromages fabriqués en hiver, au printemps et en été avec du lait cru et pasteurisé.

Ripening day	Raw	Pasteurized
<b>Winter</b>		
1	2 272 ± 1 221 <sup>a</sup>	1 535 ± 218 <sup>a</sup>
90	8 820 ± 1 427 <sup>a</sup>	7 298 ± 1 688 <sup>a</sup>
180	15 543 ± 1 949 <sup>a</sup>	10 812 ± 2 251 <sup>b</sup>
<b>Spring</b>		
1	1 112 ± 78 <sup>a</sup>	1 018 ± 152 <sup>a</sup>
90	5 410 ± 927 <sup>a</sup>	4 237 ± 610 <sup>b</sup>
180	8 298 ± 425 <sup>a</sup>	6 526 ± 1 034 <sup>b</sup>
<b>Summer</b>		
1	1 352 ± 401 <sup>a</sup>	1 855 ± 803 <sup>a</sup>
90	8 668 ± 2 237 <sup>a</sup>	10 341 ± 4657 <sup>a</sup>
180	11 436 ± 1 532 <sup>a</sup>	13 827 ± 5 783 <sup>a</sup>

Amounts represent the mean ± standard deviation of the 12 values obtained for the three batches made at each time of the year on each ripening day. Different superscripts within each time of the year and for each ripening day represent statistically significant differences ( $P < 0.05$ ) between raw and pasteurized milk cheeses.

Les valeurs représentent les moyennes ± écart-type de 12 résultats obtenus pour les trois lots fabriqués à chaque saison de l'année et pour chaque période d'affinage. La présence de lettres différentes représente l'existence de différences statistiquement significatives ( $p < 0,05$ ) entre les fromages fabriqués à partir de lait cru et pasteurisé.

milk counterparts, as reported herein. In contrast, Sousa and Malcata [38] report higher levels of lipolysis in cheeses made with pasteurized sheep's milk than in those made with raw milk and attribute the differences to the higher percentage of fat retained in the pasteurized milk curd [21]. In our case both types of cheeses made in winter and spring had approximately 53% fat, whereas those made in summer had approximately 56% [27], indicating that pasteurization did not significantly affect the level of fat in cheese.

**Table III.** Rate of total FFA accumulation ( $\mu\text{mol FFA}\cdot\text{kg}^{-1}\text{ cheese}\cdot\text{d}^{-1}$ ) and correlation coefficient ( $r$ ) for batches 2 (winter), 5 (spring) and 8 (summer).

**Tableau III.** Accumulation d'acides gras libres totaux ( $\mu\text{mol AGL}\cdot\text{kg}^{-1}\text{ fromage}\cdot\text{j}^{-1}$ ) et coefficient de corrélation ( $r$ ) pour les différents lots 2 (hiver), 5 (printemps) et 8 (été).

Type of Cheese	Winter	Spring	Summer
Raw milk	67.9 ( $r = 0.997$ )	38.0 ( $r = 0.998$ )	51.0 ( $r = 0.956$ )
Pasteurized milk	40.5 ( $r = 0.997$ )	22.7 ( $r = 0.984$ )	62.1 ( $r = 0.986$ )

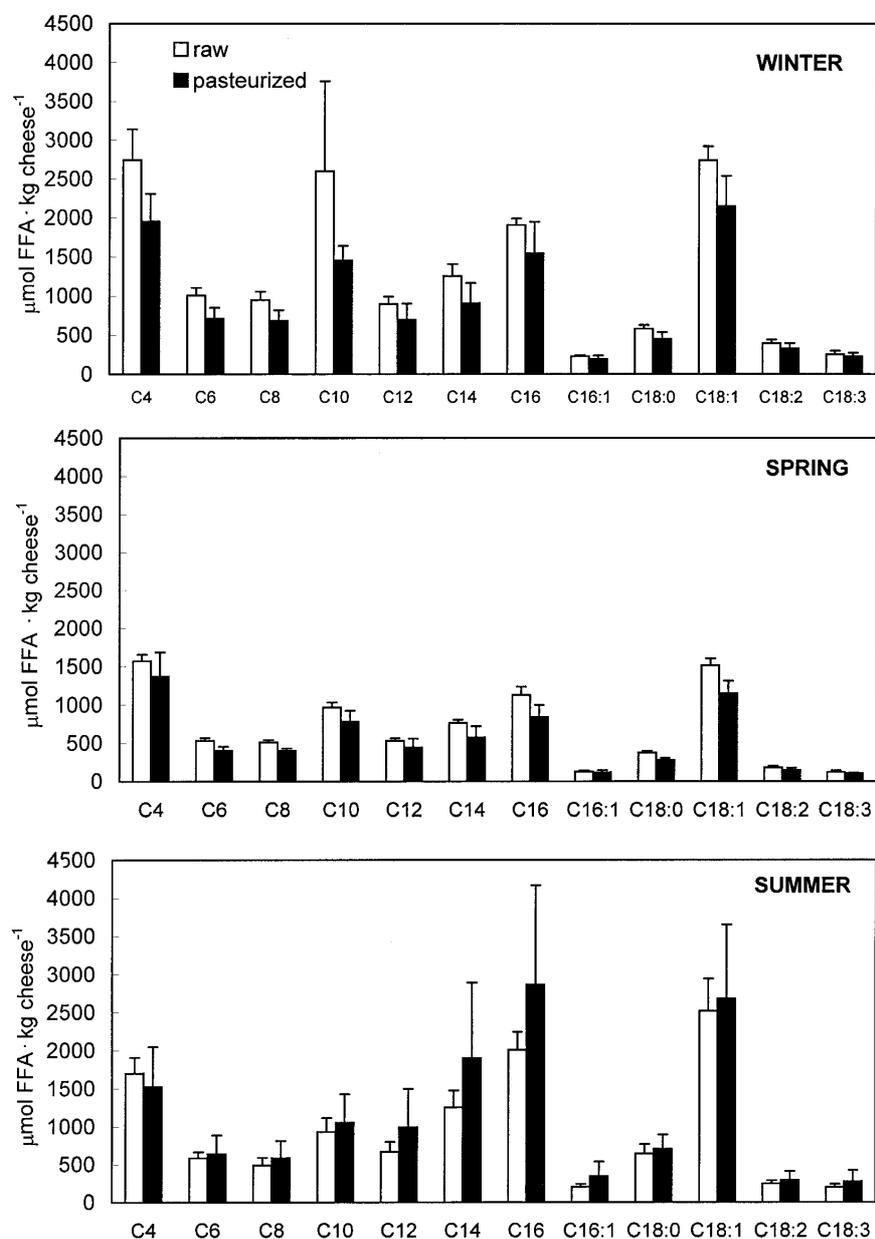
Ovine milk has been reported to have a higher fat content towards the end of the lactation period [3, 30, 32], a fact that would agree with the higher fat content of summer cheeses. Ovine milk lipoprotein lipase activity exhibited its highest levels of activity at the beginning of lactation and it decreased substantially as the lactation period progressed [12], with significantly lower values obtained from milk samples collected in summer, when most flocks are at the end of their lactation period. Likewise, pasteurization has been shown to reduce sheep's milk lipoprotein lipase activity by 75–90% [12].

Although lipolysis was assessed routinely after 1, 90 and 180 ripening days, batches 2 (winter), 5 (spring) and 8 (summer) were monitored also after 45, 60 and 120 ripening days to study the rate of FFA accumulation. In all cases, the values obtained fitted a linear regression curve for the entire ripening period studied (Tab. III). In pasteurized milk cheeses, FFA accumulated at a lower rate than in raw milk cheeses in winter and spring, consistent with the lower levels of lipolysis presented.

The levels of individual FFA were significantly affected by the factors 'season of the year' and 'ripening time', whereas 'milk pasteurization' significantly affected the levels of short-chain FFA and, to a lesser extent, those of palmitoleic, stearic and oleic acids (Tab. I). Figure 1 depicts the levels of individual FFA in raw and pasteurized cheeses made in winter, spring and summer after 180 ripening days. In pasteurized milk cheeses made in winter, the predominant

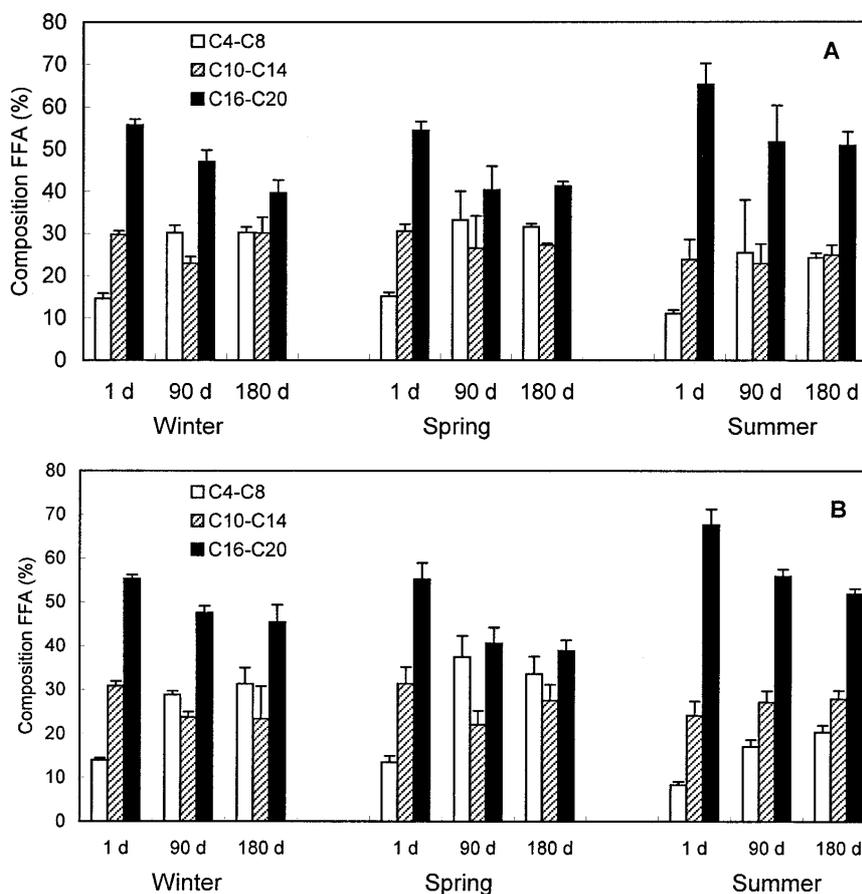
FFA were C18:1 ( $2\,152 \pm 386 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese), C4 ( $1\,954 \pm 354 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese), C16 ( $1\,541 \pm 406 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) and C10 ( $1\,452 \pm 188 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese). In spring, when total lipolysis levels were significantly lower than in winter or summer, the major FFA in pasteurized milk cheeses were C4 ( $1\,368 \pm 318 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) and C18:1 ( $1\,148 \pm 161 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese). However, the FFA profile of summer cheeses made with pasteurized milk was very different, with a clear predominance of the long chain FFA C16 ( $2\,860 \pm 1\,305 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) and C18:1 ( $2\,677 \pm 973 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) over C14 ( $1\,901 \pm 986 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) and C4 ( $1\,526 \pm 524 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese). The two-fold increase in C14 (with respect to its value in cheeses made in winter), in particular, was quite remarkable.

The profiles of FFA in raw milk cheeses after 180 ripening days were comparable to those of pasteurized milk cheeses for the three seasons of the year, with significantly higher levels of C4 ( $2\,742 \pm 400 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese), C18:1 ( $2\,739 \pm 181 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) and C10 ( $2\,597 \pm 1\,162 \mu\text{mol}\cdot\text{kg}^{-1}$  cheese) in winter cheeses (Fig. 1). The main differences found between these two types of cheese was a lower relative amount of C14 in summer cheeses and an almost double amount of C10 in winter cheeses made with raw milk. The dramatic reduction in the amount of C10 from winter to summer cheeses was comparable to that reported by Chávarri et al. [13] for raw milk cheeses with no starter culture added.



**Figure 1.** Individual FFA ( $\mu\text{mol}\cdot\text{kg}^{-1}$  cheese) accumulated during ripening of cheeses made with raw ( $\square$ ) and pasteurized ( $\blacksquare$ ) milk after 180 ripening days in winter, spring and summer. Student's *t*-test was used to determine whether or not the differences between the amounts of individual FFAs were significant.

**Figure 1.** Acides gras libres ( $\mu\text{mol}\cdot\text{kg}^{-1}$  fromage) accumulés pendant l'affinage des fromages fabriqués à partir de lait cru ( $\square$ ) et pasteurisé ( $\blacksquare$ ) après 180 j d'affinage, en hiver, au printemps et en été. L'analyse statistique des résultats a été faite en employant le test *t* de Student.



**Figure 2.** Changes in the percent composition of short (C4-C10), medium (C12-C14) and long ( $\geq$  C16) FFA during ripening of cheeses manufactured with pasteurized (A) and raw (B) milk.

**Figure 2.** Modifications des pourcentages d'acides gras à chaîne courte (C4-C10), moyenne (C12-C14) et longue ( $\geq$  C16) pendant l'affinage des fromages fabriqués à partir de lait pasteurisé (A) et cru (B).

Although the amounts of all FFA increased during ripening of the raw and pasteurized milk cheeses, their relative increases were different (Fig. 2). As a result, the percent composition of short (C4 to C10), medium (C12 and C14) and long ( $\geq$  C16) FFA changed during ripening, significantly affecting the short-chain FFA. In both types of cheese, as ripening progressed, the percentage of short chain FFA was observed to increase (from approximately

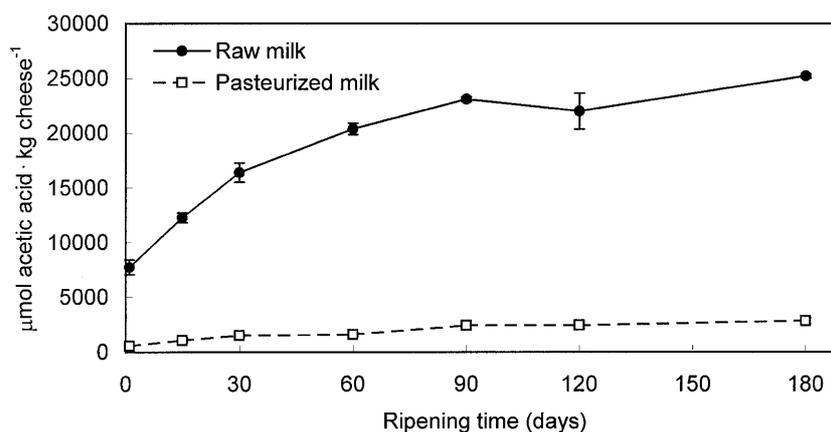
25% to approximately 40 to 45%), whereas that of long chain FFA decreased (from approximately 55% to 40 to 45%), particularly in winter and spring. The percent composition of winter and spring cheeses was significantly different from that of summer cheeses in which, after 180 ripening days, long chain FFA represented approximately 50% and short-chain FFA represented approximately 25 to 30%. These results most likely reflect the changes

observed in the composition of ovine milk fat as lactation progressed [33]: in winter and spring the percentage of short and medium chain fatty acids (between 4 and 12 carbon atoms) was significantly higher than in summer, whereas in summer that of long chain and unsaturated fatty acids was significantly higher than in the other two seasons. Alternatively, these results could also be due to the presence of a microbial lipolytic activity (or activities) with a different substrate specificity.

The concentration of acetic acid was significantly affected by all the three factors studied with 'milk pasteurization' having the largest size effect on this variable (Tab. I). Because acetic acid could result from various metabolic routes and it has not been reported in the fatty acid composition of the triacylglycerol fraction [31, 37], the accumulation of this acid during ripening is presented separately (Fig. 3). Pasteurization of the milk killed the acetic acid producing species present in the raw milk cheeses (as yet unidentified). Similar results for pasteurized milk cheeses have been reported for Cheddar cheese [35] and for Swiss-type cheeses [5, 8]. Beuvier et al. found that adding the retentate from microfiltered raw

milk to pasteurized milk caused the same rate of production of acetic acid during ripening as was observed in the raw milk cheeses.

Sensory analysis of these cheeses [27] indicated that raw milk cheeses had a significantly higher total score (as determined by an expert panel) than pasteurized milk cheeses in winter and spring. In fact, pasteurized milk cheeses made in spring had the lowest total scores of all cheeses, being rated as 'rather mild'. However, in summer, pasteurized milk cheeses had significantly higher total scores than raw milk cheeses after 90 d of ripening and were considered as 'better'. No differences were observed between the two types of cheeses after 180 ripening days in summer. It is interesting to note that pasteurized milk cheeses made in spring had the lowest levels of lipolysis. In cheeses manufactured with raw milk and no starter culture added [13] it was also found that both the highest levels of lipolysis and highest total scores in the sensory analysis corresponded to winter cheeses, suggesting that perhaps a minimum level of lipolysis is necessary for Idiazabal cheese to develop its characteristic flavour.



**Figure 3.** Accumulation of acetic acid during ripening of raw (●) and pasteurized (□) milk cheeses made in winter.

**Figure 3.** Accumulation d'acide acétique pendant l'affinage des fromages fabriqués en hiver à partir de lait cru (●) et pasteurisé (□).

#### 4. CONCLUSION

The three effects studied, 'milk pasteurization', 'season of the year' and 'ripening time', significantly affected all the FFA accumulated during ripening. Milk pasteurization decreased the total level of lipolysis (as compared to raw milk cheeses) in cheeses made in winter and spring, but it did not alter the proportions of short, medium and long chain FFA at these times of the year. Yet, results of the sensory analysis indicated that pasteurized cheese was different from raw cheese and suggested that higher levels of lipolysis could be important for the development of the characteristic flavour of Idiazabal cheese.

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Abbreviations used: FFA: free fatty acids; C4: butyric acid; C6: caproic acid; C8: caprylic acid; C10: capric acid; C12: lauric acid; C14: myristic acid; C16: palmitic acid; C16:1: palmitoleic acid; C18: stearic acid; C18:1: oleic acid (it includes any *trans* isomers that could be present); C18:2: linoleic acid; C18:3:  $\gamma$ -linolenic acid.