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Changes in lipid fractions and sensory properties of Idiazabal cheese induced by lipase addition

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This work studied the addition of an adequate lipase to enhance lipolysis reactions and the development of piquant flavour and sharp odour in Idiazabal cheese, as an alternative to the use of lamb rennet paste. Cheeses were manufactured from bulk raw ewes' milk in 50 l vats with commercial bovine rennet and 80 lipase units of pregastric or 180 lipase units of fungal lipase and ripened for 180 days. A higher lipolytic activity was induced by lipase addition promoting strong changes in odour and flavour attributes. Both fungal and pregastric lipases increased the content of total free fatty acids (FFA), but the fungal lipase released mainly medium- and long-chain FFA. In contrast, the pregastric lipase preferably released short-chain FFA. Diglyceride (DG) content was considerably higher in cheeses made with added pregastric lipase compared with those made with fungal lipase or with no lipase. Monoglycerides (MG) were detected only in cheeses made with either lipase added, reaching comparable concentrations after ripening for 180 days. The cheeses made with pregastric lipase had the highest scores for odour and flavour intensity, and sharp and rennet odours, desirable attributes for the Idiazabal cheese made with lamb rennet paste. None of the texture attributes were significantly influenced by the concentrations of MG and DG in the cheeses made with either lipase. Thus, the pregastric lipase was more appropriate than the fungal lipase to develop a more traditionally-flavoured Idiazabal cheese.

Keywords: Lipase, cheese ripening, free fatty acid, partial glyceride, cholesterol, sensory, flavour, odour, Idiazabal cheese.

Enzymatic hydrolysis of triglycerides to free fatty acids (FFA), monoglycerides (MG), or diglycerides (DG) is essential for flavour development in many cheese varieties (Foda et al. 1974; Woo et al. 1984; Farkye & Fox, 1990; Gripon, 1993). Most papers have been focused on FFA (Fox et al. 1993; Macedo et al. 1996; Chávarri et al. 1999; Buffa et al. 2001) but very few studies have dealt with changes in partial glycerides during cheese ripening. DG have been shown to be present in higher concentrations than MG (Vujicic & deMan, 1967; Precht & Abd El Salam, 1985; Contarini & Topino, 1995), and 1,2-DG are usually present in higher concentration than 1,3-DG (Conte et al. 1987; Koprivnjak et al. 1997b). Contarini & Topino (1995) indicated no specific trend changes in concentration of either MG or DG during ripening of Gorgonzola cheese, but Conte et al. (1997) reported an increase in diglyceride concentration with ripening in the same type of cheese. Other authors also found that the content of MG and DG increased as ripening progressed in Domiati cheese (Precht & Abd El-Salam, 1985). There is very little information about the contribution of partial glycerides to the sensory properties of cheese. Some authors have indicated that the emulsifying capacity of MG and DG may have an impact on the properties of the paste (Adda et al. 1982; Catalano et al. 1985). DeMan (1966) reported that the MG in a

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rancid cheese were characterized by high contents of short-chain fatty acids. No information on the effect of lipase addition on the cholesterol concentration in cheese has been found in the literature.

Lipase addition has been often used to develop the characteristic flavour of some cheese varieties (Nelson et al. 1977; Law & Goodenough, 1991; Aydemir et al. 2001; Hernández et al. 2001). It is well known that animal pregastric lipase specifically releases short-chain fatty acids, particularly, butyric acid (Arnold et al. 1975; Nelson et al. 1977; Moskowitz & Noelck, 1987). Different microbial lipases, especially fungal lipases, have been used for cheesemaking, although their effect on the sensory characteristics of the cheese was not always comparable with that of the animal pregastric lipases (Jolly & Kosikowski, 1978; De Felice et al. 1991).

Idiazabal cheese is a traditional raw ewes' milk cheese manufactured in the Basque Region of Northern Spain and protected by the European Union (Diario Oficial de las Comunidades Europeas, 1996). It is usually commercialized at between 3 and 6 months of ripening. Lipolysis is essential for the development of the piquant flavour and sharp odour of this raw ewes' cheese (Nájera et al. 1994; Chávarri et al. 1999, 2000; Larráyoz et al. 1999; Hernández et al. 2001), highly appreciated by taste panellists and consumers (Bárcenas et al. 2001a, b). Traditionally, the piquant flavour and sharp odour of Idiazabal cheese have been associated with the use of artisanally produced lamb rennet pastes (Virto et al. 2003) in which lipolytic activity has been detected (Bustamante et al. 2000). Reglamento de la Denominación de Origen Idiazabal allows the use of any animal rennet as coagulant (Boletín Oficial del Estado, 1993). However, owing to the lack of a standardized lamb rennet paste preparation procedure, cheese manufacturers have been gradually avoiding its use in recent years in favour of commercial bovine rennets in which no lipase activity is detectable. Thus, this piquant flavour and sharp odour are gradually being lost. The addition of an adequate lipase to enhance lipolysis reactions and the development of piquant flavour and sharp odour in cheeses made with commercial bovine rennet could be an alternative to the use of lamb rennet paste. Therefore, the aim of the present work was to investigate the changes induced by the addition of two different commercial lipases on lipid fractions and sensory properties of Idiazabal cheese made with commercial bovine rennet, to aid in the selection of the more adequate lipase.

Materials and Methods

Cheese manufacture

Cheeses were made in 50 l vats in the pilot plant of Queserías Araia (Araia, Alava, Spain) from bulk raw ewes' milk according to the method approved by the Denominación de Origen Idiazabal (Boletín Oficial del Estado, 1993). The bulk raw ewes' milk was a mixture of milk collected from several farmhouses on the same day and kept refrigerated at 4 °C for up to 48 h. Three batches of cheese were made using commercial bovine rennet (Marschall powder, Rhône-Poulenc Texel, Dangé St. Romain, France) on each day: to one vat no lipase was added; and to another vat 80 lipase units of lamb pregastric lipase Capalase L (SKW Biosystems, Barcelona, Spain) was added; to a third vat 180 lipase units of fungal lipase Palatase 20 000L from *Mucor miehei* (Novo Nordisk Bioindustrial, Madrid, Spain) was added. Lipase activity of the commercial preparations was assayed with the same assay using tributyrin (Sigma–Aldrich, Alcobendas, Spain) as substrate as described by Bustamante et al. (2000) to both ascertain and ensure the number of activity units in each case. A homofermentative starter culture (EZAL, Rhône-Poulenc Texel) was added to all vats. Rennet and lipase were added to the vat after heating the milk at 30 °C. After moulding and pressing, cheeses were brined for 24 h at 10–12 °C in a saturated solution of salt. Cheeses were ripened at 8–10 °C and a relative humidity of around 85% for 180 d. Cheeses were made in January and duplicate sets of fabrications were made in consecutive weeks. After ripening for 90 and 180 d two whole cheeses from each vat were taken for analysis of the lipid fraction. Cheese samples were cut into triangular sections weighing around 100 g each, the sections were wrapped in plastic film and aluminium foil and frozen at −35 °C until analysed. Before analysis of the lipid fraction, cheese sections were defrosted for 24 h at 7–8 °C. Then, the rind (approx. 0·5 cm) was removed from the cheese portions, which were comminuted to a uniform grain size at 8–10 °C in a model A10 grinder (Janke & Funkel, Staufen, Germany). FFA, partial glycerides and free cholesterol analyses were made in duplicate.

FFA analysis

FFA were analysed underivatized by gas-liquid chromatography in a model 5890 series II gas chromatograph (Hewlett Packard, Las Rozas, Spain) equipped with a flame ionization detector (FID), on a fused silica capillary column (25 m × 0·32 mm) coated with FFA phase (cross-linked polyethylene glycol, 0·52 μm layer thickness), essentially as described previously (Chávarri et al. 1997). FFA were quantified on the basis of their chromatographic peak areas using pentanoic, nonanoic and heptadecanoic acids (99% purity, Sigma–Aldrich) as internal standards added to the cheese sample. The amounts of FFA in the cheese samples (μmol/kg) are reported as total FFA, short-chain (C_4 to C_{10}), medium chain (C_{11} to C_{14}) and long-chain ($>C_{15}$) FFA.

Partial glycerides and free cholesterol analysis

A modified method of Caboni et al. (1990) was used to extract partial glycerides and free cholesterol. Briefly, ground cheese was dispersed in chloroform : hydrochloric

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acid (100 : 3; Panreac, Barcelona, Spain) at 0 °C in an ice bath, after adding naringenin (95% purity, Sigma–Aldrich) dissolved in 2-propanol (Panreac) as internal standard. The organic phase was separated by centrifugation and passed through a column containing sodium sulphate to remove the residual water. After this, the solvent was driven off under vacuum and the lipid residue was redissolved in n-hexane (Merck, Darmstadt, Germany). The lipid extract was purified using LC-Diol solid-phase extraction (SPE) cartridges (Supelco, Bellefonte, USA) as described (Pérez-Camino et al. 1996) to obtain a fraction containing partial glycerides and free cholesterol. This fraction was finally dissolved in n-hexane : 2-propanol (9 : 1; Merck) and analysed by HPLC. The equipment comprised two 422 and 422S pumps (Kontron, Milan, Italy), a 7161 injector (Rheodyne, Cotati, USA), and a Sedex 45 evaporative light-scattering detector (Sedere, Alfortville, France). The HPLC analysis was as described by Liu et al. (1993) with some modifications using a stainless steel column (15 cm × 4·6 mm) packed with 5 µm Kromasil Silica 60. A non-linear gradient of n-hexane in n-hexane : 2-propanol : ethyl acetate : 10% formic acid in 2-propanol (80 : 10 : 10 : 1) was used as mobile-phase. All solvents used in the mobile-phase were HPLC grade (Romil, Cambridge, UK). The flow-rate was 2 ml/min and the column was held at 40 °C during the analysis. Partial glycerides and free cholesterol were quantified on the basis of their chromatographic peak areas using naringenin as internal standard. The results were expressed as mg/100 g for (1,2+2,3)-DG, 1,3-DG, total MG and free cholesterol. Mean response factors were used for each of the partial glyceride groups and free cholesterol, and the response factors were calculated from five replicate analyses of 1·0 mg/ml of the pure compounds 1,2-dimyristin, 1,3-dimyristin, 1,2-dipalmitin, 1,3-dipalmitin, 1,2-diolein, 1,3-diolein, 1-monopalmitin, 1-monoolein, 2-monopalmitin, and cholesterol (99% purity, Sigma–Aldrich) in n-hexane : 2-propanol (9 : 1; Merck).

Sensory analysis

One whole cheese from each vat was used for sensory analyses after 90 and 180 d ripening. Cheese sample preparation was as described by Bárcenas et al. (2001b). Samples were assessed by a team of ten assessors who had previously been selected and trained in the sensory characterization of ewes' milk cheeses (Bárcenas et al. 2000). The following sensory attributes were assessed using a seven-point scale; seven odour terms: intensity, sharp, milky, brine, rennet, buttery and toasty; ten flavour terms: intensity, butyric acid, nutty, buttery, acidic, sweet, salty, bitter, rennet and pungent; and seven texture terms: surface roughness, surface moisture, elasticity, firmness, friability, adhesiveness, graininess, solubility and humidity in mouth (Bárcenas et al. 1999). Sensory assessment was performed in standard individual tasting booths following the Spanish standard UNE 87-004 (AENOR, 1979).

Statistical analysis

The SPSS statistical software, version 11.0 (SPSS, Chicago, USA), was used for the statistical analysis. Two-way analysis of variance (ANOVA) was done to establish the presence or absence of significant differences in the lipid fractions and sensory attributes among the cheeses according to the factors 'type of lipase' (L) and 'ripening time' (T). When the interaction term (L × T) was significant ($P \leqslant 0{\cdot}05$), Student's *t*-test was used to evaluate the significance of differences between samples according to the factor 'ripening time' for each type of cheese. Also, one-way ANOVA was used to evaluate the differences among samples according to the factor 'type of lipase' for the same 'ripening time'. Pearson bivariate correlations were applied for lipid fractions and sensory attributes of the cheeses.

Results and Discussion

Lipid fraction composition

Table 1 shows the changes with ripening in the concentrations of total FFA, short-, medium- and long-chain FFA in cheeses made with either of the lipases and with no lipase. Significance levels for the effects 'type of lipase' and 'ripening time' in the concentrations of FFA are shown in Table 2. As expected, the concentration of total FFA was highest ($P \leqslant 0{\cdot}001$) in cheeses made with fungal lipase (Table 1). The amount of fungal lipase added was 2·25-fold higher than that of pregastric lipase because in a previous study, in which 80–90 lipase units of fungal lipase were added, no effect on the total FFA or in the sensory characteristics of the cheese was observed (Hernández et al. 2001). Other authors found that the development of a desired flavour in one specific type of cheese required a 5-fold greater addition of a similar fungal lipase based on lipolytic activity than of pregastric lipase (Peppler et al. 1976). Remarkable differences were found when FFA were grouped according to their chain length and their amounts were compared among the cheeses. The concentration of short-chain FFA was highest ($P \leqslant 0{\cdot}001$) in cheeses made with pregastric lipase throughout the ripening period. In contrast, the concentrations of medium- and long-chain FFA were highest ($P \leqslant 0{\cdot}001$) in cheeses made with fungal lipase (Table 1). In cheeses made with pregastric lipase, short-chain FFA comprised approximately 70% of the total, whereas they represented around 30% of the total FFA in cheeses made with no lipase or with fungal lipase. The latter cheeses exhibited the largest percentage (over 51%) of long-chain FFA. Virto et al. (2003) found that in Idiazabal cheeses made with artisanally-produced lamb rennet paste containing lipolytic activity, short-chain FFA comprised 70% of the total FFA, but in cheeses made with commercial bovine rennet only 35% of the total FFA were short-chain fatty acids, both after 90 and 180 d of ripening. Thus, the

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Table 1. Concentrations (μmol/kg) of total, short-, medium- and long-chain free fatty acids (FFA) in Idiazabal cheeses made with pregastric or fungal lipase and with no lipase, using commercial bovine rennet as coagulant

Values are means ± SD for $n = 8$

	90 d			180 d		
	Pregastric	Fungal	No lipase	Pregastric	Fungal	No lipase
Total FFA	26 313·81 ± 2691·66^{a}	33 471·33 ± 4086·33^{b}	8683·69 ± 842·36^{c}	34 065·50 ± 3358·46^{a}	44 130·96 ± 6315·01^{b}	10 991·86 ± 576·65^{c}
Short-chain FFA	19 154·75 ± 2844·22^{a}	8973·19 ± 909·15^{b}	2734·81 ± 168·60^{c}	23 710·89 ± 1984·46^{a}	13 955·72 ± 1494·20^{b}	3989·74 ± 265·30^{c}
Medium-chain FFA	2519·18 ± 328·85^{a}	6811·60 ± 866·97^{b}	1353·39 ± 176·61^{c}	3149·45 ± 333·76^{a}	8873·61 ± 1696·86^{b}	1592·13 ± 114·69^{c}
Long-chain FFA	4627·27 ± 442·22^{a}	17 634·84 ± 2329·18^{b}	4580·28 ± 540·21^{a}	5923·66 ± 263·22^{a}	21 323·00 ± 3147·37^{b}	5352·29 ± 500·90^{a}

Means within rows without a common superscript are significantly different ($P \leqslant 0{\cdot}05$) for the same ripening day

Table 2. Significance levels (P) for the factors 'type of lipase' (pregastric or fungal lipase) and 'ripening time' (90 or 180 d) in the concentrations of free fatty acids (FFA), diglycerides (DG), monoglycerides (MG) and free cholesterol in Idiazabal cheeses

	L	R	L x R
Short-chain FFA	***	***	**
Medium-chain FFA	***	***	**
Long-chain FFA	***	***	*
Total FFA	***	***	**
(1,2+2,3)-DG	***	***	***
1,3-DG	***	***	***
Total MG	***	***	***
(1,2+2,3)/1,3-DG	***	NS	**
Free cholesterol	***	NS	***

***$P \leqslant 0{\cdot}001$, **$P \leqslant 0{\cdot}010$, *$P \leqslant 0{\cdot}050$; NS, not significant; L, type of lipase; R, ripening time

FFA profile in cheeses made with pregastric lipase was comparable to that of cheeses made with lamb rennet paste, but quite different from the FFA profile of the other batches.

Table 3 shows the changes with ripening in the concentrations of partial glycerides. Significance levels for the effects 'type of lipase' and 'ripening time' in the concentrations of partial glycerides are shown in Table 2. The concentrations of (1,2+2,3)-DG and 1,3-DG in cheeses made with pregastric lipase were significantly higher ($P \leqslant 0{\cdot}001$) than in cheeses made with fungal lipase or with no lipase after 180 d ripening (Table 3). As other authors have reported (Koprivnjak et al. 1997a), the release of (1,2+2,3)-DG was greater than that of 1,3-DG during cheese ripening. The ratio (1,2+2,3)/1,3-DG was highest ($P \leqslant 0{\cdot}001$) in cheeses made with either of the lipases, but in cheeses made with pregastric lipase the ratio increased significantly ($P \leqslant 0{\cdot}05$) during ripening (Table 3). These results were in agreement with the activity of pregastric lipase on the *sn*-3 position of the triglyceride molecule (Christie, 1986; Ha & Lindsay, 1993). As indicated before, pregastric lipase released primarily short-chain FFA, which are located, to a large extent, at the *sn*-3 position of the glycerol molecule in milk fat, whereas fungal lipase from *Mucor miehei* released primarily long-chain FFA which are distributed into the three *sn*-positions of glycerol (Christie, 1986). Lipolysis observed in cheeses made with no lipase can be attributed to milk lipoprotein lipase and microbial lipases (Deeth & Fitz-Gerald, 1983). Psychrotrophic bacteria are the most common sources of microbial lipases in ripened hard cheeses (Martín-Hernández, 1991; Litopoulou-Tzanetaki & Vafopoulou-Mastrojannaki, 1995). However, it has also been reported that lipases of lactic acid bacteria, usually used as starter culture, exhibit certain activity to hydrolyze partial glycerides (Stadhouders & Veringa, 1973; Urbach, 1997). This observation could help to explain the decrease observed in the concentration of DG during the ripening of cheeses made with no lipase (Table 3).

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Table 3. Concentrations (mg/100 g) of diglycerides (DG), monoglycerides (MG) and free cholesterol in Idiazabal cheeses made with pregastric or fungal lipase and with no lipase, using commercial bovine rennet as coagulant

Values are means ± SD for $n = 8$

	90 d			180 d		
	Pregastric	Fungal	No lipase	Pregastric	Fungal	No lipase
(1,2+2,3)-DG	236·00 ± 20·28^{a}	199·00 ± 5·78^{b}	200·11 ± 14·31^{b}	369·75 ± 16·96^{a}	219·86 ± 20·60^{b}	155·50 ± 8·69^{c}
1,3-DG	133·40 ± 6·82^{a}	99·43 ± 2·32^{b}	133·14 ± 10·08^{a}	182·83 ± 22·36^{a}	109·57 ± 5·50^{b}	114·43 ± 9·11^{b}
Total MG	ND	40·43 ± 3·38	ND	45·75 ± 1·86^{a}	47·63 ± 4·21^{a}	ND
Free cholesterol	102·67 ± 2·93^{a}	117·43 ± 3·38^{b}	147·20 ± 15·72^{c}	127·00 ± 5·51^{ab}	132·43 ± 4·84^{b}	120·71 ± 6·68^{a}
(1,2+2,3)/1,3-DG	1·77 ± 0·19^{a}	2·00 ± 0·07^{b}	1·51 ± 0·14^{c}	2·05 ± 0·28^{a}	2·01 ± 0·21^{a}	1·34 ± 0·16^{b}

Means within rows without a common superscript are significantly different ($P \leqslant 0{\cdot}05$) for the same ripening day
ND: not detected

MG were only detected in cheeses made with fungal lipase after 90 d ripening and in cheeses made with both lipase after 180 d ripening (Table 3). At this latter stage, non-significant difference ($P > 0{\cdot}05$) was found for the concentration of total MG between cheeses with either lipase added. After 90 d ripening, the concentration of free cholesterol was highest ($P \leqslant 0{\cdot}001$) in cheeses made with commercial bovine rennet and no lipase (Table 3). Because lipolytic enzymes catalyze the hydrolysis of fatty acid cholesteryl esters and/or the esterification of free cholesterol with FFA (Ghandi, 1997), it is reasonable to suggest that the fungal or the pregastric lipases used might be involved in esterification reactions with cholesterol, causing the observed low content in free cholesterol in cheeses made with the lipases. After 180 d ripening, these lipases could preferentially catalyze the hydrolysis of cholesteryl esters, causing the observed increase in free cholesterol content (Table 3). However, other entities and/or physical factors apart from the lipases could also have been involved when such a small number of samples is under consideration.

Sensory analysis

The mean scores awarded by the assessors to each of the sensory attributes evaluated in the cheese samples are shown in Table 4. The statistical results showed that non-significant ($P > 0{\cdot}05$) interaction was found between factors 'type of lipase' and 'ripening time' for all the sensory attributes. Odour intensity, sharp and rennet odour scores were highest ($P \leqslant 0{\cdot}05$) in cheeses made with pregastric lipase, whereas milky and buttery odour scores were highest ($P \leqslant 0{\cdot}05$) in cheeses made with no lipase. In cheeses made with pregastric lipase the highest scores ($P \leqslant 0{\cdot}05$) for brine odour and flavour intensity were assessed (Table 4). Butyric acid flavour was higher ($P \leqslant 0{\cdot}05$) in cheeses made with lipase than in cheeses made with no lipase, but difference between cheeses made with fungal and pregastric lipase was non-significant ($P > 0{\cdot}05$). High scores for the attributes odour and flavour intensities, sharp odour, rennet odour and flavour, and pungent flavour are considered desirables for Idiazabal cheese (Urarte et al. 1998). As recently shown by Virto et al. (2003), Idiazabal cheeses made with lamb rennet paste had higher intensity scores for these attributes than cheeses made with commercial bovine rennet.

None of the texture attributes, toasty odour, buttery, nutty, acidic, sweet, salty, bitter, rennet and pungent flavours were significantly affected by the factor 'type of lipase' (Table 4). The factor 'ripening time' significantly ($P \leqslant 0{\cdot}05$) affected odour attributes, such as odour intensity and brine odour; flavour attributes, such as flavour intensity, butyric acid, nutty, buttery, bitter and pungent flavour; and texture attributes, such as surface roughness, elasticity, firmness and friability. Odour intensity, brine odour, flavour intensity, butyric acid, pungent, nutty and bitter flavours increased from 90 to 180 d ripening in the three types of cheeses. In contrast, buttery flavour decreased ($P \leqslant 0{\cdot}05$) as ripening progressed (Table 4). Cheeses ripened for 180 d were characterized by higher ($P \leqslant 0{\cdot}05$) scores for surface roughness, firmness and friability. Likewise, elasticity was significantly ($P \leqslant 0{\cdot}001$) lower in the longest ripened cheeses (Table 4).

Relationship between sensory attributes and lipid composition

Pearson bivariate correlations were applied between the concentrations of FFA in the cheese samples, and the scores for the odour and flavour attributes which were significantly affected by the factors 'type of lipase' and 'ripening time'. Butyric acid flavour was positively correlated ($P \leqslant 0{\cdot}05$) with the concentration of total FFA and short-chain FFA (Table 5). Flavour intensity was also positively correlated ($P \leqslant 0{\cdot}05$) with the concentration of total FFA, but non-significant differences ($P > 0{\cdot}05$) in flavour intensity were found between the cheeses with the highest and the lowest concentration of total FFA (Table 1). Positive bivariate correlations ($P \leqslant 0{\cdot}05$) were found between the concentration of short-chain FFA and odour intensity, sharp and rennet odours. Brine odour has been associated with odour intensity in ewe's cheeses (Bárcenas et al.

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Table 4. Mean values and SD of the odour, flavour and texture attributes evaluated in Idiazabal cheeses made with pregastric or fungal lipase and with no lipase, using commercial bovine rennet as coagulant

Values are means ± SD for $n = 20$

	90 d			180 d		
	Pregastric	Fungal	No lipase	Pregastric	Fungal	No lipase
Odour intensity	4·4 ± 1·2^{a}	3·8 ± 0·7^{b}	3·4 ± 1·0^{b}	4·9 ± 1·0^{a}	4·1 ± 0·9^{b}	3·7 ± 1·0^{b}
Sharp odour	4·3 ± 1·3^{a}	3·5 ± 1·3^{b}	3·0 ± 1·1^{b}	4·6 ± 1·3^{a}	3·9 ± 1·3^{b}	3·2 ± 1·4^{b}
Milky odour	2·0 ± 1·0^{a}	2·2 ± 1·0^{a}	2·6 ± 0·9^{b}	1·9 ± 0·8^{a}	2·3 ± 1·1^{a}	2·9 ± 1·1^{b}
Brine odour	2·5 ± 1·0^{a}	2·2 ± 0·8^{ab}	1·7 ± 0·8^{b}	3·1 ± 1·4^{a}	2·8 ± 1·0^{ab}	2·4 ± 0·9^{b}
Rennet odour	2·9 ± 1·0^{a}	2·1 ± 0·9^{b}	2·1 ± 1·0^{b}	3·0 ± 1·4^{a}	2·5 ± 0·8^{b}	2·2 ± 0·9^{b}
Buttery odour	2·0 ± 0·9^{a}	2·6 ± 1·4^{ab}	2·7 ± 1·3^{b}	2·0 ± 0·9^{a}	2·2 ± 1·1^{ab}	2·8 ± 1·1^{b}
Toasty odour	2·0 ± 0·8	2·2 ± 1·0	2·7 ± 1·3	2·3 ± 1·4	1·8 ± 0·7	2·5 ± 1·1
Flavour intensity	4·4 ± 0·7^{a}	4·1 ± 1·1^{ab}	3·9 ± 1·0^{b}	5·1 ± 0·9^{a}	4·9 ± 1·0^{ab}	4·4 ± 0·9^{b}
Butyric acid flavour	4·1 ± 0·8^{a}	4·2 ± 1·3^{a}	2·7 ± 1·1^{b}	4·3 ± 1·3^{a}	4·6 ± 1·5^{a}	3·5 ± 1·3^{b}
Buttery flavour	2·3 ± 1·1	2·1 ± 0·9	2·5 ± 1·0	2·4 ± 1·4	2·4 ± 1·5	2·6 ± 1·5
Nutty flavour	1·8 ± 1·0	1·8 ± 0·9	2·0 ± 0·8	2·2 ± 1·5	2·3 ± 1·6	2·6 ± 1·5
Acidic flavour	4·1 ± 0·9	4·2 ± 1·1	3·3 ± 1·3	3·5 ± 1·2	3·8 ± 1·4	3·6 ± 1·2
Sweet flavour	2·4 ± 1·0	1·9 ± 0·7	3·0 ± 1·1	2·3 ± 1·0	2·2 ± 1·0	2·3 ± 1·0
Salty flavour	3·8 ± 0·6	4·1 ± 1·3	3·5 ± 0·9	3·8 ± 0·9	4·6 ± 1·0	3·9 ± 1·0
Bitter flavour	2·7 ± 1·0	2·9 ± 1·4	2·5 ± 1·1	3·4 ± 1·3	3·5 ± 1·6	2·8 ± 1·3
Rennet flavour	3·2 ± 1·6	2·5 ± 1·1	2·4 ± 1·1	3·1 ± 1·3	2·8 ± 1·3	2·7 ± 1·4
Pungent flavour	3·2 ± 1·6	2·4 ± 1·2	2·2 ± 0·8	3·3 ± 1·3	3·4 ± 1·2	2·8 ± 1·4
Surface roughness	3·3 ± 1·0	4·0 ± 1·3	4·0 ± 0·9	4·1 ± 0·7	4·3 ± 1·1	4·3 ± 1·0
Surface moisture	3·7 ± 0·7	3·6 ± 1·1	3·8 ± 0·9	3·8 ± 1·1	3·8 ± 0·9	3·9 ± 0·5
Elasticity	4·2 ± 1·1	4·0 ± 1·0	4·2 ± 1·0	3·4 ± 1·1	3·3 ± 0·9	3·6 ± 0·9
Firmness	3·2 ± 0·8	4·1 ± 0·8	3·7 ± 0·9	4·3 ± 1·2	4·2 ± 1·2	4·2 ± 1·2
Friability	3·6 ± 0·8	3·7 ± 1·0	3·7 ± 0·8	3·9 ± 0·9	3·8 ± 0·8	4·1 ± 0·8
Adhesiveness	3·5 ± 0·6	3·3 ± 0·6	3·1 ± 0·7	3·7 ± 1·0	3·5 ± 1·0	3·4 ± 0·9
Graininess	4·0 ± 1·2	4·5 ± 1·2	4·1 ± 1·1	4·1 ± 0·9	4·5 ± 1·0	4·2 ± 0·8
Solubility	4·2 ± 1·1	3·7 ± 0·7	3·9 ± 0·8	3·8 ± 0·8	3·5 ± 0·9	3·7 ± 0·8
Humidity in mouth	3·4 ± 1·0	2·7 ± 0·8	3·0 ± 0·9	3·1 ± 1·0	2·7 ± 1·0	2·9 ± 0·9

Means within rows without a common superscript are significantly different ($P \leqslant 0{\cdot}05$) for the same ripening day

Table 5. Pearson bivariate correlations between free fatty acids (FFA), and odour and flavour attributes of the Idiazabal cheeses

	Short-chain FFA	Medium-chain FFA	Long-chain FFA	Total FFA
Odour intensity	0·401**	0·022†	−0·054†	0·210†
Sharp odour	0·517**	0·021†	−0·102†	0·243†
Milky odour	−0·265†	−0·203†	−0·175†	−0·280†
Brine odour	0·392**	0·077†	−0·003†	0·242†
Rennet odour	0·462**	0·062†	−0·032†	0·264†
Buttery odour	−0·217†	−0·215†	−0·181†	−0·267†
Flavour intensity	0·261†	0·263†	0·223†	0·322*
Butyric acid flavour	0·293*	0·279†	0·232†	0·357*
Pungent flavour	0·339*	0·231†	0·171†	0·326*
Nutty flavour	−0·006†	0·147†	0·122†	0·106†
Buttery flavour	0·073†	−0·004†	−0·035†	0·041†
Bitter flavour	0·206†	0·219†	0·176†	0·267†

**$P \leqslant 0{\cdot}010$, *$P \leqslant 0{\cdot}05$
† not significant

2001b), and was also positively correlated ($P \leqslant 0{\cdot}05$) with the concentration of short-chain FFA. Pungent flavour was positively correlated ($P \leqslant 0{\cdot}05$) with the concentration of total FFA and short-chain FFA (Table 5). Therefore, the highest scores assessed for odour intensity, brine odour, flavour intensity, butyric acid and pungent flavours in the cheeses ripened for 180 d (Table 4) were probably due to the increase in the concentration of total FFA and short-chain FFA (Urbach, 1997; McSweeney & Sousa, 2000). Bustamante et al. (2003) showed that the use of

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artisanally-produced lamb rennet paste during Idiazabal cheese manufacturing was strongly correlated with a high concentration of short-chain FFA and high scores for the sensory attributes flavour and odour intensity, sharp odour, rennet odour and flavour, and pungent flavour. These observations would indicate that cheeses made with the pregastric lipase but not with the fungal lipase are comparable to cheeses made with lamb rennet paste.

Correlations between the concentrations of FFA, and milky and nutty odours, buttery odour and flavour, and bitter flavour were found to be non-significant ($P>0{\cdot}05$; Table 5). It has been reported that carbonyl compounds derived from the metabolism of lactose, lactate and citrate contribute to milky and buttery odours and flavours (Badings, 1991; McSweeney & Sousa, 2000). Nutty odour has been associated with volatile compounds derived from the metabolism of fatty acids and with esterification reactions (Lawlor et al. 2002), and bitter flavour with proteolytic processes which release peptides and free amino acids during cheese ripening (Grappin et al. 1985; Gómez et al. 1997).

Very little information of the effect of partial glycerides on sensory properties of cheeses has been reported in the literature. Several authors have indicated that the emulsifying capacity of MG and DG may have an impact on the properties of the paste (Adda et al. 1982; Catalano et al. 1985). However, none of the texture attributes were significantly influenced by the concentrations of MG and DG probably due to the low concentrations of these compounds found in the cheese samples (Table 3). The higher scores assessed for the texture attributes, such as surface roughness, firmness and friability in the cheeses ripened for 180 d (Table 4), were probably due to dehydratation and paste compacting during ripening (Bárcenas et al. 2001b). Likewise, the lower score assessed for elasticity in the longest ripened cheeses (Table 4) was primarily caused by loss of moisture (Jack & Paterson, 1992).

In conclusion, a higher level of lipolysis was induced in cheeses made with added lipase, resulting in important changes in odour and flavour attributes of the Idiazabal cheeses. The concentration of short-chain FFA, as well as those of (1,2+2,3)-DG and 1,3-DG, were higher in cheeses made with the pregastric lipase than in cheeses made with the fungal lipase. The cheeses made with the pregastric lipase also had the highest scores for odour and flavour intensity, and sharp and rennet odours, desirable attributes for Idiazabal cheese (Urarte et al. 1998). In addition, the concentration of short-chain FFA correlated with these sensory attributes, as described for Idiazabal cheeses made with lamb rennet paste (Bustamante et al. 2003). Thus, the pregastric lipase was more appropiate than the fungal lipase to develop a more traditionally flavoured Idiazabal cheese.

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