Changes in Triacylglycerols during the Ripening of Idiazabal Cheese

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The changes in triglyceride (TG) composition taking place in Idiazabal cheese over a ripening period of 360 days were studied using HPLC. The partition numbers (PNs) of TGs ranged between 22 and 53, the groupings of TG peaks with PN values of 34, 36, and 38 being the main contributors. Polynomial and nonlinear regression analyses were used to fit the experimental data over the ripening period. Cheese TGs change with ripening time as a function of the chain length and degree of unsaturation of the constituent fatty acids. The unsaturated TGs increased slightly during the first 3 months of ripening, whereas the saturated TGs decreased slowly during that same period. The short-chain TGs exhibited considerable decreases during the first 2 months of ripening, whereas the long-chain TGs increased during that time, reaching a maximum around that same date. The medium-chain TGs increased rapidly during the first 60 days of ripening and followed a slightly rising trend at the end of the ripening.

Keywords: *Ewe's-milk cheese; ripening; triglycerides*

INTRODUCTION

The composition of cheese fat and the changes taking place during ripening exert a strong influence on cheese quality (Prentice, 1987; Jameson, 1990; Olson and Johnson, 1990; Hennequin and Hardy, 1993). In considering the effect of the composition of cheese fat on the structural and rheological properties of cheese, one must keep in mind that although cheese firmness is primarily determined by the protein content, it is also affected by the melting interval of the fat, dependent upon temperature and the triglyceride (TG) composition (Prentice, 1987). A number of researchers have reported that cheese fat contributes to adhesion, thereby improving cheese homogeneity and helping to impart a smooth texture to the cheese (Jameson, 1990; Stampanoni and Noble, 1991; Hennequin and Hardy, 1993; Tunick et al., 1993a,b). Furthermore, a minimum fat content appears to be necessary, with levels below that limit strongly affecting cheese texture and lowering sensory quality (Banks et al., 1989; Jack et al., 1993a,b).

In addition, the fat exerts an influence on cheese aroma formation, because hydrolysis of the TGs increases the concentration of free fatty acids during ripening (Fontecha et al., 1990a,b; Olson and Johnson, 1990; Macedo and Malcata, 1996; Sousa et al., 1997). The free fatty acids thus liberated are the precursors of substances that play an important role in aroma development (esters, methyl ketones), whereas short-chain fatty acids themselves contribute to the aroma of ripened cheeses (Farkye and Fox, 1990; Ha and Lindsay, 1991; Urbach, 1991; Nájera et al., 1994). On the other hand, both the possible interactions between the free fatty acids and proteins and/or peptides as well as the emulsifying capacity of partial glycerides may have a certain effect on cheese texture (Schwartz et al., 1963; Adda et al., 1982; Catalano et al., 1985).

Most work undertaken on cheese fat has centered on the total and free fatty acid composition. A review of the literature has not disclosed any publications describing changes in the TG fraction with ripening, except for a few studies that have quantified the partial glyceride fractions in work on cheese ripening and lipolytic activity (Precht and Abd El-Salam, 1985; Vema and Anand, 1987; Hassan and El-Deeb, 1988; Contarini and Toppino, 1995; Koprivnjak et al., 1997).

Most papers dealing with the TG composition of milk fat have focused on describing the types of TGs present in terms of molecular weight, degree of unsaturation, and total number of carbon atoms in the constituent fatty acids (Arumughan and Narayanan, 1982; Caboni et al., 1982; Parodi, 1982; Lund, 1988; Barron et al., 1990; Assenat, 1991). Other researchers have classified the TGs according to their partition number (PN) (Parodi, 1980; Caboni et al., 1982; Frede and Thiele, 1987; Lund, 1988; Weber et al., 1988; Barron et al., 1990). This variable characterizes molecules on the basis of both the carbon number (CN) and the number of double bonds (ND), two parameters responsible for the separation of TGs in reverse-phase high-performance liquid chromatography ($PN = CN - 2ND$) (Wada et al., 1977).

The objective of this study was to characterize the changes in the TGs taking place during the ripening of Idiazabal cheese.

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MATERIALS AND METHODS

Cheese Samples. The cheeses were manufactured from the raw milk of ewes of the Lacha breed collected in March and natural lamb rennet in accordance with the procedures approved by the Regulatory Board of the Idiazabal Cheese Appellation of Origin (Ministerio de Agricultura, Pesca y Alimentación, 1993).

All cheeses were manufacturated from the same volume of raw milk in a single vat. After molding and pressing, the cheeses were brined in a saturated solution of 1.2 g of sodium chloride/mL at 13 °C for 24 h. Cheeses were ~13 cm in diameter and ~10 cm in height and between 1.0 and 1.2 kg in weight. Ripening took place at a temperature of 8−10 °C and a relative humidity of ~85%.

Of the total of rounds of cheese obtained from the same batch, two different cheeses were selected on each sampling date. The samplings were performed on 1, 15, 30, 60, 90, 120, 180, 270, and 360 days of ripening. Two samples of each cheese were taken for TG analysis. The HPLC analyses were made in duplicate.

TG Analysis. The cheese TGs were extracted in accordance with Standard 32 of the International Dairy Federation (1965) using *n*-pentane (Panreac, Barcelona, Spain) as the extraction solvent. The solvent was then driven off under vacuum at a temperature of <35 °C, and dilutions with a concentration of 30 mg/mL in *n*-hexane (Merck, Darmstadt, Germany) were prepared.

The TGs were analyzed by HPLC. The equipment employed consisted of one model 422M and one model 422S pump (Kontron, Milan, Italy), a model 7161 injector (Rheodyne, Cotati, CA), and a model Sedex 45 light scattering detector (Sedere, Alfortville, France) equipped with a model Compact 106 automatic air compressor (Cedime, Arrancudiaga, Spain). The system was operated by a work station running MT 450 software (Kontron). Column temperature was 30 °C, regulated by a model Precisterm thermostated hot water bath (Selecta, Barcelona, Spain).

The analysis was performed using two Nucleosil 120 C-18 (Machery Nagel, Düren, Germany) columns (Symta, Madrid, Spain), each 20 cm × 4.6 mm, connected in series, with a particle size of 3 μm. The chromatographic procedure employed gradient elution using a mobile phase of 0% acetone in acetonitrile (HPLC grade, Scharlau, Barcelona, Spain) increasing to 35% acetone over 50 min, followed by isocratic conditions for 20 min, a new gradient increasing the proportion of acetone to 80% over 75 min, and then isocratic conditions once again for the final 10 min. Detector conditions were a temperature of 40 °C and a compressed air pressure of 2.2 bar. An amount of 10 μL of fat extract was injected after filtering through a Durapore filter (Millipore, Milford, MA) with a pore diameter of 0.22 μm and warming to 30 °C in a hot water bath.

The TGs were quantified on the basis of their chromatographic peak areas using tristearin (purity 99%, Sigma Chemical, St. Louis, MO) as internal standard. The response factors were calculated by means of five replicate analyses of 0.5 mg/mL of the pure (99%) TGs tricaproin (CoCoCo), tricaprylin (ClClCl), trinonanoin (NoNoNo), tricaprin (CaCaCa), trilinolenin (LnLnLn), trimyristolein (MiMiMi), trilaurin (LaLaLa), 1,2-dilauroylmyristin (LaLaM), tritridecanoin (DeDeDe), 1,2-dimyristoyllaurin (MMLa), trilinolein (LLL), trimyristin (MMM), 1,2-dilinoleoylolein (LLO), 1,2-dimyristoylolein (MMO), 1,2-dimyristoylpalmitin (MMP), tripentadecanoin (PnPnPn), 1-myristoyl-2-oleoylpalmitin (MOP), 1,2-dipalmitoylmyristin (PPM), triolein (OOO), 1,2-dioleoylpalmitin (OOP), 1,2-dipalmitoylolein (PPO), tripalmitin (PPP), 1,3-dioleoylstearin (OSO), 1-palmitoyl-2-oleoylstearin (POS), 1,2-distearoylmyristin (SSM), triheptadecanoin (HeHeHe), 1,3-stearoylolein (SOS), 1,2-distearoylpalmitin (SSP), and tristearin (SSS) (Sigma) in *n*-hexane (Merck).

The response factor for each peak in the chromatograms for the cheese samples was estimated on the basis of the PN. The PN value for each peak was calculated using the equation obtained by linear regression of the independent chromatographic variable, log k', found experimentally by analyzing known pure TGs, on the PNs for those same known TGs.

The TGs were classified acccording to the PN value of the HPLC peaks. For each group of peaks with the same PN value, subgroupings were established on the basis of the equivalent carbon number (ECN) estimated for the HPLC peaks. ECN = CN − 2ND − 0.2NUFA, where NUFA was the number of constituent unsaturated fatty acids in the TG. The ECN values for the HPLC peaks were calculated by employing the same method used to calculate the PN values.

The quantitative results were expressed as grams of triglyceride per 100 g of cheese dry weight (DW).

Statistical Analysis. The experimental data were fit by means of polynomial and nonlinear regression using the 5R and 3R programs from BMDP Statistical Software, Inc. (Los Angeles, CA). The 5R program determines the degree of the polynomials by means of a goodness-of-fit test. This is a test of the inadequacy of the model at each degree, indicated by a tail probability >0.05, relative to the residual mean square from fitting the polynomial of highest degree. The 3R program estimates the parameters of regression by an iterative algorithm looking for the smallest residual sum of squares value.

RESULTS AND DISCUSSION

The peaks in the HPLC analyses of the TGs from the Idiazabal cheeses had PN values ranging from 22 to 53. Changes during ripening were evaluated for only those TG groupings by PN value making percentage contributions to the total TGs >1%. This included all of the groupings except those with odd PN values and those with the even PN values of 22, 24, 26, and 52. In addition, the TGs were grouped on the basis of the degree of saturation as saturated TGs, that is, all HPLC peaks with an integer ECN value, and unsaturated TGs, that is, all HPLC peaks with a noninteger ECN value. Last, pursuant to the work of Ruiz-Sala et al. (1996) on the identification of the TGs in milk fat, the TGs were also classified as short-chain TGs if they contained mainly short-chain fatty acids ($\leq C_{10}$), that is, all HPLC peaks with an ECN value of <39.8; medium-chain TGs if they contained mainly medium-chain fatty acids (C_{12} and C_{14}), that is, all HPLC peaks with an ECN value of from 39.8 to 42.0; and long-chain TGs if they contained mainly long-chain fatty acids ($\geq C_{16}$), that is, all HPLC peaks with an ECN value >42.0.

Regression analysis was employed to describe the behavior of the different TG groupings from the Idiazabal cheeses over the ripening period. The changes were fit by either polynomial (eq 1) or nonlinear (eq 2) regression

$$c = q + p_1 t + p_2 t^2 + \ldots + p_n t^n \quad (1)$$

$$c = p_1 e^{p2t} + p_3 e^{p4t} + \ldots + p_{n-1} e^{pnt} \quad (2)$$

where c is the TG content, t, the ripening time, q, a constant, and p_i, the regression coefficients.

Table 1 shows the R^2 determination coefficient (explained variance percentage), degree of freedom (DF), standard error of the estimate (SE), and number of parameters (p_i) for the regression equations obtained for the different TG groups studied. As can be observed, most of the TG groupings were fit by polynomial equations showing explained variance percentages ≥83.76%. All of these TG groupings required high degrees of polynomials, indicating complex evolutions with different stages over the ripening time (Figures 1 and 2). On the other hand, the TG groupings fit by the exponential equations required three or four parameters

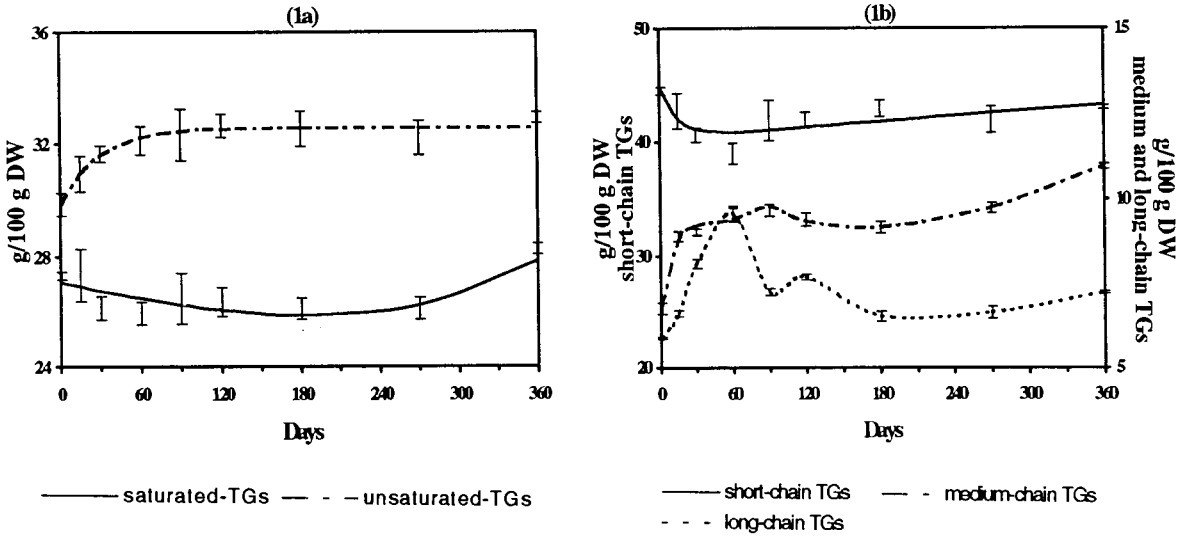

Figure 1. Contents (g of TG/100 g of DW) of the saturated, unsaturated, short-chain, medium-chain, and long-chain TGs during the ripening of Idiazabal cheese. Vertical lines represent the standard error of the mean value.

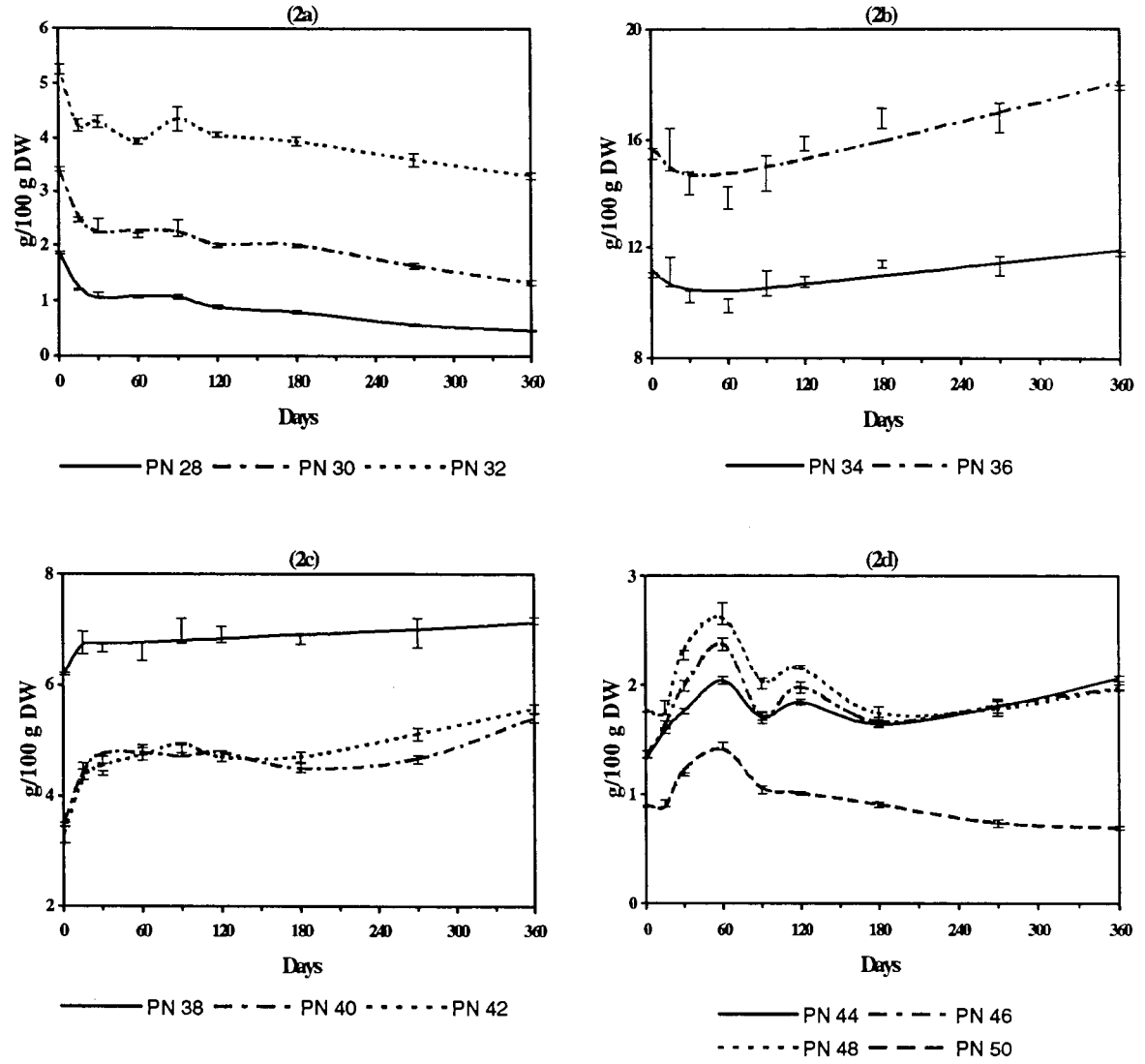

Figure 2. Contents (g of TG/100 g of DW) of the TG groupings by PN during the ripening of Idiazabal cheese. Vertical lines represent the standard error of the mean value.

of the regression, indicating more clearly trends with fewer oscillations over the ripening period (Figures 1 and 2). In all cases, the residuals followed a normal distribution, being randomly distributed around the mean value zero.

The mean total TG content over the ripening period

Table 1. Regression Analyses for the Changes in the TG Content (c) in Idiazabal Cheese with Ripening Time (t)[a]

TG group	regression model	p_i	SE	DF	R^2 (%)
saturated TGs	nonlinear	4	1.2453	32	
unsaturated TGs	nonlinear	3	1.1622	33	
short-chain TGs	nonlinear	4	2.3422	32	
medium-chain TGs	polynomial	7	0.3691	28	90.90
long-chain TGs	polynomial	8	0.2997	27	93.95
PN 28	polynomial	6	0.0696	29	97.42
PN 30	polynomial	6	0.1872	29	91.41
PN 32	polynomial	8	0.2610	27	83.76
PN 34	nonlinear	4	0.7003	32	
PN 36	nonlinear	4	1.1463	32	
PN 38	nonlinear	4	0.3478	33	
PN 40	polynomial	6	0.1971	29	87.21
PN 42	polynomial	7	0.2308	28	89.12
PN 44	polynomial	8	0.0821	27	90.12
PN 46	polynomial	8	0.1039	27	90.15
PN 48	polynomial	7	0.1352	28	85.19
PN 50	polynomial	7	0.0614	28	93.86

[a] p_i, number of parameters; SE, standard error of the estimate; DF, degree of freedom; R^2, coefficient of the determination. Polynomial model: $c = q + p_1t + p_2t^2 + \ldots + p_nt^n$. Nonlinear model: $c = p_1e^{p2t} + p_3e^{p4t} + \ldots + p_{n-1}e^{pnt}$.

was ~58 g/100 g of DW, with unsaturated TGs contributing ~55% and saturated TGs contributing 45%. Short-chain TGs made up ~72% of the total TGs over the ripening period, medium-chain TGs ~16%, and long-chain TGs ~12%.

Figure 1 depicts the changes in the contents of the saturated TGs, unsaturated TGs, short-chain TGs, medium-chain TGs, and long-chain TGs with ripening. The content of the unsaturated TGs increased slightly during the first 3 months of ripening, approximately, while the saturated TGs decreased slowly during that same period. Also, the saturated TGs exhibited an increasing trend at the end of the ripening period, whereas the unsaturated TG content was constant at ~33 g/100 g of DW during the last 9 months of ripening (Figure 1a). The short-chain TGs underwent a drop during the first 2 months and followed a slightly rising trend until the end of ripening (Figure 1b). On the contrary, long-chain TGs increased during the first 2 months, reaching a maximum at ~day 60 (Figure 1b). The medium-chain TGs also increased rapidly during the first 30 days of ripening and followed a slightly rising trend at the end of the ripening period studied (Figure 1b).

During the ripening period considered, the most important TGs were those for the TG groupings with PN values of 36, 34, and 38, which made percentage contributions of 27, 19, and 12, respectively, to the total TG content. As already mentioned above, the TG groupings with odd PNs and those with the even PNs of 22, 24, 26, and 52 made contributions of <1% each; hence, changes in those groupings over the ripening period were not evaluated in this study.

Figure 2 graphically illustrates the changes in the content of the different TG groupings according to PN value during the ripening of the Idiazabal cheeses. The groupings with PN values of 28, 30, and 32 underwent gradual declines over the entire ripening period, particulary emphasized during the first 30 days (Figure 2a). The groupings with PN values of 34 and 36 exhibited slight decreases during the first month of ripening and followed a slowly rising trend until the end of the ripening (Figure 2b), analogous to the trend for the short-chain TGs (Figure 1b). The trends for the groupings with PN values of 38, 40, and 42 were quite similar to the trend for the medium-chain TGs, with a slightly rising trend at the end of the ripening, particulary for PN values 40 and 42 (Figure 2c). The behavior of the groupings with PN values from 44 to 50 was rather similar to that of the long-chain TGs, with a maximum around day 60, followed by a downward trend until day 180. After that date, their behaviors diversified: the groupings with PN values of 44, 46, and 48 followed a slightly rising trend, and the grouping with the PN value of 50 continued to decrease until the end of ripening (Figure 2d).

In a previous work carried out in our laboratory (Nájera et al., 1994) a sharp increase in lypolytic activity was recorded in the Idiazabal cheese during the early months of ripening, with the liberation of substantial quantities of short-chain fatty acids tending to level off around a constant value toward the end of ripening. These findings are consistent with the decrease in the contents of the TG groupings of PN from 28 to 32 over the ripening period and with the decrease of those of PN 34 and 36 during the first month of ripening.

In conclusion, the TGs in Idiazabal cheese followed differing patterns of behavior depending upon the chain length and degree of unsaturation of their constituent fatty acids. The short-chain TGs of PN 28−32 decreased their contents over the ripening period, indicating that these short-chain TGs could be involved in a hydrolysis process to produce short-chain free fatty acids. The same observation can be made for the short-chain TGs of PN 34 and 36 during the first month of ripening.

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