SHORT COMMUNICATION

Fat Modification in Afuega'l Pitu Cheese During Ripening by Capillary Gas Chromatography

L. Alonso*

Instituto de Productos Lacteos de Asturias (CSIC), Asturias, Spain

Free and esterified fatty acids and the major groups of triacylglycerol in Afuega'l Pitu cheese have been identified and quantitated over a maturation period of 60 d by capillary gas chromatography. Levels of lipolysis were slight at the end of ripening, with a total free fatty acid content of 5604 mg/kg of cheese. At the same time, the short-chain esterified fatty acids and short-chain triglyceride contents decreased slightly.

KEY WORDS: Afuega'l Pitu cheese, fatty acid methyl ester, free fatty acids, gas chromatography analysis, milk fat, programmed temperature injection (PTV), triacylglycerol.

Milk fat represents one of the most complex mixtures of natural fatty acids as glycerol esters. The great variability in composition is mainly a function of feeding and lactation. The fatty acids range in chainlength from C2 to C24, with odd carbon numbers and unsaturations from one to four double bonds, including *cis* and *trans* isomers (1-3).

Traditional farmhouses have developed a number of different cheese varieties in Spain, including soft and hard cheeses, acid- or enzyme-coagulated, and cheeses ripened by molds or lactic-acid bacteria (4). Afuega'l Pitu cheese is a semisoft cheese produced by artisanal methods in the north of Spain (Asturias).

Interesting data have been published about blue (5,6) and goat cheeses (4,7) concerning free and esterified fatty acids in artisanal Spanish cheeses. However, work in relation to the triglyceride fractions is lacking.

In the present study, free and esterified fatty acids and the major groups of triacylglycerols have been identified and quantitated in Afuega'l Pitu cheese during ripening by capillary gas-liquid chromatography columns (GLC) and programmed temperature injection.

MATERIALS AND METHODS

Cheese samples. Four batches of artisanal Afuega'l Pitu cheese were made according to traditional cheese-making methods at two different farms in Asturias, Spain. Raw cow's milk was coagulated by means of natural acidification and addition of calf rennet during an 18-20-h period. The curd is placed in cotton to drain and then is mixed with salt, molded without applying pressure and ripened at room temperature (18-20°C) for 60 d. Cheeses were removed for analysis at 3, 7, 15, 30 and 60 d of ripening.

Milk fat. Fats for determination of free and esterified fatty acids in cheeses were extracted according to the method of Martín-Hernández *et al.* (8).

Fat samples for triglyceride analysis were extracted with hexane, filtered with sodium sulfate and concentrated on a rotary evaporator at 40-50°C. A solution (0.2 μL) of 0.05% in hexane (wt/vol) was injected for gas chromatographic analysis.

Chromatographic analysis. Preparation of the methyl esters of fatty acids of the glycerides and the free fatty acid (FFA) fractions was performed according to Martínez Castro *et al.* (9). The GLC analysis was carried out by programmed GLC with a Perkin-Elmer 8600 gas chromatograph (Norwalk, CT), a FFAP-CB fused-silica column (25 m × 0.25 mm, df: 0.30 μm) (Chrompack Int., Middelburg, Netherlands), a flame-ionization detector and a programmed temperature injector (PTV) and flow splitter. N_2 was used as carrier gas.

Triglyceride analyses by carbon number were performed in the same equipment with a WCOT fused-silica capillary column (25 × 0.25 mm) coated with TAB-CB (df: 0, 10 μm) (Chrompack). Experimental chromatographic conditions were initial column temperature 280°C, hold for 1 min rising to 350°C at 3°C/min. Initial PTV split injector temperature was 50°C, raised to 350°C in 10 s. Helium was used as carrier gas.

RESULTS AND DISCUSSION

Separation of total triglycerides has been accomplished directly by GLC of the fat. Figure 1 shows the GLC profile of milk fat triglycerides of Afuega'l Pitu cheeses, which were essentially resolved by acyl carbon number. The complex chromatographic elution patterns of the triacylglycerols into several groups of molecular species is due to the characteristic compositions of the milk fat and the molecular associations of the fatty acids. The reproducibility of the analytical triglyceride analysis was studied by repeating the gas chromatographic analysis five times from a cheese sample. The coefficients of variation (CV) of the different groups of triglycerides are shown in Table 1. There are some differences in the CV between triglycerides with short, medium (C26-C46) and long carbon chains (C48-C54); those differences can be attributed to losses of triglycerides by thermal degradation of the unsaturated fatty acids (10). The application of response factors permits a more accurate quantitation of triglycerides. Triglyceride compositions of Afuega'l Pitu cheeses after 3, 30 and 60 d of ripening are listed in Table 2. The triglyceride groups C26 to C34 showed a small decrease during ripening. The group C34 had the largest decrease, with values from 7.11 to 6.56 at the end of the ripening period.

Table 2 shows the average FFA composition for four batches of Afuega'l Pitu cheese during ripening. Total FFA increased from 1528 mg/kg in the 3-day-old cheese to 5661 mg/kg at the end of ripening; 25% of the total FFA consisted of short and medium fatty acids (C4-C12). The FFA contents were slightly higher than those reported by Woo *et al.* (11) in soft and semisoft cheeses, but considerably lower than those observed in mold-ripened cheeses (5,6) and in artisanal Spanish hard goat cheeses, which displayed values of 32,000 mg/kg at the end of ripening (4). This result was attributable to the utilization of unpurified

*Address correspondence at Instituto de Productos Lacteos de Asturias (CSIC), 33300 Villaviciosa, Asturias, Spain.

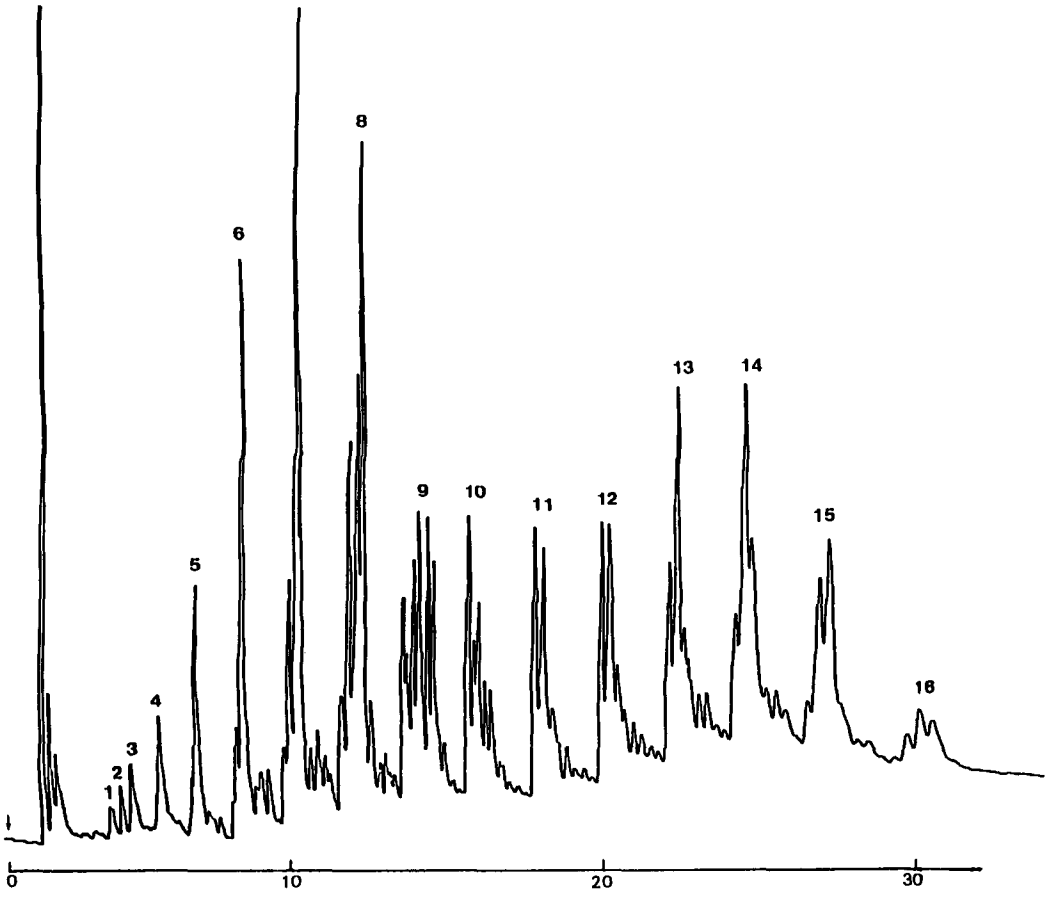

FIG. 1. Capillary gas–liquid chromatography (GLC) profile of major groups of triglycerides in Afuega'l Pitu cheese. Peaks are identified by their carbon number. GLC conditions: see text. 1 = C26; 2 = Cholesterol; 3 = C28; 4 = C30; 5 = C32; 6 = C34; 7 = C36; 8 = C39; 9 = C40; 10 = C42; 11 = C44; 12 = C46; 13 = C48; 14 = C50; 15 = C52; 16 = C54.

TABLE 1

Average Triglyceride Composition (C26–C54) and Coefficient of Variation (CV) in Afuega'l Pitu Cheese Fat During Ripening (3, 30 and 60 d)

	Component (%)														
Days	C26	C28	C30	C32	C34	C36	C38	C40	C42	C44	C46	C48	C50	C52	C54
3	0.30	0.70	1.43	3.22	7.11	11.46	13.08	9.14	6.82	7.26	7.75	9.14	10.43	8.42	3.74
30	0.28	0.66	1.34	3.10	6.72	11.37	12.98	9.30	7.02	7.38	7.86	9.28	10.61	8.40	3.60
60	0.24	0.63	1.28	3.05	6.56	11.40	13.02	9.35	7.09	7.42	7.81	9.40	10.51	8.58	3.66
CV	1.2	1.8	1.5	2.7	2.6	3.1	2.4	3.1	2.9	1.9	2.8	6.7	4.0	4.1	5.6

rennet pastes obtained by macerating stomachs containing high amounts of pregastric esterases (12). Table 3 shows the percentage of fatty acids as methyl esters determined by GLC in Afuega'l Pitu cheese during ripening. The short-chain fatty acids' content decreased slightly during the different stages of ripening. Butyric and caproic acids showed values of 3.91 and 2.65% at the beginning of ripening and reached a percentage of 3.34 to 2.16 at the end. Capric acid decreased from 1.74 to 1.34 during the same period.

From the present study, it can be concluded that, in this type of cheese made from raw milk, the lipid fraction experienced slight hydrolysis, due mainly to the action of the bacterial lipases or lipases present in the commercial preparations of milk-clotting enzymes, which yield a limited amount of FFA.

TABLE 2

Average Free Fatty Acid Contents (mg/kg) in Afuega'l Pitu Cheese During Ripening (3–60 d)

Components	Ripening (days)				
	3	7	15	30	60
C4:0	90	117	182	342	434
C6:0	65	79	110	141	228
C8:0	78	94	119	168	194
C10:0	81	109	154	254	329
C12:0	83	98	136	210	219
C14:0	226	288	466	496	886
C14:1	37	42	44	66	58
C16:0	303	373	551	1009	1187
C16:1	51	54	59	78	80
C18:0	119	170	241	517	603
C18:1	327	417	577	117	1233
C18:2	68	74	91	113	153
Total	1528	1915	2730	3511	5604

TABLE 3

Fatty Acid Composition (%) in Afuega'l Pitu Cheese During Ripening (3–60 d)

Fatty acid	Ripening days				
	3	7	15	30	60
C4:0	3.91	3.79	3.63	3.54	3.34
C6:0	2.65	2.60	2.49	2.33	2.16
C8:0	1.74	1.70	1.65	1.49	1.34
C10:0	3.06	2.97	2.85	2.80	2.57
C10:1	0.46	0.37	0.40	20.54	0.56
C12:0	3.75	3.60	3.51	3.39	3.30
C14:0	14.06	14.10	13.97	13.83	13.69
C14:1	0.68	0.82	0.94	1.07	1.04
*ai*C15	0.49	0.40	0.51	0.60	0.74
C15:0	1.05	1.21	1.11	1.23	1.24
*i*C16	0.39	0.39	0.42	0.58	0.69
C16:0	27.79	27.22	27.69	27.43	27.76
C16:1	1.12	1.14	1.10	1.14	1.18
*i*C17	0.72	0.82	0.96	1.04	1.04
*ai*C17	0.63	0.63	0.75	0.81	0.87
C17:0	0.66	0.78	0.85	0.85	0.86
C17:1	0.53	0.65	0.72	0.74	0.76
C18:0	10.49	10.36	10.24	10.26	9.95
C18:1	21.69	21.79	21.42	21.34	21.78
C18:2	1.69	1.65	1.63	1.65	1.63
C18:3	0.44	0.51	0.64	0.66	0.66
C18:*ctc*[a]	0.74	0.81	0.89	0.89	0.89
C20:1	0.69	0.89	0.91	0.99	0.97

[a]*ctc* = *cis-trans* conjugated.

ACKNOWLEDGMENTS

The author acknowledges the research project (CICYT: ALI 90-0233-CO2-02). The author also thanks H. Pazo for technical assistance.

REFERENCES

1. Hinrichs, J., U. Heinemann and H.G. Kessler, *Milchwissenschaft 47*:495 (1992).
2. Precht, D., *Z. Lebensm. Unters Forsch. 194*:1 (1992).
3. Myher, J.J., A. Kuksis and L. Marai, *J. Chromatogr. 452*:93 (1988).
4. Fontecha, J., C. Peláez, M. Juárez, T. Requena, R. Gómez and M. Ramos, *J. Dairy Sci. 73*:1150 (1990).
5. Alonso, L., M. Juárez, M. Ramos and P.J. Martín-Alvarez, *Z. Lebensm. Unters Forsch. 185*:481 (1987).
6. González de llano, D., M. Ramos, A. Rodriguez, A. Montilla and M. Juárez, *Int. Dairy J. 2*:121 (1992).
7. Martín-Hernández, M.C., M. Juárez and M. Ramos, *J. Dairy Sci. 75*:1747 (1992).
8. Martín-Hernández, M.C., L. Alonso, M. Juárez and J. Fontecha, *Chromatograph. 25*:87 (1988).
9. Martínez Castro, I., L. Alonso and M. Juárez, *Ibid. 21*:37 (1986).
10. Hinshaw, J.V., and W. Seferovic, *J. High Resol. Chrom. 9*:731 (1986).
11. Woo, A.H., S. Kollodge and R.C. Lindsay, *J. Dairy Sci. 67*:874 (1984).
12. Nelson, J.H., R.G. Jensen and R.E. Pitas, *Ibid. 60*:327 (1977).

[Received April 24, 1993; accepted August 5, 1993]