CHANGES IN THE COMPOSITION OF NEUTRAL LIPIDS IN SUBCUTANEOUS AND MUSCLE FAT DURING THE ELABORATION PROCESS OF SPANISH CURED HAM

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INTRODUCTION

Cured ham is an important meat product in Spain. The development of typical organoleptical characteristics requires a long time procedure (6 to 18 months). During this process the lipids suffer degradative changes that are related with a part of the organoleptical properties of cured ham. The evaluation of these changes has been carried out by different ways, mainly by the evaluation of the changes in the fatty acid composition of the different lipid fractions (neutral, free fatty acid and polar) obtained from back fat and muscle samples (Cantoni et al. 1970; Cantoni et al. 1971 ; Flores et al. 1985, 1986). The results reported do not show important changes among the elaboration procedure (Flores et al. 1987 , 1988), affecting to more insaturate fatty acids (C18:2). However the real composition of neutral lipids is reflected by the triglyceride profile, in consequence the direct determination of these compounds togheter with the mono & diglycerides can offer a more accurate picture of lipid changes during the elaboartion of cured hams, also the statement of the reological aspects can be explored in a more realistic way than on the basis of fatty acid composition.

In the last years the difficulties to analyze triglycerides in fats and oils have been surpassed. At present it is possible to determine these compounds by HPLC (Geeraert & De Scheeper, 1982 ; Podlaha & Toregard, 1982 ; Hersloff & Kindsmark, 1985 ; Geeraert & De Schepper, 1983 ; Stolylhwo et al. 1985) and CGC (capillary gas chromatography)(Geeraert & Sandra, 1985 ; Termonia et al. 1987 Hinshäw & Seferovic, 1986). CGC allows the separation of triglyceride according to NUFA (number of unsaturated fatty acids) or according to carbon number (CN) and to NDB (number of double bounds in the triglyceride molecule). HPLC requires the use of gradient combined with light scattering detector to obtain a similar separation with a more long analysis time; UV detector can be used but only with a few mobile phases, presenting quantitative problems due to the different responses of sample triglycerides.

Inthis work a methodology to determine neutral lipids in back fat and muscle is presented. This procedure was applied to evaluate the changes in the elaboration process of cured ham.

MATERIAL AND METHODS

70 animals were sampled. Hams were refrigerated (2 days) and they were cured with a mixture of salt (40 g/Kg) and nitrate in a ratio of 100:1. Fifteen days later the hams were washed and hung at 5ºC for 30 days, and then the temperature increases 1.5 ºC weekly until the sixth month. Samples of back fat and semimembranosus and biceps femoris muscles were taken for analysis at different stages of elaboration procedure (fres ham, salting, 1, 2, 4 & 6 months).

Lipid analysis

Extraction: lipids were extracted form back fat and muscle samples with chloroform:methanol mixture (2:1) (Folch et al. 1957). The extract was evaporated to dryness with a rotatory evaporator.

Fractionation of lipids: The residue was redissolved in 1 ml of chloro-

form, this solution was applied to aminopropyl silica minicolumn (Analytichem). Neutral lipids were recovered with 3 ml chloroform:isopropanol (2:1); free fatty acids with 3 ml 2% CH_3COOH in diethyl ether and phospholipids with 6 ml methanol 100 %.

Neutral lipid analysis: a) triglyceride determination: a1) HPLC , a Lichrospher RP-18 column (250 x 4 mm) was used at ambient temperature; mobile phase A: acetonitrile:isopropanol 55:45 ; B: isooctane 100% gradient 0% to 10% in 40 min. at 0.8 ml/min; detector UV photodiode array (Waters, Millipore) at a wavelength range 200-240 nm. a2) CGC, a FSOT column 25 m x 0.2 mm coated with phenylmethylsilicone (Rescom, Belgium) was used , carrier gas hydrogen at 60 cm/sec, temperature program: 280ºC - 10ºC/min- 340ºC - 1ºC/min - 365ºC - 5´, FID detector at 380ºC, cold split injection via a PTV injector (Gerstel, W. Germany), 70ºC - 12ºC/sec - 350ºC, was used. b) Mono, di & triglyceride determination: A FSOT capillary column (25 m x 0.25 mm) coated with OV-1 (Rescom, Belgium) was used, carrier gas : helium at 65 cm/sec, temperature program: 200ºC - 5ºC/min - 365ºC- 10´ , FID detector at 380ºC, cold split injection was used (60ºC - 360ºC) via a PTV injector (Dani, Italy).

Fatty acid composition: Neutral lipids were saponified with 0.3 N NaOH and the methyl esthers (FAMES) were obtained adding 14% BF_3/MeOH, FAMES were extracted with hexane and injected and analyzed under the following CGC conditions: FSOT capillary column (25 m x 0.25 mm) 90% cyanopropylsilicone (rescom, Belgium), carrier gas hydrogen at 30 cm/sec, temperature program: 120ºC - 4ºC/min - 220ºC, FID detector at 300ºC, split injection via a spli/splitless injector (Carlo Erba, Italy).

RESULTS & DISCUSSION

Analytical procedure:

HPLC combined with UV photodiode array detector allowed the simultaneous determination of triglycerides and the evalaution of oxidation products (fig. 1) by acquisition of chromatograms at 205, 210 and 230 nm. The resolution of triglycerides by the procedure described was performed in basis to ECN (equivalent carbon number), but the quantitation was disturbed by the different responses of triglycerides in the UV region used for detection; however it is possible to obtain a relative percentages to study the evolution of triglycerides among the elaboration process. Also UV detection does not allow the use of more appropiate mobile phases (ex. gradient of dichloromethane in acetonitrile) to achieve a more complete resolution; this can be obtained using a light scattering detector.

CGC determination of triglycerides was the best method to evaluate the evolution of these compounds (fig. 2) during the elaboration process of cured ham. It was possible to obtain more resolution than with HPLC; with a % sd $\leq$ 5 for all triglycerides studied (García-Regueiro et al. 1989). The identification was made based on retention times of standards and other triglyceride profiles well established (ex.: olive oil, cocoa butter,...), but the assignments of different peaks can only be considered as a tentative identification. The fatty acid composition was determined to facilitate the above mentioned identification; the statistical distribution of triglycerides, on the basis of fatty acid percentages, will be, in back fat of fresh ham, POO (21.2 %), PSO (8.2 %), OOL (8.2%), POL (6.8 %), SOO (9.2 %), OOO (16.7 %). The determination of triglycerides, mono & diglycerides was performed by CGC using an armoured OV-1 capillary column (fig. 3); it was not necessary to obtain TMS derivatives, despite of a small tailing in the peaks of mono&diglycerides.

Neutral lipids changes:

The most important differences between back fat and intramuscular fat triglycerides were the percentages corresponding to peaks 4, 5, 7, 11 and 14 (see table 1 and fig. 2). Free cholesterol was detected in muscle fat (fig. 2), there was a slight increase of free cholesterol among the elaboration process. The degradation of triglycerides in back fat was more intense than in muscle fat, but in both cases it was small, only peak 14 (fig. 2) was completely degraded, in back fat, showing the more unsaturated triglycerides a higher decrease in percentages in back fat during the elaboration process. Oxidation products were detected at salting stage but mono & diglycerides appeared at the 4th month at significative concentrations, being diglycerides with a CN of 36 the most important.

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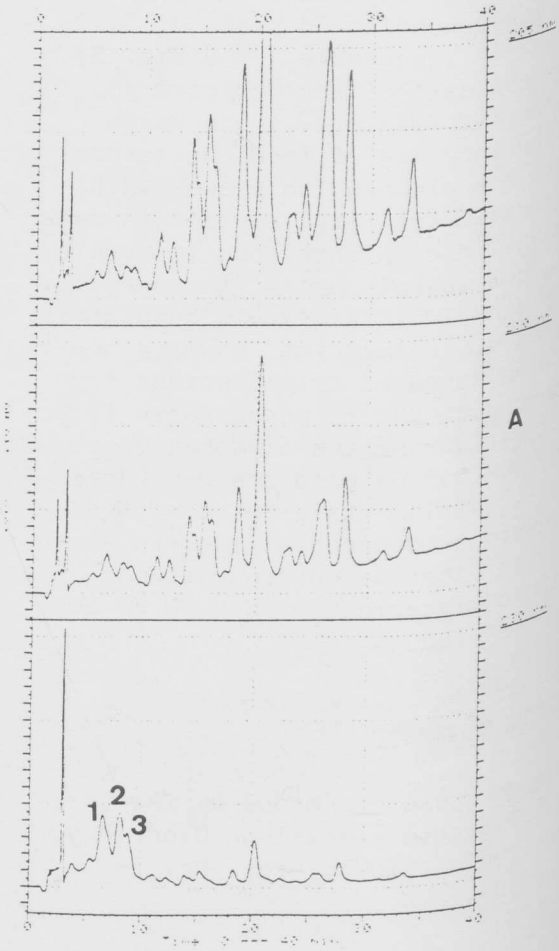

Fig. 1. HPLC chromatograms of back fat from cured ham. 1st fraction of aminopropyl silica column. A: chromatograms at 205, 210 & 230 nm. B: UV spectra of the peaks 1, 2 & 3.

TABLE 1. TRIGLYCERIDE PERCENTAGES IN BACK FAT AND MUSCLE FAT OF CURED HAM

MUSCLE FAT

	1	4	5	6	7	10	11	13	14
TØ	6.63	15.54	31.46	7.90	12.87	4.13	7.16	3.35	1.82
T1	7.00	13.64	32.52	6.85	12.06	4.49	8.99	2.93	2.44
T2	8.76	16.78	30.80	7.90	9.72	3.84	6.14	2.28	0.77
T3	7.56	15.37	30.33	7.85	10.52	4.57	6.97	2.89	1.79
T4	6.99	15.31	31.33	8.10	12.32	4.46	6.83	3.11	1.98
T6	8.44	12.95	29.35	7.73	12.55	3.43	6.27	3.17	2.60

BACK FAT

	1	4	5	6	7	10	11	12	13	14
TØ	8.63	16.65	28.41	6.40	14.22	4.87	5.89	2.57	3.69	1.0
T1	7.14	17.98	31.62	6.29	12.31	5.28	5.87	2.19	2.35	0.50
T2	9.70	20.63	30.07	6.66	12.40	3.90	4.10	1.77	1.44	0.16
T3	8.55	19.16	30.95	6.94	11.06	4.68	4.91	2.03	2.07	0.20
T4	7.86	19.70	29.95	6.76	14.32	3.75	4.42	2.11	1.95	0.00
T6	10.33	21.88	28.34	7.00	10.33	4.00	4.59	1.63	1.61	0.00

Mean of three samples (n=3)

Peaks identification: see fig. 2.

TØ: fresh fresh ham; T1: salting; T2: 2nd month; T3: 3rd month; T4: 4th mont
T6: 6th month.

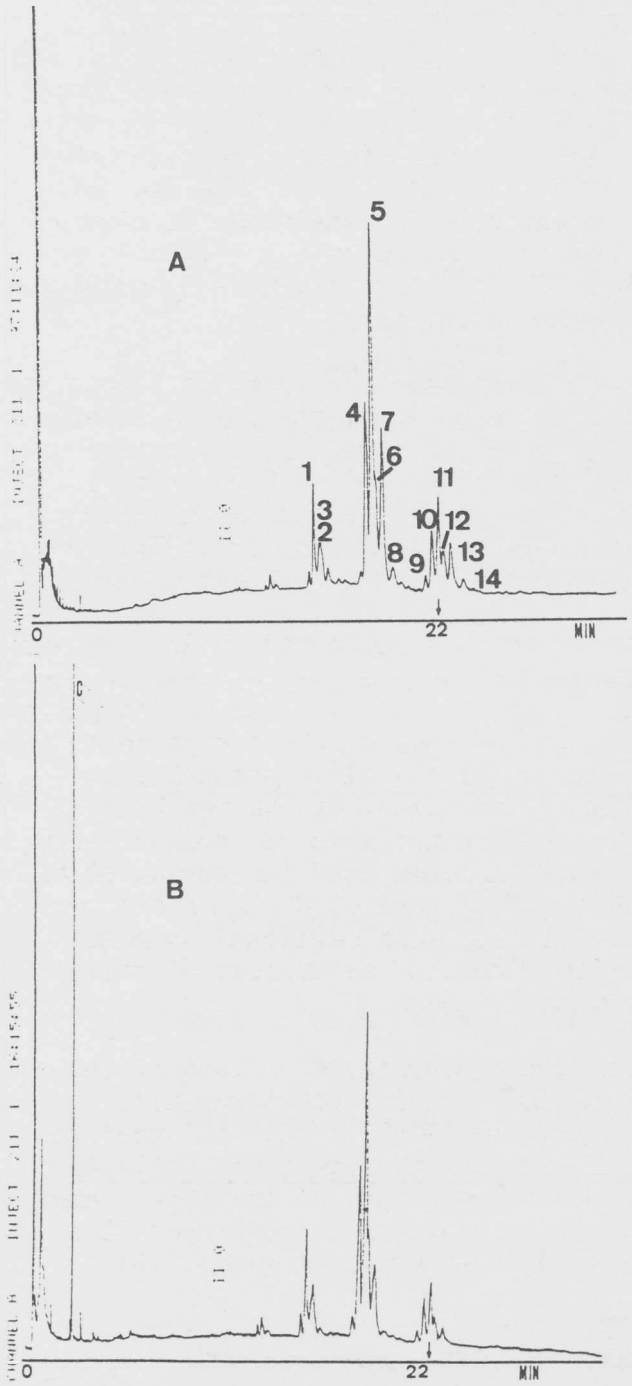

Fig. 2 CGC chromatograms of triglycerides from back fat (A) ans muscle fat (B). 1st fraction of aminopropylsilica. Conditions: see text. Peaks: 1.PPO 2+3. PP_oO+PPL, 4.POS, 5. POO,6.PLS, 7.PLO, 9. SSO, 10. SOO, 11. OOO, 12.SLO, 13.OOL, 14.OLL;C.Cholesterol.

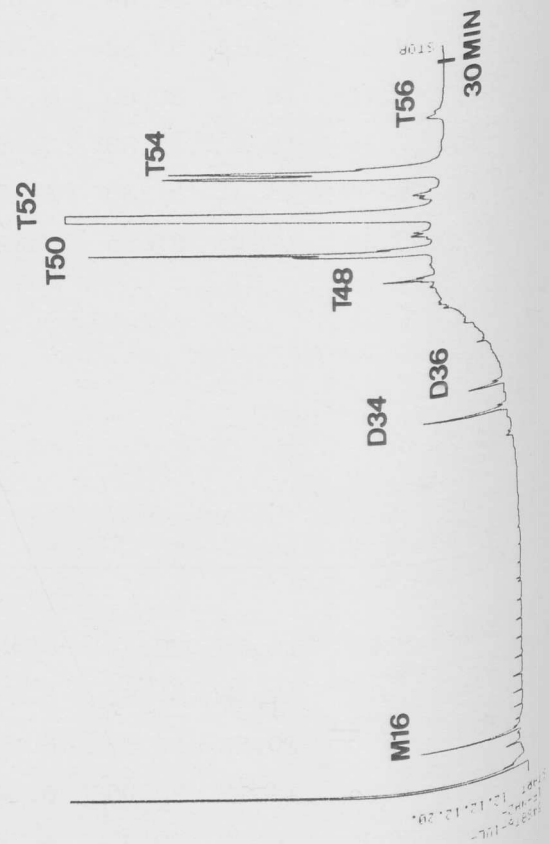

Fig. 3. Chromatograms of mono, di & triglycerides from back fat of cured ham. 1st. fraction of aminopropylsilica. Conditions: see text.