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Effect of storage time on fatty acid composition of subcutaneous fat

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SUMMARY - Samples of subcutaneous fat from 10 Iberian pigs were kept at -20°C for 32 months. The samples were analysed, using gas chromatography, at 0, 6, 12 and 32 months and the composition in fatty acids was obtained. The variance analysis shows the similarities between the fatty acid composition along the 32 months. Small significant differences ($P<0.01$) were obtained for C18:0 after 6 and 12 months as compared to the initial month. However, those small differences could be due to a change in the chromatographic column and in the injection method (automatic versus manual) during the assay period. Furthermore, no significant differences were found between the composition in C18:0 at 32 months as compared to the zero month.

Key words: Fatty acids, Iberian pig, storage fat.

RESUME - "Effet du temps de stockage sur la composition en acides gras de la graisse sous-cutanée". La conservation des échantillons de gras extrait du tissu adipeux du porc Ibérique, devient une exigence en vue des réclamations en conflit sur les critères de classification des carcasses et produits de ce dernier. De même, cette conservation nous permet de disposer d'échantillons afin d'établir des standards de référence de qualité. 10 échantillons de gras extrait du tissu adipeux des jambons de porcs Ibériques engraisés avec un aliment commercial, ont été stockés à -20°C . Leurs compositions en acides gras ont été déterminées par chromatographie en phase gazeuse, au moment de l'extraction et à 6, 12 et 32 mois de conservation. La comparaison de la composition des acides gras montre une similarité lors des périodes considérées. Les différences les plus remarquées ont été notées pour les pourcentages en acide stéarique (C18:0) à 6 et 12 mois de conservation (14,9% et 14,5% respectivement), sachant que les pourcentages de cet acide au moment de l'extraction et à 32 mois de conservation ont été de 15,5% et de 15,9% respectivement. Cette diminution du pourcentage peut être due à une altération du processus analytique (changement de colonnes, substitution de l'injection manuelle par une injection automatique) qui a eu lieu entre le début et 32 mois de conservation. Néanmoins, cette diminution ne peut en aucun cas résulter de l'altération de la matière grasse, sachant que l'évolution de la composition des acides gras ne se maintient pas à un niveau constant, celle-ci se retrouve similaire à la composition du début de conservation.

Mots-clés : Acides gras, porc Ibérique, conservation.

Introduction

The quality of meat from Iberian pigs is related to fat composition, which, in turn, is related to feeding. Feeding, therefore, is one of the most important factors in determining the price paid for the animals (De Pedro and Casillas, 1991).

It is, however, more difficult to ascertain the type of feeding the animals have had than to distinguish between genetic types. Various methods have been used for this in the industry, such as resistance to finger pressure of the cured hams fat, slip temperature of the fat, iodine value, and gas chromatography. Recent research emphasizes the differences in free fatty acids or triacylglycerides, in the composition of the fat, depending on feeding during the finishing period (De Pedro and Secondi, 1991; De Pedro and Dousset, 1992; Osorio *et al.*, 1992; Casillas, 1994; Coutron, 1996; Diaz *et al.*, 1996; Peralta, 1996; Vidal-Aragón, 1996).

The contract recently approved by the CICI (Comisión Interprofesional del Cerdo Ibérico) in Spain, for purchasing Iberian pigs for slaughter, recognizes different fatty acid compositions of the subcutaneous fat, depending on the type of feed used during the finishing period (BOE, 1997).

Given the importance of the fatty acid component of the fat, as the key factor in characterizing the carcass, and consequently the products, it would be very useful to be able to keep samples of subcutaneous fat in a good condition for as long as possible, for two reasons. It would allow disagreements between buyers and sellers to be resolved, where classification occurs some time after slaughter, when it would be difficult to obtain fresh samples in order to confirm or reject the original results.

Secondly, samples of known composition would be available, with characteristics specific to, for example, animals finished in "montaneras" with either abundant or scarce pasture, or where special feeds are offered, which would provide a reference for particular diets during other seasons, as it is not always possible to sample all feeding systems, because of the variability between "montaneras" and the cost of carrying out the trials. These samples could be stored in a Sample Bank and used for the study and application of new analytical techniques, such as NIRS, that give a more accurate recognition of the different feeding regimes used for the animals (Hervás *et al.*, 1994; De Pedro *et al.*, 1997b).

Lipids, generally, are susceptible to different forms of deterioration (Iriarte *et al.*, 1992), of which the process of oxidative rancidity or autooxidation is the most important, especially in animal fats. Temperature is the most important factor in determining the intensity and rapidity of deterioration, since both air and high temperatures encourage the formation of peroxides (Díaz, 1977).

Refrigeration and freezing also affect the fat. The type of packaging used is important at these low temperatures. Various studies have shown that the packaging must be airtight and lightproof for long term storage (Brewer and Harbers, 1991a; Brewer and Wu, 1993; Borbolla *et al.*, 1955). Jeremiah (1980), however, concluded that packaging need not be airtight in order to slow the oxidation process, as it appears that the oxidation of lipids occurs on the surface, with very little on the inside (Borbolla *et al.*, 1955; Brewer and Harbers, 1991b).

The aim of this study is to assess the variation in composition in fatty acids of fat extracted from the subcutaneous fat depot of Iberian pig carcasses, and kept at -20°C .

Material and methods

Origin and obtaining of samples

Ten Iberian pigs were used for the experiment. They were fattened before slaughter on a diet consisting entirely of a commercial compound feed, in order to obtain samples with a more homogeneous composition. After fasting for 48 h, the animals were slaughtered and jointed. The hams were stored for 24 h at 4°C . Samples of subcutaneous fat were then taken, just before the characteristic V cut was made. For this, perpendicular and transverse cuts at opposite ends of the hoof were made, and care was taken to sample both the external and internal subcutaneous fat. These samples were stored at 4°C in well sealed plastic bags, until the fat was extracted.

Extraction of fat

After removal of the skin, the samples were chopped into small pieces, approximately 0.5 cm^3 , and placed in individual microwave resistant glass containers. These were heated in a domestic microwave oven of 700W power and 2450 MHz microwave frequency, using the method described by De Pedro *et al.* (1997a). The melted fat was then distributed between four 10 ml polyethylene tubes. The first sample was analysed immediately (sample 0) and the others were stored at -20°C for 6, 12 and 32 months.

Analysis of fatty acids

The fatty acid composition of the samples was determined by gas chromatography in the Laboratorio Agrario of the Government of Andalucía in Córdoba, using the official method, on

a Perkin Elmer SIGMA-3D chromatograph coupled to a SIGMA 10B integrator, with a flame ionization detector, FID and integrator. A stainless steel column (2 m x 3.2 mm internal diameter) was used, containing a 10% solution of Diethylene Glycol Succinate (DGS) and 1% solution of PO₄H₃ on Chromosorb W, AW 80-100 mesh, into which 0.3 ml of the solution of methyl-esters was injected. The isothermic temperature of the oven was 175°C and that of the detector and injector 250°C. The carrier gas was nitrogen, at a flow rate of 2.5 ml/min. The results were expressed as a percentage of the weight of the injected methyl-esters.

The data were analysed, using the SAS package of statistical programs (SAS, 1990). Analysis of variance was made, using the GLM (General Linear Model) program, to determine whether mean values for fatty acid composition differed with storage time. Comparisons between means was made, using Duncan's method (Steel and Torrie, 1988), within the GLM program.

Results and discussion

Table 1 shows the mean ($\pm$ s.d.) concentration for each fatty acid in the samples of subcutaneous fat stored for 0, 6, 12 and 32 months.

Table 1. Mean values ($\pm$ s.d.) of fatty acids as a percentage of total fatty acid content in the subcutaneous fat of Iberian pigs stored for 0, 6, 12 and 32 months

Fatty acid	Time (months)							
	0		6		12		32	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
C140	1.6	0.3	1.5	0.4	1.5	0.3	1.5	0.3
C160	26.0	2.1	25.7	2.7	25.7	2.6	26.1	2.2
C161	2.4 ^a	0.2	2.4 ^a	0.3	2.4 ^a	0.3	2.0 ^b	0.2
C170	0.4	0.1	0.4	0.1	0.4	0.0	0.3	0.1
C171	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1
C180	15.5 ^a	1.0	14.9 ^{bc}	0.7	14.5 ^c	0.8	15.9 ^a	1.1
C181	44.7	2.5	45.6	2.9	46.1	3.0	44.8	2.7
C182	6.6	0.5	6.6	0.7	6.5	0.7	6.2	0.5
C183 + C20	0.7	0.1	0.7	0.1	0.7	0.1	0.8	0.1
C201	1.8	0.3	1.8	0.2	1.9	0.2	1.7	0.3

a,b,c: Values in the same row with different superscript differ significantly ($P < 0.001$)

Significant differences only occurred in the percentage of palmitoleic (C16:1) and stearic (C18:0) acids. At 12 months there was a decrease in stearic and an increase in oleic acid. As these changes were not found in samples stored for 32 months, we attribute the change at 12 months to small alterations in the analytical procedure. In the long period between analyses, columns on the chromatograph were changed and automatic injection was substituted for manual injection.

These changes would not affect classification under the official purchasing contract for Iberian pigs, as samples analysed both at the time of slaughter and later would be classified as having been taken from pigs fed concentrates.

This study shows that frozen samples can be stored for long periods without alteration in analytical results. This opens the way for the storage of samples from pigs, with known records of feeding and management, to be used both for verification and in the development of new analytical techniques.

Pigs finished in "montanera" have more unsaturated fatty acids than those fattened on concentrates and there is, therefore, a greater risk of alteration of the fatty acid composition,

during cold storage, of samples from these animals. We are currently carrying out work to verify definitively the effects of long term cold storage on fatty acid composition, determined by both gas chromatography and NIRS analysis.

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