

## CHANGES IN FREE FATTY ACID COMPOSITION IN SUBCUTANEOUS ADIPOSE TISSUE DURING THE DRYING STAGE OF IBERIAN HAM.

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### Background

Dry-cured Iberian ham is a typical product of high sensorial quality. Subcutaneous adipose tissue represents a large percentage of this product and is eaten by consumers, and therefore it also contributes to the typical flavour of ham (Flores et al., 1988; Moltiva et al., 1993). The traditional processing of elaboration of this ham involves a salting period for 15 days at temperature below 5° C, followed by a post-salting period (resting for 3 months under low temperatures). When salt diffusion is completed, the hams are left in drying rooms where a period of increasing temperatures (drying stage) begin for 80 days and finally 12 additional months in a cellar environment. Many changes occur in lipids of ham during processing, such as lipolysis. The relatively high temperature reached during the drying stage (25-30°C) plays a main role in the lipolysis (Antequera et al., 1992; Martín et al., 1999). This phenomenon is very important because it constitutes the prior step of free fatty acid auto-oxidation, which gives rise numerous volatile compounds, that are responsible to a large extent of the dry-cured ham characteristic flavour (García et al., 1991; López et al., 1992; Bolzoni et al., 1996; Flores et al., 1997).

### Objectives

The objective of this study was to determine the changes in the free fatty acid composition during the drying stage in subcutaneous adipose tissue of Iberian ham, to know the evolution of lipolysis and oxidation process.

### Methods

Twenty legs were obtained from Iberian pigs. The pigs were fed on a traditional extensive system based on acorns and pasture during the fattening period. The first steps of processing, (salting and post-salting) were performed in a local industry. Drying was carried out under controlled conditions of temperature and relative humidity in a drying room for 80 days. The relative humidity was set at 75-65%. Samples of subcutaneous adipose tissue were taken at five different moments, twenty days after each temperature condition modification. The initial temperature was set at 10-15° C and it was increased 5° C up to reach 30° C, and then decreased 5° C.

Total lipids from subcutaneous tissue (10 g) were extracted with a chloroform: methanol mixture (1:2) by the method of Bligh and Dyer. Lipids obtained (approximately 0.1 g) were fractionated into free fatty acids on NH<sub>2</sub>-aminopropil minicolumns according to the method described by Kaluzny et al. Fatty acid methyl esters of the free fatty acid fraction were prepared by acidic transesterification in presence of sulphuric acid and sodium methoxide, they were analyzed (1 µl) by gas chromatography (Hewlett Packard 5890A), equipped with FID. Identification of fatty acids was performed by comparison of retention times with those of reference compounds (Sigma Chemical Co. St. Louis, Mo, USA). The free fatty acid were expressed as percentage of area.

The effect of drying conditions on the free fatty acid composition of fat from Iberian hams was assessed by analysis of variance (ANOVA) using the general linear model of SPSS and then significantly different means were compared by a Tukey test at level of  $p \leq 0.05$ .

### Results and discussion

Drying conditions caused an increase in the proportion of saturated fatty acid from 32.27 to 41.82%. In contrast, the proportion of monounsaturated and polyunsaturated fatty acids decreased from 58.63 to 50.44% and from 9.10 to 7.74%, respectively (figure 1). It looks like that drying conditions do not stimulate lipolysis since unsaturated fatty acids decrease. However it is necessary to be in account that it is possible oxidation process in this drying stage due to the high temperature reached. The relationship between lipolysis and oxidation is not established even though several authors have reported that free fatty acids are more sensitive to oxidation than corresponding triacylglycerols (Gray and Pearson, 1984; Enser, 1987). The decrease in unsaturated fatty acids such as oleic and linoleic acid (table 1) which could indicate that part of these acids were oxidised during the drying stage. However, saturated fatty free acids not oxidise as easily (Gray and Pearson, 1987), thus these fatty acids not disappear during the drying stage (figure, 1). The proportion of palmitic and stearic acids significantly increased in the free fatty acids during the drying stage (table 1), which suggests a great degradation of triacylglycerols containing these fatty acids, such as di-oleoyl-palmitoyl glycerol (POO), palmitoyl-stearoyl-oleoyl glycerol (PSO), palmitoyl-oleoyl-linoleoyl glycerol (POL) and di-oleoyl-stearoyl glycerol (SOO). These triacylglycerols are the most abundant in the fat of Iberian ham (Tejeda, 1999). In addition, these triacylglycerols contain important amount of unsaturated fatty acids. This triacylglycerols are more susceptible to undergo lipolysis because they present unsaturated fatty acids (Coutron-Gambotti and Gandemer, 1999).

These results are in agreement with those found in subcutaneous adipose tissue during dry-cured processing of Corsican ham (Coutron-Gambotti and Gandemer, 1999), where an increase of saturated and a decrease of unsaturated was also found during the first six month of processing.

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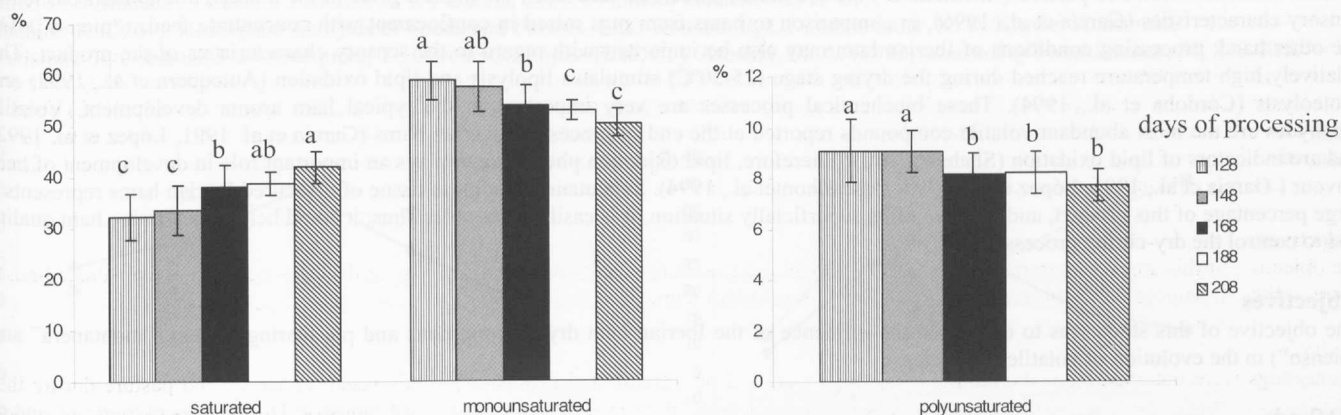


Fig. 1. Evolution of saturated, monounsaturated, and polyunsaturated fatty acids of the free fatty acid fraction during the drying stage. Different letters mean significant differences ( $p < 0.05$ ).

Table 1. Changes in free fatty acid composition in adipose tissue during the drying stage (% of methyl ester) (means  $\pm$  standard deviations).

Time (days)	128	148	168	188	208	Level of significance
Temperature	10-15°C	15-20°C	20-25°C	25-30°C	20-25°C	
<b>Saturated</b>						
16:0	23.97 $\pm$ 3.18 <sup>c</sup>	24.14 $\pm$ 3.16 <sup>bc</sup>	26.63 $\pm$ 3.25 <sup>ab</sup>	27.02 $\pm$ 2.09 <sup>a</sup>	28.85 $\pm$ 2.26 <sup>a</sup>	***
18:0	8.09 $\pm$ 1.79 <sup>b</sup>	9.18 $\pm$ 2.32 <sup>b</sup>	11.21 $\pm$ 2.02 <sup>a</sup>	11.52 $\pm$ 1.29 <sup>a</sup>	12.7 $\pm$ 1.49 <sup>a</sup>	***
20:0	0.21 $\pm$ 0.05 <sup>b</sup>	0.24 $\pm$ 0.05 <sup>ab</sup>	0.23 $\pm$ 0.09 <sup>ab</sup>	0.27 $\pm$ 0.05 <sup>a</sup>	0.27 $\pm$ 0.04 <sup>a</sup>	**
<b>Monounsaturated</b>						
16:1	2.33 $\pm$ 0.31 <sup>a</sup>	2.16 $\pm$ 0.33 <sup>ab</sup>	2.06 $\pm$ 0.51 <sup>ab</sup>	2.03 $\pm$ 0.25 <sup>ab</sup>	1.91 $\pm$ 0.27 <sup>b</sup>	**
18:1	54.42 $\pm$ 3.41 <sup>a</sup>	53.33 $\pm$ 4.51 <sup>ab</sup>	50.05 $\pm$ 3.73 <sup>b</sup>	49.14 $\pm$ 1.98 <sup>c</sup>	46.83 $\pm$ 2.60 <sup>c</sup>	***
20:1	1.88 $\pm$ 0.23	1.88 $\pm$ 0.20	1.65 $\pm$ 0.46	1.77 $\pm$ 0.19	1.70 $\pm$ 0.19	ns
<b>Polyunsaturated</b>						
18:2	8.48 $\pm$ 1.16 <sup>a</sup>	8.44 $\pm$ 0.81 <sup>a</sup>	7.65 $\pm$ 0.72 <sup>b</sup>	7.69 $\pm$ 0.77 <sup>b</sup>	7.21 $\pm$ 0.63 <sup>b</sup>	***
18:3	0.51 $\pm$ 0.10 <sup>a</sup>	0.49 $\pm$ 0.08 <sup>ab</sup>	0.42 $\pm$ 0.13 <sup>b</sup>	0.44 $\pm$ 0.07 <sup>ab</sup>	0.42 $\pm$ 0.07 <sup>b</sup>	**
20:4	0.12 $\pm$ 0.04	0.14 $\pm$ 0.05	0.11 $\pm$ 0.05	0.13 $\pm$ 0.02	0.12 $\pm$ 0.03	ns

On the same row, means with different superscripts differ significantly. Significance levels: ns, not significant; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$