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Lipolysis and oxidation in subcutaneous adipose tissue during dry-cured ham processing

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Abstract

In southern countries where the consumers eat the adipose tissue of ham slices, adipose tissue contributes to the typical flavour of ham. The aim of this study was to investigate the changes in lipids of subcutaneous adipose tissue during dry-cured ham processing. Lipid hydrolysis and oxidation were followed in the adipose tissue of 30 hams (by groups of six) at different stages of processing (green ham, 6, 12, 18 and 24 months). The results showed that: (1) Adipose tissue of the green ham contained 89.7% triacylglycerols, 5.6% water and low amounts of free fatty acids (FFA) (0.1%), diacyl- and monoacylglycerols (traces). The main triacylglycerols were di-oleoyl-palmitoyl glycerol (POO) (36.6%), palmitoyl-stearoyl-oleoyl glycerol (PSO) (19.9%) and palmitoyl-oleoyl-linoleoyl glycerol (POL) (8.0%). Free fatty acids had a higher proportion of polyunsaturated fatty acids than triacylglycerols (17.7% vs 8.3%). (2) An intense lipolysis occurred during the first 6 months and continued at a slow rate up to 24 months. The amount of triacylglycerols decreased from 89.6 to 75.8% while FFA, diacyl- and monoacylglycerol amounts increased to reach 10.5, 4.5 and 1.3%, in the 24 month old hams, respectively. A preferential hydrolysis of triacylglycerols POO, POL and OOL was observed. Free fatty acid composition showed a sharp decrease in linoleic and stearic acids and an increase in palmitic acid. The decrease in linoleic content during the process suggested that this fatty acid acid was oxidised. Both lipolytic and oxidative changes in lipids of adipose tissue could affect its sensory traits, particularly colour and taste.

1. Introduction

Adipose tissue is a component of dry-cured ham, which varies in quantity and quality according to genotypes, rearing conditions and dry-cured ham processing. Colour, firmness and thickness are essential attributes of dry-cured ham (Langlois, 1990) and largely determine the acceptance of the product by the consumer (Lo Fiego et al., 1989). In southern countries where the consumers eat the adipose tissue of ham slices, adipose tissue also contributes to the typical flavour of ham (Flores et al., 1988; Moltilva et al., 1993; Baldini et al., 1983).

During dry-cured ham processing, adipose tissue lipids are subjected to both lipolysis and oxidation (Flores et al., 1988; Diaz and Garcia-Regueiro, 1991; Melgar et al., 1990; Moltilva et al., 1993, 1994). Lipolysis mainly occurs during the first steps of ripening (Pezzani et al., 1988; Moltilva et al., 1993) and causes an increase in free fatty acids and diacylglycerols and a correlated decrease in triacylglycerols (Moltilva et al., 1993; Garcia-Regueiro and Diaz, 1989). The lipids undergo oxidation, mainly during the first 6–10 months of processing (Cantoni et al., 1976; Flores et al., 1985; Melgar et al., 1990; Moltilva et al., 1993). While a small amount of oxidation products is required to get the typical aroma of dry-cured ham, an excessive oxidation leads to off-flavour. The degree of lipid oxidation in adipose tissue is related to the unsaturation of fatty acids (Astiasaran et al., 1991), yet 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 the corresponding triacylglycerols (Gray and Pearson, 1984; Enser, 1987). Because polyunsaturated fatty acids are more sensitive to oxidation than monounsaturated ones, it is of interest to determine if lipolysis in adipose tissue

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preferentially releases polyunsaturated fatty acids. Lipolysis preferentially produces oleic and linoleic acids in dry sausages (Cerise et al., 1972; Demeyer et al., 1974; Molly et al., 1996) and in frozen adipose tissue (Girard and Bucharles, 1985). Data on the lipolysis in adipose tissue during dry-cured ham processing support the hypothesis of a non specific hydrolysis of triacylglycerols. Recent studies suggest that lipolysis in adipose tissue is mainly related to the activity of endogenous lipases such as lipoprotein lipase and hormone-sensitive lipase (Toldra et al., 1991; Moltilva et al., 1993), but the specificity of these lipases has not been studied yet (Toldra and Verplaetse, 1994).

Among the studies devoted to lipolysis and oxidation in adipose tissue of dry-cured ham, most deal with the changes in free fatty acid amount and composition, fatty acid composition of triacylglycerols and in oxidation during processing. A few have been devoted to the changes in diacylglycerols and monoacylglycerols or in the composition of triacylglycerols (Diaz and Garcia-Regueiro, 1991; Garcia-Regueiro and Diaz, 1989). But none simultaneously take into account all the lipid fractions which could be helpful in showing a specific hydrolysis of triacylglycerols. Most of the investigations were performed on dry-cured hams processed for 12 months (Cantoni et al., 1970b, 1976; Flores et al., 1985) and limited results are available on the long term process (12–24 months) used in the traditional production of dry-cured ham in southern European countries (Moltilva et al., 1993).

We, therefore, studied the changes in lipids of adipose tissue during dry-cured ham processing. We followed these changes by measuring the amounts of total lipids, triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids during 24 months. The fatty acid composition of free fatty acids and total lipids and the composition of triacylglycerols were determined. Lipid oxidation was estimated by measuring the ratio of the absorbance of lipids at 232 and 215 and 270 and 215, and the TBA reactive substance amount.

2. Material and methods

2.1. Material

Thirty Corsican pigs were grown in the traditional extensive system in Corsica. Twenty month old pigs were slaughtered at 141 kg ± 15. Carcass and ham weights were 115.5 kg ± 14 and 11.5 kg ± 1.1, respectively. Hams were removed from the carcasses after 24 h refrigerated storage at 4°C. They were thoroughly rubbed with dry salt and were buried in a pile of salt at 4°C for 30 to 40 days. After washing for 24 h to remove salt from the surface, they were hung for 6 to 9 months in a drying room at 8–10°C and at 75–90% relative humidity (RH). Then, the hams were matured under environmental conditions in a cellar (temperature range of 14–18°C and RH of 75–80%) until the end of the ripening. The thirty hams were divided into five groups of six hams for analyses. The subcutaneous adipose tissue covering the *Biceps femoris* muscle was removed from six hams at the following stages of processing: green ham, 6, 12, 18 and 24 months. The samples were stored at −20°C until analyses.

2.2. Analytical methods

2.2.1. Water content determination

Two grains of adipose tissue were dried at 103°C during 24 h. After cooling to ambient temperature in a desiccator, the sample was weighed again and the water content calculated as the difference between the weights before and after drying.

2.2.2. Lipid extraction

The lipids were extracted from 1 g of adipose tissue with chloroform/methanol (2:1) according to the method of Folch et al. (1957). The extracts were dried under vacuum on a rotary evaporator. Total lipid content was determined by weighing and was expressed in g 100 g^{-1} of adipose tissue.

2.2.3. Lipolysis measurement

Triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids were quantified according to a method adapted from that of Myher and Kuksis (1984). An aliquot of 0.5 to 1 mg of the total lipid extracts was introduced into a conic vial. The solvent was evaporated under N_2 and the lipids were silylated using 250 µl of a mixture of BSTFA:TMCS (80/20) (composed of pyridine, trimethylchlorosilane (TMCS) and bis-trimethyl-silyl-trifluoro-acetamide (BSTFA)) (Pierce Chem., Co., Rockford, USA). The vial was immediately closed with a Teflon cap. The reaction was achieved in 30 min at room temperature. The mixture was evaporated under N_2 and the derivatives were dissolved in 2 ml of hexane. The silylated components (TMS) were then analysed by gas chromatography. The chromatograph was a Delsi DI 700 equipped with a flame ionisation detector and an 'on-column' injector (SGE). One microlitre of the sample was injected into a capillary column (DB5, SGE, 7 m length, 0.32 mm internal diameter, 0.1 µm film thickness) containing a non-polar stationary phase (5% phenylmethyl–95% siloxane). The oven temperature was maintained at 120°C for 5 min, increased from 120°C to 230°C at 20°C min^{-1} and from 230 to 330°C at 15°C min^{-1} and then held at 335°C for 5 min. The detector temperature was maintained at 350°C. The flow of the carrier gas (H_2) maintained at 2 ml min^{-1}. The silylated compounds of the sample were eluted according to their molecular weight in less than

25 min. Response coefficients of the compounds were determined according to an internal standard, the tricaprin, for which the coefficient was set at 1. Data were collected with a system including an 'acquisition box', software and a computer (Apex, Stang, France). Results were expressed as mg 100 g of subcutaneous adipose tissue.

2.2.4. Triacylglycerol composition determination

The analysis was performed by reverse phase by high performance liquid chromatography using a Gilson binary delivery system and a rheodyne valve injection equipped with a 20 µl loop. We used two columns (25 cm long, 4.4 mm i.d.) packed with silica (3 µm Superspher 100, RP18, Merck). Triacylglycerols were quantified with a light scattering detector (DDL 10, Cunow, France). It was paired with a suitable computer and software (Apex). The main triacylglycerols were eluted in 60 min using a gradient mode. The eluants A and B were a mixture containing acetone–acetonitrile 1/1, v/v and pure chloroform respectively. The amount of eluant B increased from 0 to 20% in 60 min. The flow rate was 1 ml min^{-1}. The results were expressed in percent of total triacylglycerol content injected.

2.2.5. Free fatty acid purification

Free fatty acids were purified from the total lipids using an anionic exchange resin (Amberlyst A26) as described by Gandemer et al. (1991). An aliquot of 50 mg to 100 mg of total lipids were dissolved in 15 ml of a mixture of acetone/methanol 2:1 (v/v). After addition of 100 mg to 200 mg of the resin and heptadecanoic acid (internal standard), the mixture was shaken for 30 min. Non-resin bound lipids were removed by washing the resin with acetone/methanol 2:1 (v/v). Resin was then transferred into a dry tube for methylation.

2.2.6. Fatty acid composition determination

The fatty acid composition of total lipids and free fatty acids were determined by gas liquid chromatography of methyl esters prepared as described by Morrison and Smith (1964). The gas chromatograph was a Hewlett Packard apparatus (HP5890). It was equipped with a flame ionisation detector. We used a capillary column (DB 225, J&W, 30 m long, 0.32 mm i.d., 0.25 µm film thickness) containing a polar stationary phase (cyanopropylphenyl-methylpolysiloxane). The flow rate of the gas carrier was 2 ml min^{-1} and the detector temperature was 250°C. For free fatty acids we used the on-column injection mode. The oven temperature was held at 50°C for 3 min and then programmed from 50 to 180°C at 10°C min^{-1}, held at 180°C for 10 min, increased from 180 to 210°C at 20°C min^{-1} and then maintained at 210°C for 10 min. For triacylglycerols we used the split injection mode and the split flow rate was set at 30 ml min^{-1}. The oven temperature was held at 150°C for 3 min, increased to 190°C at 10°C min^{-1}, held at 190°C for 10 min, increased from 190 to 210°C at 20°C min^{-1}, and then maintained at 210°C for 10 min. The injector temperature was 250°C. The individual fatty acid peaks were identified by their retention times. The results were expressed in percent of the amount of methyl esters present and in mg 100 g^{-1} of adipose tissue.

2.2.7. Lipid oxidation measurement

Lipid oxidation was evaluated by the ratio of the absorbance at 232 nm to the absorbance at 215 nm and by the ratio of the absorbance at 275 nm to the absorbance at 215 nm, from a sample of lipids (200 µg ml^{-1} of cyclohexane) according to the method of Klein (1970). An increase of the ratio A_{232}/A_{215} corresponds to an increase in the concentration of conjugated dienes. An increase of the ratio A_{275}/A_{215} is related to an increase in carbonyl compounds. TBA test was performed according to the method of IUPAC WG 1-82. A 50 mg to 200 mg sample of total lipids were dried under N_2. Then they were dissolved in 25 ml of butanol. Five millilitres of the mixture were combined with 5 ml of TBA (2 mg ml^{-1}). The solution was mixed thoroughly and heated for 2 h in a boiling water bath. A blank was prepared with 5 ml of TBA (2 mg ml^{-1}) and 5 ml of butanol. After cooling, the absorbance of the sample and the blank was determined at 530 nm against distilled water. The results were expressed as ng of MDA mg^{-1} of adipose tissue.

2.2.8. Statistical analysis

The comparison of the lipid characteristics at the different stages of processing was performed by a one way-variance analysis (Snedecor and Cochran, 1981).

3. Results

3.1. Compositional traits of adipose tissue of green ham

The subcutaneous adipose tissue of green ham contained 5.6% water and 89.7% lipids (Table 1). Triacylglycerols accounted for more than 99% of total lipids. Free fatty acids were present in a low amount (0.1%) and diacyl- and monoacylglycerols in traces. Expressed in g 100 g^{-1} of adipose tissue, the main triacylglycerols of adipose tissue were di-oleoyl-palmitoyl glycerol (POO) (36.6) and palmitoyl-stearoyl-oleoyl glycerol (PSO) (19.9). The proportions of palmitoyl-oleoyl-linoleoyl glycerol (POL) (8.0), di-palmitoyl-oleoyl glycerol (PPO) (5.9) and tri-oleoyl glycerol (OOO) (5.6) were lower. The saturated triacylglycerols were present in a very low proportion (2.3) (Table 2). Fatty acid composition of total lipids was characterised by a high proportion of monounsaturated (54.4%) and a low proportion of PUFA (8.3%) (Table 3). The main

fatty acids were oleic acid (50.0%), palmitic acid (23.3%) and stearic acid (12.2%), linoleic acid accounting for only 7.4%. These results are in good agreement with those previously published on adipose tissue of Corsican pigs reared in extensive system (Secondi et al., 1992). The free fatty acid composition largely differed from that of the triacylglycerols because it exhibited a higher proportion of PUFA (17.7 vs 8.3%) and a lower proportion of saturated fatty acids (25.0 vs 37.3%) (Table 4).

3.2. Changes in adipose tissue during processing

The water content of adipose tissue decreased during processing from 5.6 to 2.1%. The water loss was mainly observed during the first 6 months of processing and the water content of adipose tissue remained roughly constant from 6 to 24 months of processing (Table 1).

The total lipid content did not change significantly during processing. During the first six months, the tri-

Table 1
Changes in lipid and water contents and in oxidation and lipolysis of adipose tissue during processing (g 100 g^{-1} of adipose tissue)

Time (months) Number of hams	0 ($n=6$)	6 ($n=6$)	12 ($n=6$)	18 ($n=6$)	24 ($n=6$)	Level of significance
Water	5.6 ^{a} (0.85)	2.7 ^{bc} (0.50)	3.5 ^{b} (0.41)	2.1 ^{c} (0.72)	2.5 ^{bc} (0.50)	***
Total lipids	89.7 (1.47)	91.8 (3.57)	92.2 (1.31)	91.1 (2.94)	91.9 (2.14)	ns
Lipolysis						
Triacylglycerols	89.6 ^{a} (0.56)	78.0 ^{bc} (2.02)	79.1 ^{b} (2.00)	75.8 ^{c} (1.39)	75.8 ^{c} (0.43)	***
Monoacylglycerols	0.0 ^{d} (0.08)	1.2 ^{ab} (0.19)	0.7 ^{c} (0.16)	1.0 ^{bc} (0.21)	1.3 ^{a} (0.21)	***
Diacylglycerols	0.0 ^{b} (0.34)	4.5 ^{a} (0.78)	4.6 ^{a} (0.51)	4.3 ^{a} (0.27)	4.5 ^{a} (0.37)	***
Free fatty acids	0.1 ^{c} (0.40)	8.1 ^{b} (1.19)	7.8 ^{b} (1.44)	10.1 ^{a} (1.04)	10.5 ^{a} (0.23)	***
Oxidation						
A232/A215	0.71 ^{b} (0.05)	0.79 ^{a} (0.05)	0.69 ^{b} (0.06)	0.81 ^{a} (0.07)	0.61 ^{b} (0.01)	***
A270/A215	0.06 ^{b} (0.02)	0.11 ^{a} (0.02)	0.09 ^{ab} (0.01)	0.06 ^{b} (0.02)	0.11 ^{a} (0.02)	**
TBA†	1.49 ^{ab} (0.14)	1.73 ^{a} (0.13)	1.63 ^{ab} (0.13)	1.67 ^{a} (0.09)	1.41 ^{b} (0.16)	*

On the same row, means with different superscripts differ significantly. Significance levels: ns, not significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$. Values in the brackets are standard deviations.

† ng of malonaldehyde mg^{-1} of adipose tissue.

Table 2
Changes in triacylglycerol composition in adipose tissue during processing (g 100 g^{-1} of adipose tissue)

Time (months) Number of hams	0 ($n=6$)	6 ($n=6$)	12 ($n=6$)	18 ($n=6$)	24 ($n=6$)	Level of significance
Triacylglycerols						
PSS	0.6 (0.14)	0.5 (0.08)	0.6 (0.17)	0.6 (0.13)	0.5 (0.07)	ns
PPS	0.6 (0.11)	0.6 (0.14)	0.5 (0.08)	0.7 (0.15)	0.5 (0.09)	ns
PPP	1.6 (0.27)	1.6 (0.42)	1.1 (0.16)	1.5 (0.28)	1.3 (0.23)	ns
Total saturated	2.8 (0.23)	2.8 (0.57)	2.1 (0.24)	2.8 (0.44)	2.3 (0.32)	ns
PPO	5.9 (0.32)	5.2 (0.53)	6.8 (1.22)	6.1 (1.07)	5.8 (0.69)	ns
PSO	19.9 (1.32)	19.2 (0.55)	19.2 (1.49)	16.2 (2.45)	18.4 (1.94)	ns
SSO	0.3 ^{b} (0.10)	0.3 ^{b} (0.08)	0.4 ^{ab} (0.10)	0.4 ^{ab} (0.04)	0.6 ^{a} (0.15)	*
Total monounsaturated	26.1 (1.12)	24.7 (0.97)	26.3 (1.91)	22.7 (3.23)	24.7 (2.36)	ns
SOO	2.7 ^{ab} (0.48)	2.4 ^{ab} (0.29)	3.0 ^{a} (0.22)	2.3 ^{b} (0.27)	2.3 ^{b} (0.11)	*
POO	36.6 ^{a} (1.03)	31.9 ^{b} (0.67)	28.8 ^{c} (1.47)	27.4 ^{c} (2.08)	31.2 ^{b} (1.36)	***
PPoO	2.2 ^{a} (0.24)	1.4 ^{c} (0.30)	1.7 ^{bc} (0.22)	2.0 ^{ab} (0.23)	1.8 ^{abc} (0.13)	**
PSL	2.1 ^{a} (0.19)	1.5 ^{b} (0.62)	1.6 ^{ab} (0.32)	1.8 ^{ab} (0.29)	1.7 ^{ab} (0.12)	*
Total diunsaturated	43.6 ^{a} (0.98)	37.3 ^{b} (0.86)	35.1 ^{bc} (1.32)	33.6 ^{c} (2.43)	37.0 ^{b} (1.41)	***
OOO	5.6 (0.50)	5.2 (0.25)	5.7 (0.81)	4.8 (0.42)	4.5 (0.90)	ns
POL	8.0 ^{a} (0.69)	5.5 ^{b} (0.78)	6.4 ^{b} (1.36)	5.4 ^{b} (0.71)	4.7 ^{b} (0.69)	***
PoOO	1.1 (0.25)	0.9 (0.04)	1.0 (0.19)	1.3 (0.28)	0.9 (0.15)	ns
Total triunsaturated	14.7 ^{a} (1.20)	11.6 ^{bc} (0.58)	13.1 ^{ab} (2.27)	11.5 ^{bc} (1.26)	10.1 ^{c} (1.50)	**
OOL	2.3 ^{a} (0.29)	1.7 ^{bc} (0.21)	2.1 ^{ab} (0.48)	1.7 ^{bc} (0.26)	1.5 ^{c} (0.29)	**

On the same row, means with different superscripts differ significantly. Significance levels: ns, not significant; * = $p<0.05$; ** = $p<0.01$. Values in the brackets are standard deviations.

P: palmitic acid; S: stearic acid; O: Oleic acid; Po: Palmitoleic acid; L: linoleic acid.

acylglycerol amount decreased sharply from 89.6 to 78.0% and then more slowly between 6 and 24 months from 78.0 to 75.8%. In contrast, the amounts of diacylglycerols and monoacylglycerols increased rapidly during the first six months (from 0 to 4.5% and from 0 to 1.2%, respectively) and remained steady until the end of the process.

Changes in individual triacylglycerol amounts during processing affected POO, POL and OOL. Their amounts (expressed in g $100\,g^{-1}$ of adipose tissue) significantly decreased from 36.6 to 31.2 for POO, from 8.2 to 4.7 for POL and from 2.3 to 1.5 for OOL (Table 2).

Free fatty acids rapidly increased during the first 6 months (from 0.1 to 8.1%) and at a slower rate between 6 and 24 months (from 8.1 to 10.5%) (Table 1). Whereas the fatty acid composition of total lipids was roughly constant during processing (Table 3), large changes were observed in free fatty acid composition (Table 4). The proportion of PUFA decreased from 17.7 to 13.6% because of the decrease in the proportion of linoleic acid from 16.1 to 11.8%. This decrease was marked during the first 6 months. In contrast the proportion of saturated fatty acids increased from 25.0 to 29.9% because palmitic acid sharply increased from 14.7 to 21.9%. The proportion of stearic acid continuously decreased during processing from 8.2 to 5.7%. Compared with total lipids, free fatty acids contained a higher proportion of linoleic acid, a lower

Table 3
Changes in fatty acid composition of total lipids in adipose tissue during processing (% of methyl esters)

Time (months) Number of hams	0 ($n=6$)	6 ($n=6$)	12 ($n=6$)	18 ($n=6$)	24 ($n=6$)	Level of significance
Fatty acids						
14:0	1.3 (0.13)	1.3 (0.15)	1.4 (0.08)	1.4 (0.10)	1.5 (0.01)	ns
16:0	23.3 (0.81)	23.6 (1.08)	24.8 (1.40)	24.8 (0.62)	24.6 (0.76)	ns
17:0	0.3 (0.04)	0.3 (0.06)	0.3 (0.06)	0.3 (0.06)	0.3 (0.05)	ns
18:0	12.2 (0.86)	12.7 (0.66)	12.1 (1.60)	12.2 (0.54)	12.3 (1.42)	ns
20:0	0.2 (0.06)	0.2 (0.04)	0.2 (0.02)	0.2 (0.02)	0.2 (0.02)	ns
Total saturated	37.3 (0.44)	38.1 (0.96)	38.8 (1.52)	38.9 (0.84)	38.9 (2.19)	ns
16:1	2.6 (0.27)	2.5 (0.07)	2.8 (0.61)	2.9 (0.24)	3.0 (0.29)	ns
17:1	0.3 (0.01)	0.3 (0.05)	0.3 (0.01)	0.3 (0.01)	0.3 (0.04)	ns
18:1	50.0 (0.55)	50.0 (1.27)	49.0 (1.49)	49.3 0.53)	48.9 (1.56)	ns
20:1	1.5 ^{a} (0.18)	1.6 ^{a} (0.16)	1.5 ^{a} (0.24)	1.5 ^{a} (0.15)	1.2 ^{b} (0.05)	*
Total monounsaturated	54.4 (0.52)	54.4 (1.31)	53.7 (1.58)	54.0 (0.76)	53.5 (1.73)	ns
18:2 *n*-6	7.4 ^{a} (0.21)	6.6 ^{ab} (0.40)	6.7 ^{ab} (0.30)	6.3 ^{b} (0.63)	6.9 ^{ab} (0.57)	*
18:3 *n*-3	0.9 ^{a} (0.08)	0.8 ^{ab} (0.03)	0.8 ^{ab} (0.06)	0.7 ^{b} (0.09)	0.8 ^{ab} (0.12)	*
Total polyunsaturated	8.3 ^{a} (0.19)	7.4 ^{ab} (0.43)	7.6 ^{ab} (0.34)	7.0 ^{b} (0.72)	7.7 ^{ab} (0.61)	*

On the same row, means with different superscripts differ significantly. Significance levels: ns, not significant; * = $p < 0.05$. Values in the brackets are standard deviations.

Table 4
Changes in free fatty acid composition in adipose tissue during processing (% of methyl esters)

Time (months) Number of hams	0 ($n=6$)	6 ($n=6$)	12 ($n=6$)	18 ($n=6$)	24 ($n=6$)	Level of significance
Fatty acids						
14:0	2.0 ^{b} (0.20)	2.0 ^{b} (0.17)	1.8 ^{b} (0.43)	2.3 ^{a} (0.17)	2.3 ^{a} (0.26)	*
16:0	14.7 ^{d} (1.20)	18.3 ^{c} (0.76)	19.6 ^{bc} (0.81)	20.3 ^{b} (0.84)	21.9 ^{a} (1.12)	***
18:0	8.2 ^{a} (1.09)	7.4 ^{a} (0.83)	6.1 ^{b} (0.46)	5.4 ^{b} (0.48)	5.7 ^{b} (0.47)	***
Total saturated	25.0 ^{b} (2.18)	27.7 ^{a} (1.39)	27.6 ^{a} (1.41)	28.0 ^{a} (0.91)	29.9 ^{a} (1.12)	**
16:1	5.0 ^{a} (0.72)	3.4 ^{b} (0.27)	4.0 ^{ab} (0.54)	4.4 ^{ab} (0.70)	4.5 ^{ab} (0.55)	*
17:1	0.4 (0.14)	0.5 (0.04)	0.4 (0.16)	0.5 (0.10)	0.5 (0.06)	ns
18:1	51.0 (0.96)	52.1 (1.60)	52.0 (1.54)	51.1 (1.49)	49.9 (2.03)	ns
20:1	0.9 ^{b} (0.19)	2.0 ^{a} (0.23)	1.7 ^{b} (0.21)	1.8 ^{ab} (0.31)	1.6 ^{b} (0.26)	***
Total monounsaturated	57.3 (1.74)	58.0 (1.95)	58.1 (1.39)	57.9 (1.92)	56.8 (1.72)	ns
18:2	16.1 ^{a} (1.14)	12.8 ^{b} (2.10)	12.8 ^{b} (0.45)	12.1 ^{b} (1.02)	11.8 ^{b} (0.48)	**
18:3	1.7 (0.24)	1.4 (0.47)	1.6 (0.16)	2.0 (0.72)	1.7 (0.61)	ns
Total polyunsaturated	17.7 ^{a} (1.14)	14.2 ^{b} (2.54)	14.3 ^{b} (0.44)	14.1 ^{b} (1.62)	13.6 ^{b} (0.67)	**

On the same row, means with different superscripts differ significantly. Significance levels: ns, not significant, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Values in the brackets are standard deviations.

proportion of saturated fatty acids and a similar proportion of oleic acid.

Variance analysis of the data obtained in the three oxidation tests performed in our study showed a significant effect of processing time. Moreover these differences were not related to the processing time but to fluctuations from one stage of processing to another (Table 1).

4. Discussion

4.1. Time-course of lipolysis in adipose tissue of Corsican dry-cured ham

The lipids of the adipose tissue of Corsican ham were subjected to a tremendous hydrolysis during processing. The rate of lipolysis was extremely high during the first 6 months of processing causing a correlative increase of FFA, diacylglycerols and, to a lesser extent of monoacylglycerols. These results are in good agreement with those previously published on Italian and Spanish hams (Cantoni et al., 1970a; Melgar et al., 1990; Moltilva et al., 1993). The intense lipolysis is attributed to the activity of both basic and neutral lipases, which were probably lipoprotein and hormone-sensitive lipases respectively.

In Corsican ham, lipolysis remained active up to 24 months as suggested by the slow increase in free fatty acid content. The slower rate of lipolysis after 6 months of processing could be explained either by a disappearance of lipoprotein lipase activity at 5 months or by a drop in neutral lipase activity (Moltilva et al., 1993). Note that the amount of diacylglycerols produced during lipolysis is far higher than that of monoacylglycerols, confirming previous results (Moltilva et al., 1993). These results demonstrate that the activity of triacylglycerol lipases is the limiting step of lipolysis in adipose tissue. Belfrage et al. (1984) note that hormone sensitive lipases, which hydrolyse both triacylglycerols and diacylglycerols, exhibit a lower affinity for the latter.

4.2. Evidence of preferential hydrolysis of triacylglycerols

The proportion of stearic acid decreased in the FFA during processing suggesting that it was poorly hydrolysed from triacylglycerols. In contrast, FFA fraction became richer in palmitic acid. This is consistent with the data obtained on triacylglycerols which showed preferential losses of PPO, OOL and POL during processing. No data are available on the specificity of endogenous lipases of adipose tissue according to chain length and unsaturation of fatty acids (Toldra and Verplaetse, 1994). We can postulate that the physical state of triacylglycerols could affect their rate of lipolysis. Indeed, the triacylglycerols containing two saturated fatty acids such as PSO, PPO, are solid at the temperature of dry-cured ham ripening (14–18°C) (Gunstone et al., 1994) which limits their hydrolysis by lipases. In contrast, the triacylglycerols such as POL, OOL and POO are in liquid state at 14–18°C (Gunstone et al., 1994) which would favour the action of lipases at the water-oil interface.

The decrease in POL proportion during processing suggested a preferential hydrolysis of polyunsaturated fatty acids in adipose tissue confirming the results of several authors (Flores et al., 1988; Melgar et al., 1990; Moltilva et al., 1993). But the proportion of linoleic acid decreased in FFA during processing which suggests that part of the linoleic acid was oxidised during processing. This hypothesis is supported by the fact that polyunsaturated fatty acids are more subject to oxidation than saturated and monounsaturated ones (Frankel, 1984; Gandemer, 1990) and that FFA are more sensitive to oxidation than the corresponding triacylglycerols (Nawar, 1996). The latter explanation is corroborated by the results obtained in this study which indicated that the decrease in linoleic acid proportion was marked in FFA while it remained unchanged in total lipids. The absence of change in the results given by the three oxidation tests is not in contradiction with the decrease in linoleic acid during processing. Indeed, all the previous studies indicated that the level of lipid oxidation in adipose tissue increased during the first 6 months of processing and remained steady or decreased up to the end of the process (Flores et al., 1985). In fact, the decrease of linoleic acid in adipose tissue during processing indicates how much this fatty acid was oxidised, while the data from the oxidation tests give a finger print of the oxidation products in the adipose tissue at the moment of the measurement. Oxidation products of fatty acids have a short life because some of them are volatiles (carbonyls) (Antequera et al., 1992) and/or can react with other components of ham such as proteins (Pokorny, 1981).

5. Conclusion

The subcutaneous adipose tissue of dry-cured ham was subjected to intense lipolysis during the 24 months of processing, especially in the first 6 months. The intense lipolysis could promote lipid oxidation as shown by the decrease in linoleic acid during processing. Both lipolysis and oxidation cause large changes in physical and chemical traits of adipose tissue, which could affect its sensory properties, mainly colour and flavour. Coutron (1996) reported that fat of Corsican dry-cured ham became yellow and had a rancid aroma and taste at the end of a 24 month process.

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