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Changes in the Fatty Acid and Triacylglycerol Profiles in the Subcutaneous Fat of Iberian Ham during the Dry-Curing Process

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In this study, we have evaluated the changes that occur in the profiles of total fatty acids and triacylglycerols during the dry-curing process (730 days) of Iberian ham. The subcutaneous adipose tissues of six hams obtained from three Iberian pigs fed on acorns were analyzed periodically during the processing time (from the raw to the dry-cured samples), including postsalting, drying, and ripening stages. The environmental conditions were also registered. The curing process significantly decreased ($p < 0.01$) the relative percentages of total polyunsaturated fatty acids, including C18:2n-6 and C18:3n-3 and, therefore, significantly increased ($p < 0.05$) the level of monounsaturated fatty acids. The triglycerides containing 0−2 double bonds showed an increase during the curing process. On the contrary, the more unsaturated ones (3−5 double bonds) suffered a significant decrease. We have postulated that these changes could also be due to polymerization and oxidation reactions that affect the triacylglycerols and besides the fatty acids. In general, most fatty acids and triacylglycerols reversed the trend by about 500−600 days of processing.

KEYWORDS: Iberian dry-cured hams; subcutaneous fat; fatty acids; triacylglycerols

INTRODUCTION

Iberian dry-cured ham is a high-fat content product submitted to a long period of dry curing (from 1 to 2 years). Dry curing is a typical way of manufacturing raw ham in the Mediterranean area, which includes a dry salting stage at low temperature and a long ripening stage at high temperature. A detailed description of the curing process including the main changes affecting the protein and lipid fractions can be found elsewhere (*1*). Among the chemical changes occurring during this process, those affecting lipids are of great relevance, since they play a key role in the nutritional and sensory properties of the final product (*2*). The subcutaneous fat of Iberian dry-cured ham contains 75−80% lipids, 5−15% water, and small proportions of protein and collagen. The lipid fraction is mainly composed of triacylglycerols (TGs), a small amount of cholesterol, and degradation products of TGs (*2*). The lipid fraction is mainly affected by hydrolysis and oxidation reactions, which cause the degradation of the adipose tissue lipid fraction (*3, 4*). Hydrolysis mainly affects TGs and diacylglycerols and to a lesser extent to monoacylglycerols and phospholipids (*5*). The activity of adipose tissue lipases has been reported to be restricted by the process temperature (*6*). On the other hand, chemical oxidation of fatty acids results in oxidized compounds that have a short life because some of the lipid-derived compounds are volatiles, and some of them transfer to the ambient contributing to the aroma in cellar but others are in the product (*7*). The lipolytic and oxidative changes, and finally the sensory quality of the final product, can also be affected by the length of the maturing process (*8, 9*) and by the quantitative and qualitative composition of lipids in muscular and adipose tissue, which vary according to the rearing system of the animals (*10*).

Several studies have been conducted to describe the main changes occurring in the lipid fraction of the intramuscular fat of Iberian pigs during the dry-curing process (*10–14*); however, few have been conducted on subcutaneous fat (*3, 5, 6, 11, 13*). In this sense, Delgado et al. (*11*) described the composition of the fresh and the corresponding dry-cured hams from Hairless Mexican Pigs, a species developed from the Iberian pig. They found that curing increased saturated fatty acids and decreased the monounsaturated and polyunsaturated fatty acids (PFAs). Moreover, they found that the percentage of TGs underwent a reduction during the curing process. Coutron-Gambotti and Gandemer (*3*) investigated the changes in lipids of subcutaneous adipose tissue during the ripening of Corsican dry-cured ham. The TGs, determined by high-performance liquid chromatography (HPLC), decreased sharply during the first 6 months and then more slowly between 6 and 24 months. A significant decrease was observed particularly for dioleoyl-palmitoyl glycerol (POO) ($p < 0.001$), palmitoyl-oleoyl-linoleoyl glycerol

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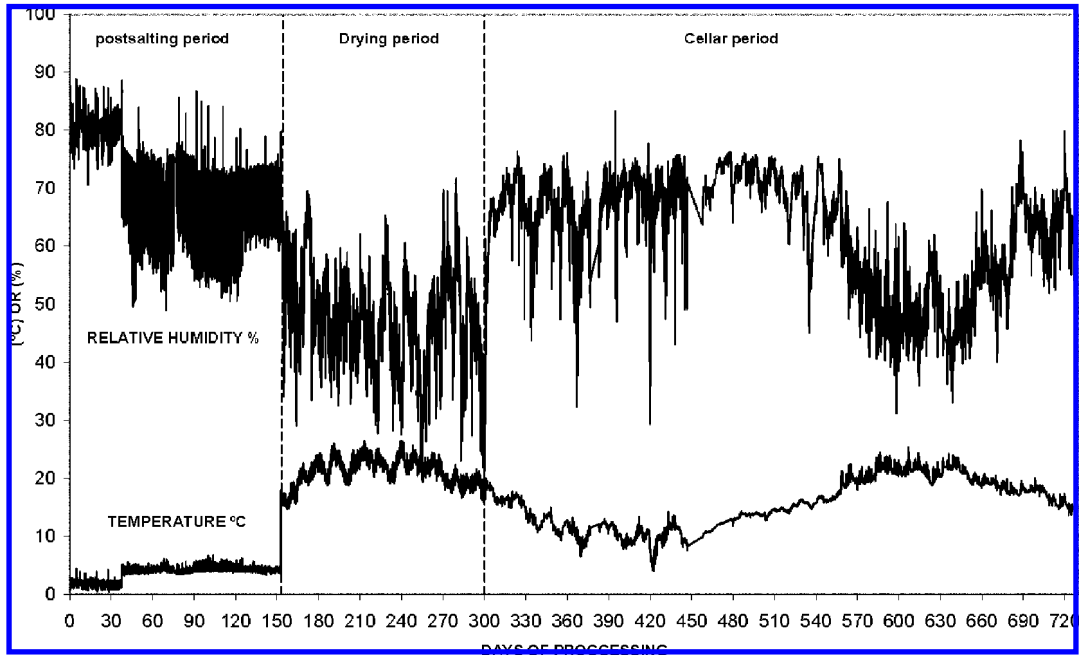

Figure 1. Evolution of the environmental temperature and relative humidity during ripening of Iberian dry-cured ham.

(POL) ($p < 0.001$), and dioleoyl-linoleoyl glycerol (OOL) ($p < 0.01$) during processing. However, the total fatty acid content, determined by gas chromatography (GC), was roughly constant during processing. The proportion of PUFAS decreased because of the decrease in the proportion of linoleic acid (C 18:2 n-6). This decrease was marked during the first 6 months. The proportion of monounsaturated fatty acids (MUFAs) decreased as well. In contrast, the proportion of saturated fatty acids (SFAs) increased because palmitic acid (C16:0) increased.

A detailed follow up of the dry-curing process, based on measurements of TGs and fatty acids, of Iberian hams produced from animals fed extensively on acorn and pasture during a long dry-curing process (2 years) can not be found in the literature. These changes are related to the final sensory properties of the dry-cured ham. In this sense, the aim of this work was to undertake a comprehensive study of the changes occurring in the fatty acid and TG fractions of the subcutaneous fat during the dry-curing process of Iberian ham.

MATERIALS AND METHODS

Processing and Sampling of Hams. Six hams (between 8.8 and 9.1 kg) were obtained from three castrated Iberian pure 14 month old male pigs, were fattened extensively with acorns and pasture for 90 days prior to slaughter, and were processed in an industry for 24 months. The stages and the procedures for dry curing have already been explained in a previous paper (*5*). The environmental conditions (temperature and relative humidity) were recorded continuously throughout the whole period of maturing (*5*), and for better comprehension, data are reproduced in **Figure 1**. Samples for analysis were taken from the subcutaneous adipose tissue as described previously (*5*) and melted in an oven microwave (*15*).

Fatty Acids and TGs Analysis. Fatty acid methyl esters (FAMEs) were analyzed by GC. FAMEs were extracted with *n*-hexane after cold methylation with 2 N KOH in methanol, following the official method (*16*). GC was performed with a Varian 3900 apparatus (Varian Co., Palo Alto, CA) using a fused silica capillary HP 88 column (100 m × 0.25 mm, 0.25 μm film thickness). The oven temperature was kept at 175 °C for 13 min and was then raised to 205 °C at a rate of 3.0 °C/min and held isothermally for 5.0 min. The injector temperature was kept at 240 °C, while the detector temperature was 250 °C. Hydrogen (131 kPa inlet pressure) was used as a carrier gas, while the makeup gas was nitrogen.

Table 1. Repeatability Results of Fatty Acid and TGs of Raw Ham Samples

	mean ($n = 6$)	SD	RSD (%)
fatty acid[a]			
C14:0	1.19	0.01	0.78
C16:0	22.54	0.10	0.45
C16:1n-6	1.88	0.07	3.63
C17:0	0.35	0.00	1.27
C17:1n-7	0.30	0.00	1.06
C18:0	11.34	0.05	0.44
$C18:1_{total}$	57.35	0.12	0.21
C18:2n-6	7.03	0.03	0.36
C18:3n-3	0.45	0.01	1.19
C20:0	0.20	0.01	3.49
C20:1n-9	1.45	0.06	4.34
TG			
PPP	0.26	0.02	7.94
MOP	0.92	0.01	1.03
PPS	0.91	0.02	2.23
POP	6.33	0.03	0.53
POPo + PLP	2.39	0.36	15.26
PLPo + MLO	0.82	0.03	3.26
PSS	1.32	0.04	2.94
PSO	17.72	0.07	0.40
POO	37.51	0.12	0.33
PLO	9.54	0.03	0.34
PLL + PoLO	0.96	0.03	2.76
SOS	1.57	0.03	2.14
SOO	6.21	0.05	0.78
OOO	7.42	0.05	0.72
SOL	2.72	0.06	2.17
OOL	3.07	0.02	0.61
OLL	0.34	0.04	12.06

[a] Expressed as %.

To identify the fatty acids, standards of each fatty acids, purchased from Sigma-Aldrich (St. Louis, MO), were used as follows: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n-6), margaric (C17:0), *cis*-10-heptadecenoic acid (C17:1n-7), stearic (C18:0), elaidic (C18:1n-9 *trans*), oleic (C18:1n-9 *cis*), linoleic (C18:2n-6), arachidic (C20:0), linolenic (C18:3n-3), and *cis*-13-eicosenoic acid (C20:1n-9). Identification of fatty acids in the samples was carried out by comparing retention times for standards and samples. The quantification of individual fatty acids was carried out by evaluating the corresponding

Table 2. Effect of Dry Curing on Fatty Acid (FA) and TG Profiles[a]

FA	raw ($n = 3$)	dry-cured ($n = 3$)	TG	DBN	raw ($n = 3$)	dry-cured ($n = 3$)	ΣDBN	raw ($n = 3$)	dry-cured ($n = 3$)
C14:0	1.22 + 0.07 b	0.96 + 0.05	PPP	0	0.09 + 0.02 b	0.25 + 0.01	DBN 0	1.19 + 0.13 a	2.32 + 0.45
C16:0	21.01 + 0.89	19.69 + 0.12	MOP	1	0.58 + 0.16	0.66 + 0.08	DBN 1	18.35 + 0.33 b	24.05 + 1.76
C16:1n-6	1.84 + 0.14	1.64 + 0.19	PPS	0	0.34 + 0.07 b	0.91 + 0.15	DBN 2	46.38 + 0.99	45.58 + 0.64
C17:0	0.26 + 0.19	0.23 + 0.01	POP	1	4.30 + 0.36 a	5.20 + 0.16	DBN 3	25.68 + 0.77 a	22.72 + 1.20
C17:1n-7	0.22 + 0.03	0.18 + 0.01	POPo + PLP	2	2.27 + 0.32 b	2.93 + 0.06	DBN 4	7.15 + 0.26 c	4.82 + 0.34
C18:0	9.21 + 0.55	10.28 + 0.63	PLPo + MLO	3	0.62 + 0.04	0.71 + 0.09			
$C18:1_{total}$	55.65 + 0.88 a	57.36 + 0.21	PSS	0	0.62 + 0.08 a	1.18 + 0.31			
C18:2n-6	8.18 + 0.30 b	7.04 + 0.13	PSO	1	10.74 + 0.23 b	16.85 + 1.68			
C18:3n-3	0.57 + 0.03 b	0.45 + 0.02	POO	2	37.73 + 1.36	36.52 + 0.65			
C20:0	0.15 + 0.02	0.18 + 0.02	PLO	3	12.22 + 0.33 a	8.80 + 0.65			
C20:1n-9	1.68 + 0.10	1.63 + 0.12	PLL + PoLO	4	0.90 + 0.10 a	0.96 + 0.12			
SFA	31.85 + 1.13	31.35 + 0.54	SOS	1	0.90 + 0.07	1.26 + 0.16			
MUFA	59.39 + 0.82 a	61.15 + 0.45	SOO	2	6.03 + 0.42	5.94 + 0.24			
PUFA	8.76 + 0.33 b	7.49 + 0.14	OOO	3	11.49 + 1.06	11.02 + 0.35			
			SOL	3	4.19 + 0.08 c	2.24 + 0.12			
			OOL	4	5.94 + 0.31 b	4.17 + 0.24			
			OLL	5	1.01 + 0.30 a	0.39 + 0.16			

[a] Data are means + SDs ($n = 3$). ΣDBN, total content of TGs with the same double bond number (DBN). Different letters indicate significant differences between raw and dry-cured data, with a for $p < 0.05$, b for $p < 0.01$, and c for $p < 0.001$.

relative percentage according to the normalization area procedure assuming an equal factor response for any species.

TGs were analyzed by GC (*17*) in a Varian 3800 (Varian Co.) using a fused silica capillary DB-17HT column (30 m × 0.32 mm i.d., 0.15 μm film thickness). The oven temperature was kept at 330 °C and was then raised to 360 °C at a rate of 2.0 °C/min and held isothermally for 10 min. The injector temperature was kept at 360 °C, while the detector temperature was 370 °C. Hydrogen (5.3 mL/min column constant flow) was used as a carrier gas, while the makeup gas was nitrogen.

The assignment of the chromatographic peaks was done by means of standards that were purchased from Sigma-Aldrich of trilinolein (LLL), triolein (OOO), tripalmitin (PPP), and tristearin (SSS). The carbon number of the components associated with each peak group as well as the difference between the retention times of the TGs was calculated as described elsewhere (*17*). The quantification of individual TGs was carried out by evaluating the corresponding relative percentage according to the normalization area procedure assuming an equal factor response for any species.

RESULTS AND DISCUSSION

Repeatability of the Methods. The repeatability of the methods was determined in a representative raw sample, not corresponding to any of the ones included in the evolution study. These results are shown in **Table 1**; they indicate a good repeatability for the assay.

Fatty Acid and TG Profiles in the Raw and Dry-Cured Ham. Table 2 shows the mean values of the fatty acid and TGs analyzed in the subcutaneous fat (as relative percentage of total fatty acid) corresponding to the raw and dry-cured stages. Several interesting observations can be inferred from these data. First, the percent of total SFA remains quite constant during the curing process. This result is in agreement with previously published data related to the subcutaneous fat (*3, 7*) and intramuscular fat during the ham-curing process (*11–14*). However, dry curing significantly decreased ($p < 0.01$) the relative percentage of myristic acid, as observed also by Larrea et al. (*13*) in the subcutaneous fat of Teruel ham. This result is not in accordance with previously published data by Motilva et al. (*6*), which reported an increase of this fatty acid after 15 months of curing. In the case of Iberian dry-cured hams, most of the published data are related to intramuscular fat (*11–14*), for which an increase of myristic acid during the curing process has been reported. Second, the dry-curing process significantly increased ($p < 0.05$) the total relative percentage of MUFA, due to a significant increase in the oleic acid ($p < 0.01$). Previously published data for MUFA in the subcutaneous fat after dry curing have reported either no significant change after 24 months of curing (*3*) or even a significant decrease after 6−8 months of dry curing (*11, 13*). Third, dry curing significantly decreased ($p < 0.01$) the total relative percentage of PUFA. This result is in agreement with previously published data for subcutaneous and intramuscular fat of ham (*3, 11–14*).

The TGs profile of the subcutaneous fat in the raw and dry-cured ham is shown in **Table 2** (as relative percentage of TGs). It can be observed that the dry-curing process increases the relative percentages of some molecular species containing two or more saturated fatty acids, such as PPP, PPS, POP, PSS, PSO, and PLP (which elutes together with POPo). On the other hand, the species PLO, SOL, OOL, and OLL decreased significantly during the dry-curing process, while other species such as MOP, PLPo + MLO, POO, SOS, SOO, and OOO do not suffer any significant change. Coutron-Gambotti and Gandemer (*3*) in Corsican ham also reported a significant decrease after 24 months of dry curing in the diunsaturated, triunsaturated, and OOL TGs. It has been suggested that the decrease in the molecular species of triacyglycerols containing linoleic acid could be due to a preferential hydrolysis of PFAs (*3, 6, 8*). In the same way, the decrease in the proportion of linoleic acid could be related to the higher oxidation rate of the fatty acids containing double bonds (*3*). In accordance to this fact, when the TGs are grouped according to the number of double bonds (DBN), as shown in **Table 2**, it can be observed that TG with DBN 0 and 1 are significantly increased at the end of the dry-curing process, while the less unsaturated DBN 2 remains unchanged, and the more unsaturated ones, DBN 3, 4, and 5, are significantly decreased.

Evolution of Fatty Acid during the Dry-Curing Process. Figure 2 shows the evolution of the individual fatty acids from the raw to the dry-cured sample. During the dry-curing process, it has been reported that the main changes occurring in the subcutaneous fat of Corsican ham are due to preferential lipolysis of unsaturated TGs and oxidative reactions occurring in unsaturated fatty acids (*3*). However, in a previous paper, we have demonstrated that in Iberian ham the hydrolytic process is not selective and it does not affect mainly the unsaturated TGs. On the other hand, it is quantitatively of scanty relevance since the level of mono- and diacylglycerols formed during the process is about 35 mg/100 g (*5*). Besides, the phospholipids are the main substrates of the lypolitic activity of enzymes

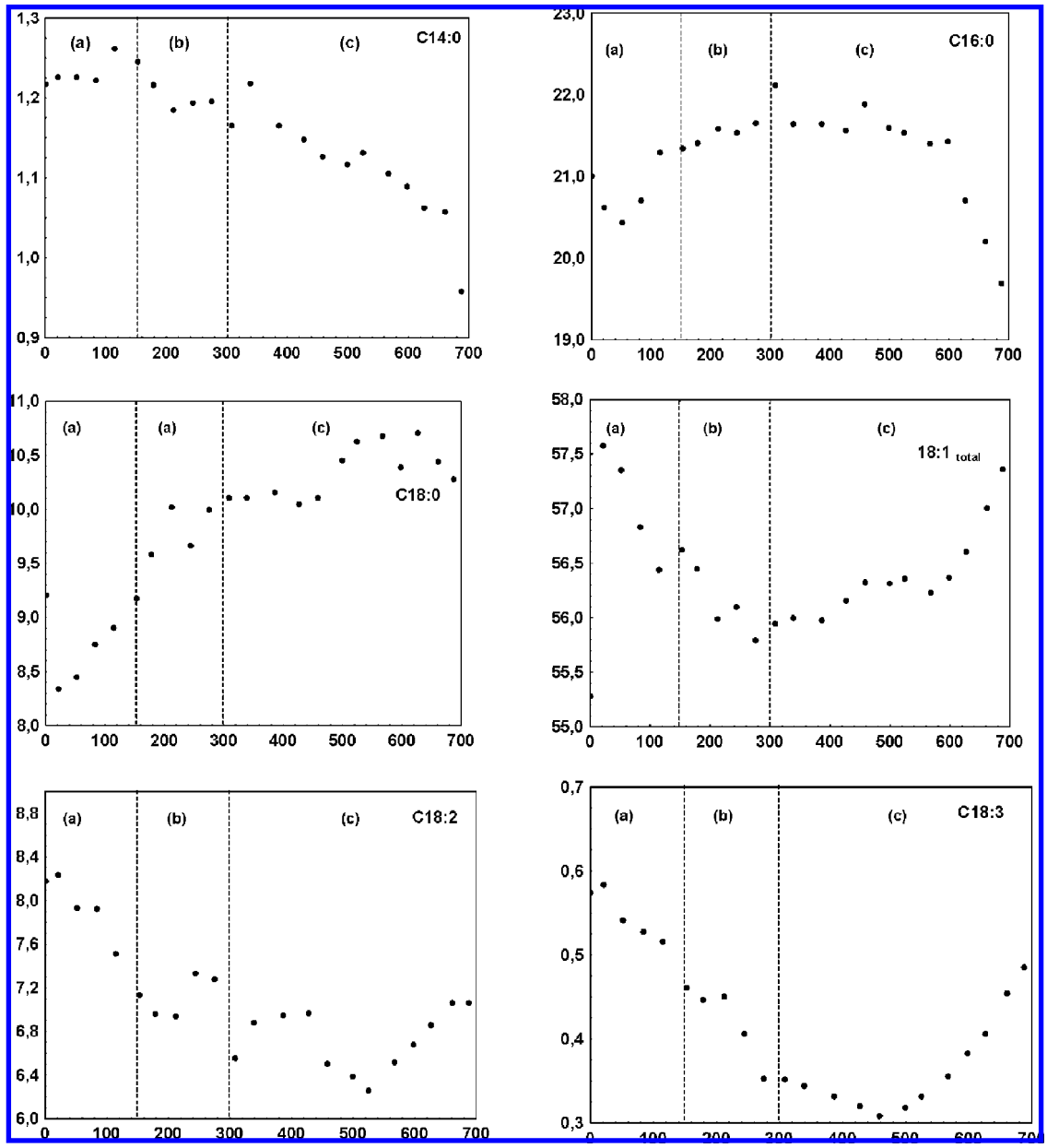

Figure 2. Evolution of different fatty acids as a relative percentage of total fatty acid vs time of dry curing (in days). (**a**) Postsalting period, (**b**) drying period, and (**c**) cellar period. The values represents the means of three replicates.

during the ripening process. They are present at low levels (0.46 g/100 g) in the subcutaneous fat, which is composed of 99% by TG (*18*). It is therefore logical to suppose that the main changes occurring in the profile of fatty acids are due to oxidation and polymerization of unsaturated fatty acids rather than to hydrolysis of TGs or changes affecting the phospholipids fraction.

Although the level of total fatty acids remains almost constant during the curing process (**Table 2**), we can observe significant changes in the profile of the main fatty acid during the three stages of the process. As shown in **Figure 2**, during the postsalting period (150 days), the saturated fatty acids (C16:0 and C18:0) show a similar increasing behavior (1.81 and 1.18%, respectively) except for C14:0, which remains constant in this period. On the contrary, the unsaturated fatty acids (C18:1_{total}, C18:2n-6, and C18:3n-3) behave in the opposite way (1.21, 1.62, and 0.18% of decrease, respectively). During the drying period (from 150 to 300 days), the same trend of the postsalting period is observed, except for the C14:0, for which a 0.05% of decrease is observed. During the cellar period (from 300 to 720 days), different behaviors are observed. The saturated C16:0 and C18:0 show the same trend described in the previous stage up to the end. In the case of C16:0, the levels remain almost constant up to 500 days; after this point, the levels show a deep decrease (1.90%). As final consequences, the SFA fraction of the subcutaneous fat remains quite stable during the curing process, in accordance with previous research by Larreat et al. (*13*). However, other authors have reported an increase in the SFA fraction of the intramuscular fat during the curing process (*12*).

The deep change in the trend observed in the period between 500 and 720 days of curing is coincident with a decrease in the % of relative humidity in the cellar and an increase in temperature (**Figure 1**). Particularly this change in the trend is also observed in the PUFAs C18:2n-6 and C18:3n-3, for which a decreasing trend is observed during the initial stages of the cellar period (from 300 to 500 days), but at the final stages in the cellar (from 500 to 720 days), this trend changes (**Figure 2**). However, at the end of the process, curing significantly decreased ($p < 0.001$) the level of PUFAs. The opposite profile

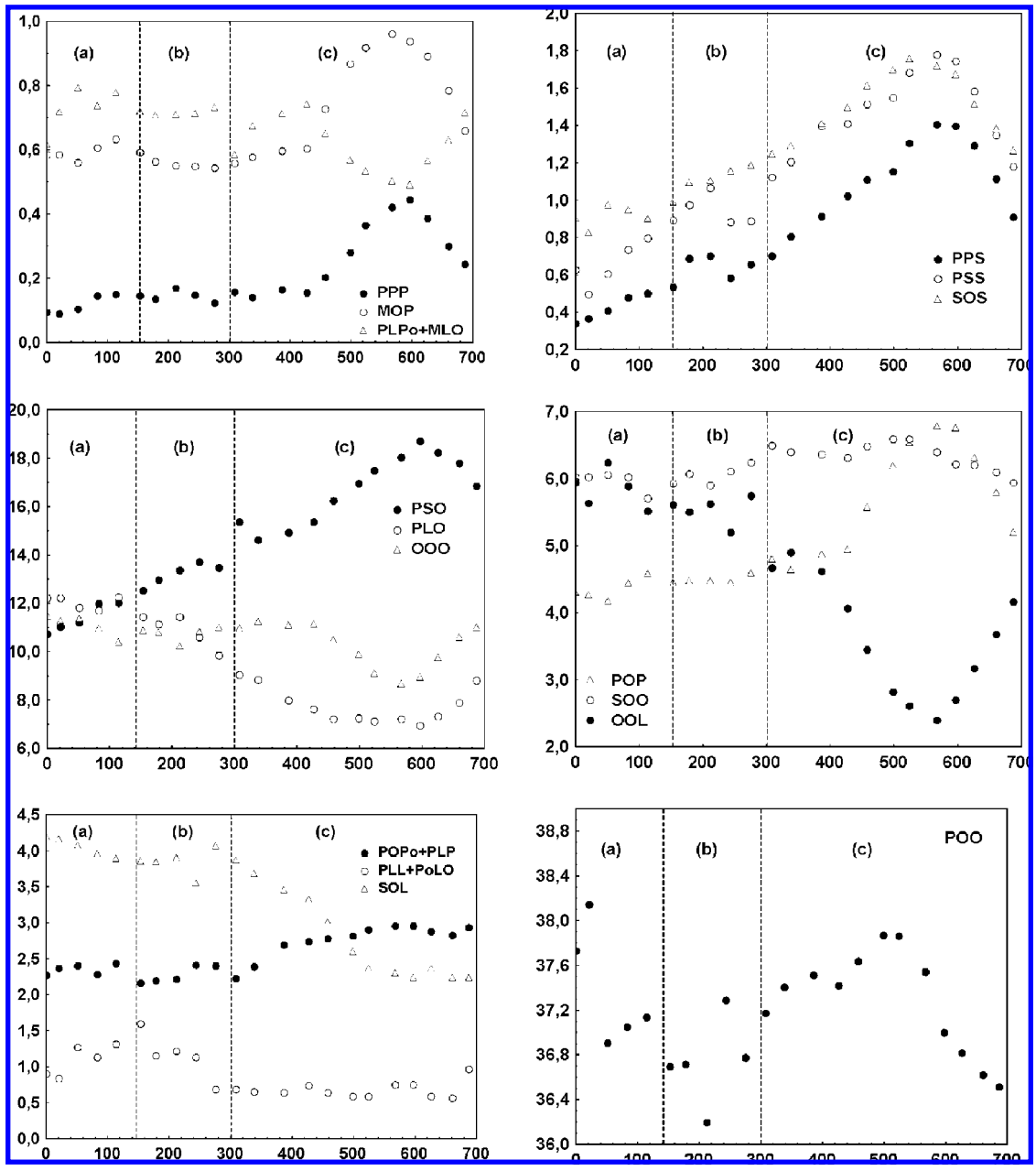

Figure 3. Evolution of different TGs as a relative percentage of total TGs vs time of dry-curing (in days). (**a**) Postsalting period, (**b**) drying period, and (**c**) cellar period. The values represents the means of three replicates.

of evolution is observed in oleic acid, which is the major monounsaturated fatty acid, whose levels are significantly increased ($p < 0.05$) by the curing process (**Table 2**).

Evolution of TGs Profiles during the Dry-Curing Process. **Figure 3** shows the evolution of the TGs during the dry-curing process. As a general remark, the individual TG profile during the two first periods (postsalting and drying) remains quite constant. This is in accordance with previous studies by Motilva et al. (*6*), indicating that during salting and postsalting stages the lipase activities are restricted by the low process temperatures. During the cellar period, as described for fatty acids, a deep change in the trend is observed in a narrower period of time from 500 to 550 days of curing, which is coincident with an increase in temperature. Similar evolution profiles are observed for PPP, MOP, PPS, POP, PSS, PSO, and SOS, with an increasing trend to reach a maximum level about 500−550 days of curing and then a decrease to levels slightly higher than the initial value. This is the same evolution observed when the TGs are grouped by DBN 0 and 1 (**Figure 4**). On the other hand, the diunsaturated PLO and SOL and the triunsaturates OOL and OLL showed the opposite profile. A decreasing trend is observed (**Figure 3**) during the whole process, reaching the lowest level between 500 and 550 days of curing, and then, the levels increased slightly; the final values were significantly lower than the initial ones. This evolution is coincident with DBN 3 to DBN 5. It has been postulated (*3*) that the lipolysis is affected by the physical state of the TGs. This could explain that saturated TGs (DBN 0, DBN 1, and DBN 2) such as PSO and POP, which are solid at the dry-curing temperature, are not affected by the lipolytic process. On the contrary, the more unsaturated ones (DBN 3 to DBN 5), which are liquid at the dry-curing temperature, are more affected by the lipolysis, suffering a significant decrease during the curing process. However, as commented previously, this fact has not been observed for Iberian ham subcutaneous fat (*5*); besides, the amount of mono- and diacylglycerols formed during the curing

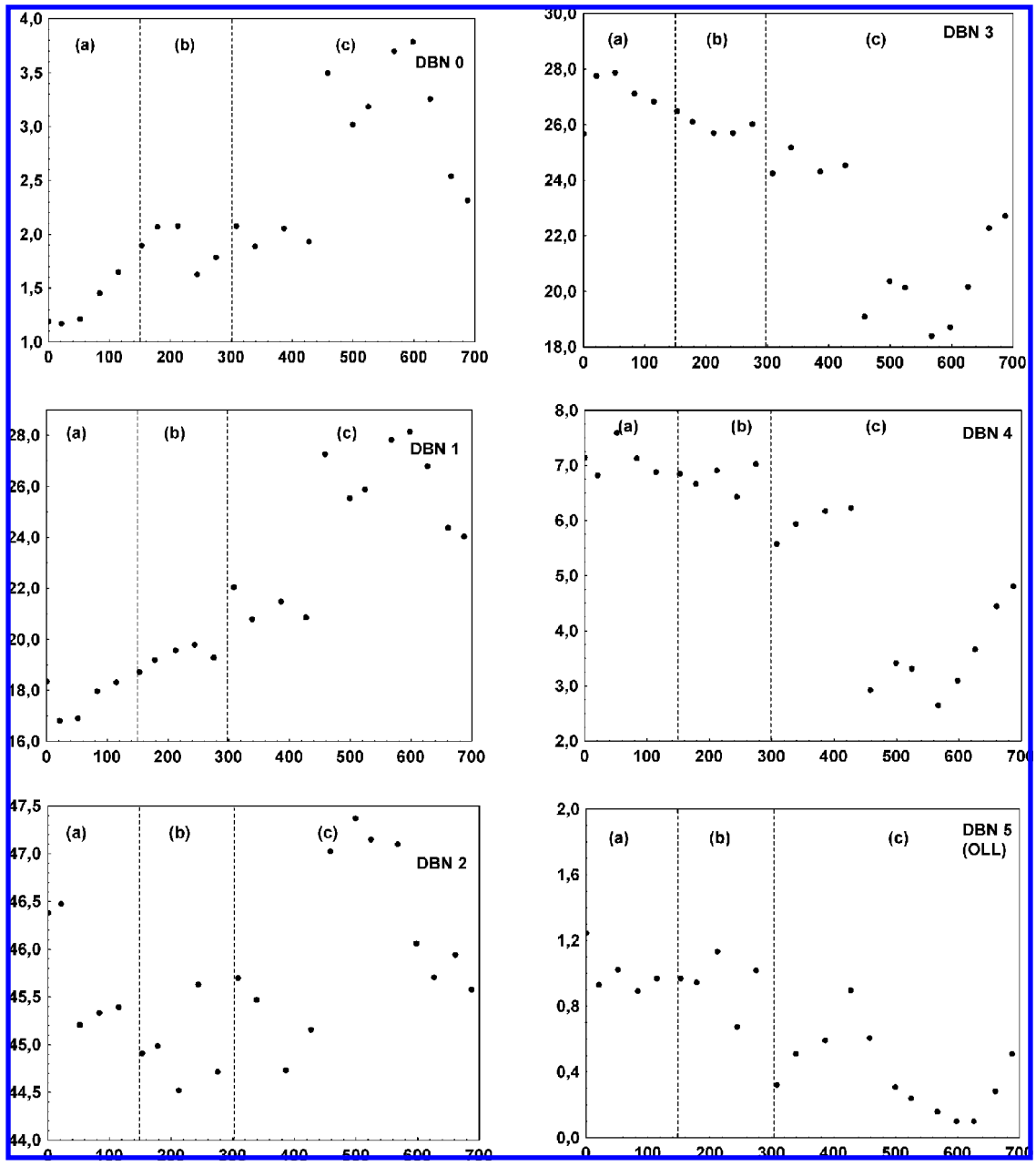

Figure 4. Evolution of different tryacylglycerols gruping as DBN vs time of dry-curing (in days). (**a**) Postsalting period, (**b**) drying period, and (**c**) cellar period. The values represents the means of three replicates.

process does not support the changes in the TGs profile. So, we have postulated that these changes could also be due to polymerization and oxidation reactions affecting not only fatty acids but also TGs.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; GC, gas chromatography; FAME, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PFA, polyunsaturated fatty acids; TG, triacylglycerols; DBN, double bond number; M, myristic; P, palmitic; Po, palmitoleic; S, stearic; O, oleic; L, linoleic.

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