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Triglyceride composition of fresh ham fat from Iberian pigs produced with different systems of animal nutrition

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A method based on triglyceride composition for evaluating the nutrition received by Iberian pigs (Ib) is presented. Triglycerides were extracted with chloroform from subcutaneous fat of *fresh ham* and triglyceride percentages were determined by reverse-phase HPLC with a light scattering detector. The main triglycerides identified were: POO, POS, OOO, POL, OOL, POP and POS (P, palmitic; S, stearic; O, oleic; L, linoleic). *Montanera* is a nutrition procedure for Iberian pigs based, during the last 2 or 3 months of life, on acorn fruits in a semi- or complete extensive mode. The intensity of *montanera* diet, which produces the most appreciated cured hams, was evaluated. Four groups of animals with different feeding systems were studied: (I) Ib produced by *montanera* exclusively; (II) Ib *montanera* supplemented with mixed feeds (850 g/day); (III) Ib *montanera* supplemented with mixed feeds (250 g/day); and (IV) Ib mixed feeds only. Significant differences were obtained between Iberian pigs with different intensities of *montanera* for triolein (OOO) and OOL. The OOL/SOO and OOO/SOO ratio indices showed clear differences between the four groups studied. The method was easy to apply and did not need saponification and the formation of methyl esters. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Iberian hams are the most appreciated cured hams produced in Spain because of their sensorial properties. Iberian pigs are produced in southwestern regions of Spain and their production is mainly of extensive mode, based on *montanera* that consists of the exploitation of grass and acorns. In this production system there are three different procedures applied about 2 months before the sacrifice of the animals: (a) exclusive consumption of grass and acorns, the animals produced by this way are referred to as *de bellota*; (b) the same system as (a) but including supplementation with mixed feeds; the animals are called, in this case, *recebo*; and (c) grass only, without acorns, with mixed feeds, the animals being referred to as *cebo*. *Montanera* applied in mode (a) is the system that produces the best quality hams and thus the pigs produced have the highest commercial value. The meat industry has shown an interest in classifying the animal nutrition received by Iberian pigs according to objective measurements in order to regulate the market. However, it is not possible to apply the *montanera* system with the same characteristics each year because the acorn production is dependent on many factors (climate, rain). It will therefore be very interesting to establish the intensity of *montanera* according to kilogrammes of acorns ingested by pigs.

Several approaches have been applied in order to determine the nutrition received by Iberian pigs. All the procedures are based on fat properties: melting and slip point, iodine value and fatty acid profiles from different fat depots. These methods are based on the fact that *montanera* produces pigs containing higher percentages of oleic acid than *recebo* and *cebo*. Osorio *et al.* (1983) determined fatty acids in samples from different fat depots; they found significant differences between nutrition and fatty acids, with a higher oleic acid content in *montanera* pigs. Díaz *et al.* (1986) reported differences between white pigs and Iberian pigs fed with the two systems: acorn + mixed feeds and mixed feeds only; the oleic acid percentage (58.4%) was higher in Iberian pigs fed with acorns, but palmitic acid showed lower values than in the other group evaluated. Flores

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et al. (1988) reported that the palmitic acid percentage was lower in *montanera* than in *recebo* and *cebo* Iberian pigs; however, oleic acid showed higher percentages (53.95%) in *montanera* pigs than in *recebo* and *cebo* pigs; through multivariate analysis it was possible to distinguish the animal nutrition but the classification of *recebo* and *cebo* animals was critical. López *et al.* (1990) studied fatty acid composition in liver, muscle and fat of Iberian pigs produced by the three systems; fatty acids (myristic, palmitic, palmitoleic, oleic and linoleic acids) showed significant differences in the liver; however, in fat and muscle, oleic and linoleic acids, respectively, did not differ; in this study, the possibility of using refractive index and total fat contents of liver samples was noted. De Pedro & Casillas (1991) and De Pedro & Secondi (1991) evaluated the quantity of *montanera* received (acorn quantity) on the basis of fatty acid percentages and they proposed a new procedure based on the NIR analysis.

Triglyceride determination has becomed as feasible by HPLC as by capillary GC (Geeraert & Sandra, 1985; Stolywho *et al.*, 1985; Herslof & Kindmark, 1985; Perrin & Prévot, 1986; García Regueiro *et al.*, 1994). The triglyceride composition could be a more appropriate system to characterize oils and fats, avoiding the use of saponification and the formation of methyl esters. In this work an alternative method for determining *montanera* intensity based on triglyceride analysis is presented.

MATERIALS AND METHODS

Animals

Table 1 shows the four groups of Iberian pigs studied and the nutrition procedures used. The main component in mixed feeds were cereals (70%), other components were: starch 6.0%, oleaginous tart 17.3%, meat meal 3.8%, minerals 1.1%, animal fat 0.6% and vitamins 0.4%. The mean weight of the four groups of animals was 160 kg and the age at the sacrifice was 15 months.

Triglyceride analysis

Fat samples were taken from subcutaneous fat of fresh ham and were cut into small pieces (5 mm, prismatic form); they were then introduced to a microwave oven (1500 W) to allow them to melt; the liquid fat collected was stored at −25°C until analysis. Five hundred milligrammes of extracted fat were extracted twice with 10 ml of chloroform and an aliquot of 2 ml was evaporated to dryness in a nitrogen stream. The residue was redissolved in 500 μl chloroform, and 5 μl of this solution was injected via a Rheodyne injector. The chromatographic conditions were: a reverse-phase column of octadecylsilica 5 μm particle, diameter (Hypersil, Shandon) 300×4 mm, at ambient temperature, mobile phase A acetonitrile and B dichloromethane with a gradient of B over A from 30 to 50% in 45 min at a flow of 1.5 ml/min. An LKB pump controlled via an LKB HPLC controller was used, gradient was formed at low pressure with a mixing valve. The detector used was a light scattering mass detector (ACS) at 70°C and an air pressure of 2.0 bar, and the chromatograms were recorded with an integrator (Spectra Physics) at an attenuation of 64. Peak identification was made using standards and ECN (equivalent carbon number; ECN = CN−2 NDB, CN carbon number and NDB number of double bounds) using the retention times and k values; where assignment of peaks was not possible they were numbered only.

Table 1. Characteristics of Iberian pig groups

Group	Nutrition[a]	Time[b]	Mixed feeds[c]
I	*bellota*	120	n.s.
II	*recebo*	128	850.
III	*recebo*	86	250
IV	*cebo*	—	—

[a]Kind of animal nutrition.
[b]Days of *montanera* duration.
[c](g of mixed feed/day/animal) supplement of mixed feeds for *montanera*.
n.s., not applied.

Fatty acids

Fat samples were extracted in the same way as for triglyceride analysis. An aliquot (50 mg) was taken to obtain methyl esters of fatty acids (FAMES) by transesterification with 0.5 M sodium methoxide in anhydrous methanol (Christie, 1989). FAMES were analysed in the following conditions: packed column coated with 10% DGS diethylglycolsuccinate and 1% H_3PO_4 on Chromosorb W, AW 80–100 mesh (2 m×3.2 mm), injector and detector temperatures 250°C, oven temperature 175°C; nitrogen was used as the carrier gas at 25 ml/min. The instrument used was a Perkin-Elmer Sigma 3D with FID detector coupled to a Sigma 10B integrator.

Statistical analysis

ANOVA was applied and the Bonferroni test was used. SAS software was used.

Abbreviations

Fatty acids: P, palmitic; S, stearic; O, oleic and L, linoleic. Nomeclature of triglycerides does not indicate the position of fatty acid in triglyceride molecule (ex.: POO, palmitodiolein, OOO triolein).

Chemicals

Triglyceride standard was purchased from Sigma (USA): POO, SOS, SOO, POP. Acetonitrile HPLC grade, dichloromethane HPLC grade and chloroform analytical grade were obtained from Merck (Germany).

Table 2. Fatty acids percentages in fresh ham fat of Iberian pigs

Fatty acid	I		II		III		IV	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
C14:0	1.16[a]	0.08	1.15[a]	0.07	1.25[a]	0.07	1.48[d]	0.12
C16:0	18.25[b]	0.82	19.44[a]	0.58	20.02[a]	0.85	24.18[c]	0.40
C18:0	2.21[a]	0.13	2.03[a]	0.13	2.30[ab]	0.21	2.58[b]	0.22
C18:1	57.13[a]	1.32	56.24[ab]	0.79	55.36[b]	0.87	47.38[c]	0.72
C18:2	9.38[a]	0.25	8.96[a]	0.52	9.18[a]	0.45	8.27[b]	0.21

$n = 10$.
Values in the same row with different superscript differ significantly ($P < 0.001$ Groups I, II, III and IV) (see Table 1).

RESULTS AND DISCUSSION

Table 2 shows the percentages of the main fatty acids of fresh ham fat of the four groups of animals. Group IV (*cebo*) showed a different composition from the other groups that were produced with different quantities of mixed feeds. In group IV palmitic and oleic acids showed the highest and lowest percentages, repectively. Group I (*bellota*), which was produced without mixed feeds, showed the highest percentage of oleic acid. These results agree with those reported previously by other authors (Flores *et al.*, 1988; Osorio *et al.*, 1992; De Pedro & Secondi, 1991). Group II, which was suplemented with 850 g of mixed feeds, showed a higher percentage of oleic acid than group III, which was supplemented with only 250 g of mixed feeds, this result may be related to the time spent in *montanera* which was longer in group II (128 days) than group III (86 days). Therefore the duration of *montanera* may be more important than the quantity of mixed feeds added. Osorio *et al.* (1992) reported a correlation between the acorn quantity and the concentration of oleic and palmitic acids. Establishment of a production system is difficult using only fatty acid percentages because of the similarity between groups I, II and III.

The analysis of other compounds, like triglycerides, could improve the classification of different groups. Figure 1 shows characteristic chromatograms of the four groups studied. The main triglycerides identified were: OOL, POL, OOO, POO, POP, SOO and POS. The analytical technique used did not allow the fatty acid position in the triglyceride molecule to be established. These identifications were based on ECN number and on similar profiles obtained with fat and ham fat from white pigs (Perrin & Prévot, 1986; García Regueiro *et al.*, 1994). Fresh ham fat from Iberian pigs

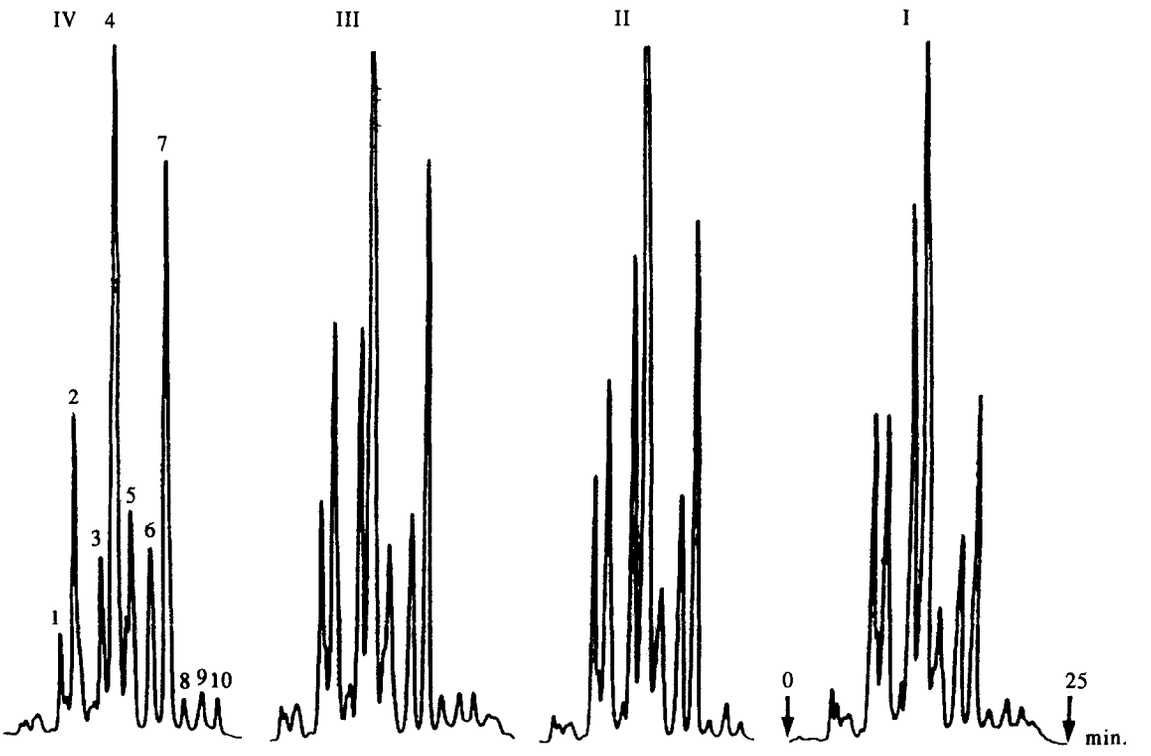

Fig. 1. HPLC chromatograms of fresh ham fat. Peaks: 1, OOL; 2, POL; 3, OOO; 4, POO; 5, POP; 6, SOO; 7, POS; 8, PPS; 9, SSO; 10, PSS. For conditions see text.

Table 3. Triglyceride percentages in fresh ham fat of Iberian pigs

Trigliceryde	I		II		III		IV	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
OOL	9.1[a]	1.0	7.5[b]	0.6	6.4[c]	0.8	3.5[d]	0.3
POL	15.1	0.5	13.9	2.4	16.3	2.3	17.3	3.9
OOO	17.7[a]	1.5	15.8[b]	0.8	11.9[c]	1.0	7.2[b]	0.7
POO	33.0	1.7	35.1	1.8	32.9	1.7	31.7	2.1
POP	3.8[a]	0.7	3.9[a]	0.3	5.9[b]	1.5	8.2[c]	1.3
SOO	7.2	1.0	6.7	0.3	6.4	0.5	6.8	0.7
POS	10.0[b]	1.3	12.9[a]	1.5	14.2[a]	1.2	19.1[c]	1.3

$n = 10$.
Values in the same row with different superscript differ significantly ($P < 0.001$ Groups I, II, III and IV) (see Table 1).

showed higher percentages of OOO and lower percentages of POS than that of white pigs. This agrees with previous data reported on fatty acid composition (Osorio *et al.*, 1983; Díaz *et al.*, 1986; Flores *et al.*, 1988; López *et al.*, 1990; De Pedro & Secondi, 1991), where an increase in oleic acid was described. One advantage of triglyceride analysis is that it is not necessary to saponify fat extract to obtain methyl esters of fatty acids, since this means a simplification of the analytical procedure and avoids the production of artifacts and sample alteration. Also, a slight decrease in the time required for complete analysis was obtained. The chromatographic conditions were established to reduce the analysis time whilst maintaining a sufficient resolution between peaks. Capillary gas chromatography is an alternative to HPLC but, to avoid the use of high temperature, HPLC was preferred in this case. HPLC analysis showed a low r.s.d. with values below 5% ($n = 5$) (García Regueiro *et al.*, 1994).

Table 3 shows the percentages of the main triglycerides identified in ham fat of Iberian pigs. Triolein and OOL showed significant differences between the four groups, with the highest values for group I, which was produced without mixed feeds during *montanera*. POP percentage was significantly different in groups III and IV; however, groups II and I did not show differences. POS presented higher values in groups III and IV, with significant differences between groups I and II; groups II and III did not show differences.

Another possibility was to express the results by a different index; thus, the ratios OOL/SOO and OOO/SOO were evaluated (Fig. 2). The OOO/SOO index showed an increase according to the use of mixed feeds in *montanera*. Group I, where mixed feeds were not used, presented the highest value; groups III and IV had clearly different values showing the effect of mixed feeds in group III. The OOL/SOO index showed similar behaviour and it could be applied to confirm the results obtained from the OOO/SOO index. Also, positive correlations were found between OOO and oleic acid (0.8898) and linoleic acid (0.6212), and negative correlations between OOO and the triglycerides POP (−0.8861), POS (−0.7354), palmitic acid (−0.9061) and stearic acid (−0.8262).

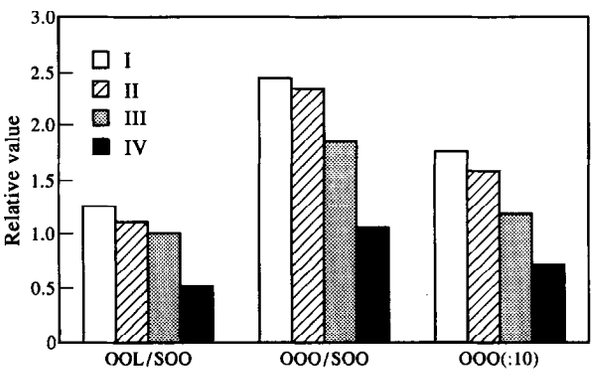

Fig. 2. Relative index obtained for the differents groups studied (see Table 1).

The results showed an increase in triolein content in *montanera* pigs related with mixed feeds intake, which agrees with previous results that showed an increase in the oleic percentage in *montanera* (Flores *et al.*, 1988; López *et al.*, 1990). However, the highest percentage of OOL in pigs produced in *montanera* systems could reflect a slight increase in linoleic acid but this effect is compensated for by a decrease in POL content; however, POP and POS, which are more saturated triglycerides, showed lower percentages in *montanera* pigs.

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