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n-Alkane content of intramuscular lipids of Iberian fresh ham from different feeding systems and crossbreeding

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Abstract

n-Alkane content of intramuscular lipids (*Biceps femoris* muscle) of the Iberian pig have been determined. Thirty-four pigs, divided into four groups, based in the feeding system (*Montanera*, fed on acorns and pasture; and *Pienso*, fed on a concentrate feed) and in the genotype (Iberian pure pigs; and Iberian crossbred with Duroc 50%) were studied. *n*-Alkane content of intramuscular lipids has not been affected by neither crossbreeding nor feeding, although the analysis of feeds administered to the pigs showed greater n-alkane values in pasture (consumed by animals in *montanera*), than in acorns and concentrate feed.

Keywords: Iberian pig; *n*-Alkanes; Intramuscular lipids; Feeding; Crossbreeding

1. Introduction

Ham from Iberian pigs is an expensive meat product with a first-rate consumer acceptance in Spain. This supposes that the Iberian ham industry is one of the most important of the meat sector (100 000 million pesetas) (Calero, Cienfuegos, & Gómez, 1999). For its production, this industry needs a raw material of great quality. The best fresh ham is originated from an Iberian pig fattened in an extensive system with acorn and pasture (*montanera*). The utilization of the crossbreeding and/or the substitution of acorn and pasture by concentrate diet in Iberian pigs produces a marked decrease in the quality of final products (García, Ventanas, Antequera, Ruiz, Cava, & Alvarez, 1996).

To discriminate the quality of raw material is very important for the Iberian pig meat industry. In this sense, the methods to differentiate Iberian carcasses and fresh hams from each feeding system have been mainly based on fatty acid analysis of fat from different tissues, as subcutaneous fat (Flores, Biron, Izquierdo, & Nieto, 1988; Ruiz, Cava, Antequera, Martín, Ventanas, & López-Bote, 1998), hepatic fat (De la Hoz, López, Cambero, Martín-Alvárez, Gallardo, & Ordóñez, 1993; Ruiz et al., 1998) and intramuscular fat (Cava et al., 1997). Advances in new methods, such as subcutaneous fat analysis by near infrared reflectance techniques (De Pedro, Garrido, Martínez, Angulo, & García Olmo, 1997), fatty acid composition of phospholipids classes of intramuscular lipids in fresh hams (Tejeda, 1999) or susceptibility to lipid oxidation in fresh meat (Cava, Ruiz, Ventanas, & Antequera, 1999), have been developed recently. However, since fatty acid composition of lipids from animals fed on an extensive system can be imitated by new dietary means (which do not produce a better quality of ham from pigs fed with such commercial feeds), until now there has not been a feasible method of analysis.

Unsaponifiable fractions could help characterize the feeding regime of Iberian pigs since hydrocarbon compounds are important components of vegetable wax (Post-Beittenmiller, 1996; Tulloch, 1976); These compounds, and specifically *n*-alkanes (parafinic hydrocarbons),

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normally occur in animal tissue and are derived from mainly plant origin. They are well absorbed at low doses by the mammalian small intestine (Tulliez & Bories, 1975); however, the absortion decreases with increase of length chain hydrocarbon (Mayes & Lamb, 1984). In this sense, differences have been found between subcutaneous fat of Iberian cured hams from pigs fed in different management systems, such as *montanera* and *pienso* (fed on a concentrate feed) (Tejeda et al., 1999). The results of this paper show the effect of the pigs' feeding regime in respect of the content of some of the *n*-alkanes identified, because the hams of pigs raised on an extensive system showed higher values than those given concentrate feed. Besides, when García, Berdagué, Antequera, López-Bote, Córdoba, and Ventanas (1991) analyzed Iberian ham volatiles, they found some compounds belonging to the unsaponifiable fraction, such as linear and branched short chain *n*-alkanes which have been described as directly deposited in pig fat from feeds (Berdagué, Denoyer, Le Quere, & Semon, 1991), and the *n*-alkane content was higher in these hams than in those from intensively farmed pigs (Berdagué & García, 1990). These results show the possibility of using the *n*-alkane profile to differentiate between feeding regimes. However, there are no studies about the *n*-alkane profiles in Iberian pig muscle. The objective of the present paper was to isolate and quantify the *n*-alkanes present in the intramuscular lipids (*Biceps femoris* muscle) of differents types of Iberian fresh hams, from two feeding systems (*montanera* and *pienso*) and genetic factors (crossbreeding of Iberian pig with Duroc pig), to contribute to developing a method to predict the quality of raw material.

2. Materials and methods

2.1. Materials

2.1.1. Animals and diets

A total of 34 Iberian pure and Iberian×Duroc (50%) crossbred pigs were used in this study. Pigs were divided into four groups following a 2×2 factorial design according to the genetic aspects (breed and crossbreed, Iberian and Iberian×Duroc) and the traditional types of feeding during the fattening period (60 days) prior to slaughter: *Montanera* [fed on acorn (*Quercus ilex*, *Q. rotundifolia* and *Q. suber*) and pasture in extensive system], and *Pienso* (fed on a concentrate feed). The studied groups were: Iberian pure pigs fed on *Montanera* ($n=10$), Iberian pure pigs fed on a concentrate feed ($n=10$), Iberian×Duroc pigs fed on *Montanera* ($n=7$) and Iberian×Duroc pigs fed on *Pienso* ($n=7$). Pigs fed on *Montanera*, at about 85–95 kg of initial weight, were slaughtered at 135–140 kg live weight; while pigs fed *Pienso*, at about 115–120 kg of initial weight, were slaughtered at 150–155 kg live weight. Diets (acorn, pasture and concentrate feed) were analyzed to determine dry matter, fat and *n*-alkane content.

After slaughtering, the *B. femoris* muscles were dissected from the carcasses and were immediately placed under vacuum in a freezer maintained at −80°C until analysis.

2.1.2. Reagents and standards

The solvents used were of the quality PRS (extra pure) grade supplied by Panreac (Barcelona, Spain). The absorbent for column chromatography was silica gel 60 (Panreac, Barcelona, Spain). Thin layer chromatography (TLC) 20×20 cm plates silica gel SIL G-50 UV_{254} (0.5 mm) (Panreac, Barcelona, Spain) were used. Standards of *n*-alkanes between heptane (n-C_7) and dotriacontane (n-C_{32}) (all from Sigma Chemical Co., St Louis, MO) were used. Eicosane (n-C_{20}) (Sigma Chemical Co., St Louis, MO) was used as an internal standard.

2.2. Methods

2.2.1. Analysis of diets

The chemical composition of the diets (moisture, protein, lipids, crude fiber, nitrogen free extractives and ash) were determined according to the Association of Official Analytical Chemists (AOAC, 1984) methods. n-Alkanes were determined according to the method of Bories and Tulliez (1977) after lipid extraction according to the method of Bligh and Dyer (1959) described below.

2.2.2. Lipid extraction

Intramuscular fat was extracted with cloroform-methanol solution (1:2 v/v) following the procedure of Bligh and Dyer (1959). After evaporation of the organic phase in a rotary evaporator and finally under a nitrogen steam, the residue (lipids) was saponified to hydrocarbon fraction analysis.

2.2.3. Saponification and hydrocarbon fraction extraction

n-Alkanes were extracted and isolated according to the method of Bories and Tulliez (1977). A total of 8 g of intramuscular lipids samples was saponified by refluxing for 2 h with 280 ml of 15% KOH ethanol solution. The warm solution was transferred to a separatory funnel, 70 ml of distilled water were added and the unsaponifiable fraction was extracted with 70 ml of hexane. The organic layer was washed three times with 50 ml of distilled water, then dried over anhydrous sodium sulphate and concentrate to 2 ml. The extract was transferred to a chromatography column (1.5 cm i.d.) that had been prepared by successively adding 2 g of silica gel and 8 g of anhydrous sodium sulphate.

Hydrocarbons were eluted with 50 ml of hexane. After direct evaporation to 1 ml under vacuum, the residue was applied as a thin band to TLC plates previously activated for 1 h at 105°C. The plate was developed with hexane for a 14-cm run and then sprayed with a 0.2% solution of 2′, 7′-dichlorofluorescein in ethanol to visualize the bands. Thin layer chromatograms of standards were run under identical conditions and were used to identifie the *n*-alkane band of the samples. This later band was scraped from the plate, transferred to a small glass column and eluted with 25 ml of hexane. The solution with *n*-alkane fraction was then evaporated to dryness under a nitrogen steam, resolved in 100μl hexane and analyzed by gas chromatography (GC).

2.2.4. Identification and quantitative determination of n-alkanes

n-Alkanes were analyzed by GC on a Hewlett Packard HP-5890A chromatograph, equiped with a flame ionization detector and a Hewlett Packard fused silica capillary column (12 m×0.2 mm i.d.) with a film thickness of 0.33 mm stationary phase of methyl silicone. Helium was used as carrier gas at a flow rate of 14.9 ml/min.

The oven temperature program was from 100 to 270°C at 6°C/min and 25 min at 270°C. Injector and detector temperatures were 260°C and 270°C, respectively. Split ratio was 1:25. Inlet pressure was 14 psi, and the sample volume injected 2μl.

Peaks of *n*-alkanes were identified by comparison of their retention times with *n*-alkane standards and confirmated by gas chromatography-mass spectrometry (GC-MS), using a Hewlett Packard HP-5890A chromatograph with a Hewlett Packard 5971A mass selective detector. The GC-MS transfer line temperature was 280°C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan/s over a range of m/z from 40 to 300. The obtained spectra were compared with those of the standards and spectra from Wiley, Hewlett Packard and the National Institute of Standards and Technology libraries. Quantitative determination was performed by adding an appropiate amount of internal standard (*n*-eicosane, n-C_{20}) to the final extract a *n*-alkane present in a small amount in the samples.

2.2.5. Statistical analysis

Results were subjected to analysis of variance (ANOVA) according to the General Lineal Model (GLM) procedure, using software Statistical Analysis System (SAS). The model used involved the feeding system and genotype. This statistical procedure was carried out using the SAS/SAT package (SAS Institute, 1989).

3. Results and discussion

The chemical composition and *n*-alkane profile of the feeds are given in Tables 1 and 2, respectively. Acorns showed higher fat content than concentrate feed and pasture (5.81 versus 4.42 and 2.61 g/100 g dry matter (DM), respectively). However, the *n*-alkane content of pasture was higher than concentrate feed and acorn (53.52 versus 8.37 and 1.62 mg/Kg DM, respectively). The higher concentrations of *n*-alkanes in pasture have been observed by several authors in a wide variety of

Table 1
Composition of feeds

	Feeds		
	Acorn	Pasture	Concentrate
Moisture[a]	35.15	72.60	10.56
Lipids[b]	5.81	2.61	4.42
Protein[b]	5.55	13.78	15.65
Crude fiber[b]	6.33	22.85	4.56
NFE[bc]	80.28	50.32	69.39
Ash[b]	2.03	10.44	5.98

[a] Expressed as percentage of fresh matter.
[b] Expressed as percentage of dry matter.
[c] NFE: nitrogen free extractives.

Table 2
n-Alkane content found in feed of pigs (values expressed in mg/Kg d.m. and mg/Kg total lipids in parentheses)

	Feeds		
n-Alkanes	Pasture	Acorn	Concentrate
n-C_{12}	1.11 (42.53)	0.06 (1.03)	0.13 (2.94)
n-C_{13}	0.09 (3.45)	0.01 (0.17)	0.02 (0.45)
n-C_{14}	1.65 (63.22)	0.30 (5.16)	0.22 (4.98)
n-C_{15}	0.33 (12.64)	0.03 (0.52)	0.05 (1.13)
n-C_{16}	1.05 (40.23)	0.20 (3.44)	0.19 (4.30)
n-C_{17}	0.21 (8.05)	0.04 (0.69)	0.07 (1.58)
n-C_{18}	0.63 (24.14)	0.13 (2.24)	0.15 (3.39)
n-C_{19}	0.45 (17.24)	0.04 (0.69)	0.07 (1.58)
n-C_{21}	1.26 (48.28)	0.07 (1.20)	0.26 (5.88)
n-C_{22}	0.51 (19.54)	0.08 (1.38)	0.14 (3.17)
n-C_{23}	0.99 (37.93)	0.05 (0.86)	0.54 (12.22)
n-C_{24}	0.21 (8.05)	0.04 (0.69)	0.16 (3.62)
n-C_{25}	2.40 (91.95)	0.07 (1.20)	0.89 (20.14)
n-C_{26}	0.21 (8.05)	0.03 (0.52)	0.15 (3.39)
n-C_{27}	4.11 (157.47)	0.09 (1.55)	0.69 (15.61)
n-C_{28}	0.81 (31.03)	0.02 (0.34)	0.25 (5.66)
n-C_{29}	13.02 (498.85)	0.27 (4.65)	2.10 (47.51)
n-C_{30}	0.69 (26.44)	0.03 (0.52)	0.16 (3.62)
n-C_{31}	12.30 (471.26)	0.03 (0.52)	2.02 (45.70)
n-C_{32}	0.66 (25.29)	0.03 (0.52)	0.11 (2.49)
n-C_{33}	10.74 (411.49)	n.d.[a] (n.d.)	n.d. (n.d.)
Total onca *n*-alkanes	45.93 (1759.77)	0.70 (12.05)	6.71 (151.81)
Total enca *n*-alkanes	7.59 (290.80)	0.92 (15.83)	1.66 (37.56)
Totals	53.52 (2050.57)	1.62 (27.88)	8.37 (189.37)

[a] n.d., non detected.

pasture, as *Leguminosae* as *Gramineae* (Dove, Mayes, & Freer, 1995; Maffei, 1996; Malossini, Piasenter, & Bovolenta, 1990; Tulloch, 1976). In our work, the same *n*-alkanes were identified in the unsaponifiable fraction of the three feeds, except for tritriacontane (*n*-C_{33}), which was only found in pasture. The analysis of feeds revealed a complete homologous series of *n*-alkanes from *n*-C_{12} to *n*-C_{32}, and *n*-C_{33} in pasture (Fig. 1). In all the samples of feeds were also found *n*-alkanes and branched hydrocarbons. Odd-numbered carbon atom (onca) *n*-alkanes concentration was more abundant than even-numbered carbon atom (enca) *n*-alkanes one, and this diference was higher in pasture than in concentrate feed, while in acorn onca and enca *n*-alkane concentration was similar. Moreover, a predominance of long chain onca *n*-alkanes (*n*-C_{27} to *n*-C_{31}) can be observed in pasture and concentrate feed, while in acorn there were slowly predominant short chain enca *n*-alkanes (*n*-C_{14} to *n*-C_{18}). The higher content of onca *n*-alkanes in pasture has been reported previously (Maffei, 1996; Malossini et al., 1990) and is one of the main characteristics of the hydrocarbon fraction of pasture species. This is the reason why the hydrocarbons, and specifically *n*-alkanes, have been proposed as internal markers for measuring herbage intake in grazing animals (Mayes & Lamb, 1984; Ohajuruka and Palmquist, 1991; Vulich, Hanrahan, & Crowley, 1995) since they are compounds which are quite inert in the digestive system and can be measured in animal faeces (Piasenter, Pison, & Bovolenta 1989). However, the faecal recovery of *n*-alkanes is incomplete, and progressively declined as carbon-chain length decreases (Dove & Mayes, 1991), so a variable proportion of *n*-alkanes are absorbed in the small intestine (Mayes, Lamb, & Colgrove, 1988). Additionally, these compounds are poorly modified during pig digestion and metabolism (Rembold, Wallner, Nite, Knollmannsberger, & Drawert, 1989; Van Straten, 1977), and are finally deposited in animal fat. So, due to Iberian pig being fed in an extensive system, with acorn and pasture, analysis of *n*-alkanes in pig fat could be used to determine if pigs had consumed pasture during the fattening period.

Table 3 shows *n*-alkane distribution in the intramuscular lipids from *B. femoris* of the Iberian pigs examined. *n*-Alkane composition in these samples is qualitatively the same, *n*-alkanes in *n*-C_{12} to *n*-C_{32} range were present in the unsaponifiable fraction of intramuscular lipids in the four groups studied. Quantities ranged from 0.13 to 7.70 mg/Kg intramuscular lipids. A similar range of *n*-alkanes have been reported by several authors in different pig and cattle tissues (Bastic, Bastic, Remberg, Skala, & Jovanovic, 1989; Bernardini, Boniforti, Citti, & Mosini, 1982; Lintas, Balduzzi, Bernardini, & Di Muccio, 1979). *n*-Alkanes with a hydrocarbon chain shorter than *n*-C_{12} were not found, because saponification and solvent evaporation processes led to a loss in short-chain *n*-alkanes (Tan & Kunton, 1993). Both enca and onca *n*-alkanes up to *n*-C_{32} were not identified. In subcutaneous fat from Iberian hams the shorter *n*-alkane found was *n*-C_{14}, and *n*-C_{29} the largest hydrocarbon chain *n*-alkane (Tejeda et al., 1999). We have observed that enca short chain *n*-alkanes (*n*-C_{12} to *n*-C_{18}) were the most abundants in all the samples, with the maximun in

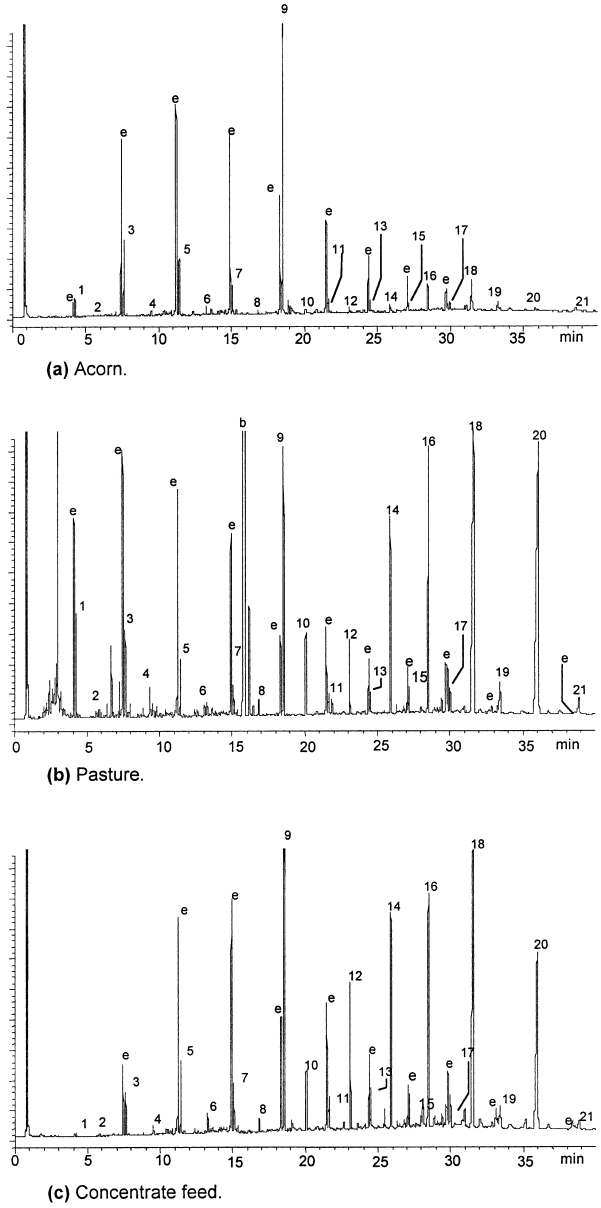

Fig. 1. Chromatograms of *n*-alkanes identified in the unsaponifiable fraction of feeds (acorn (a), pasture (b) and concentrate feed (c)) administered to pigs during fattening period. (1: Dodecane (*n*-C_{12}); 2: tridecane (*n*-C_{13}); 3: tetradecane (*n*-C_{14}); 4: pentadecane (*n*-C_{15}); 5: hexadecane (*n*-C_{16}); 6: heptadecane (*n*-C_{17}); 7: octadecane (*n*-C_{18}); 8: nonadecane (*n*-C_{19}); 9: internal standard (eicosane); 10: heneicosane (*n*-C_{21}); 11: docosane (*n*-C_{22}); 12: tricosane (*n*-C_{23}); 13: tetracosane (*n*-C_{24}); 14: pentacosane (*n*-C_{25}); 15: hexacosane (*n*-C_{26}); 16: heptacosane (*n*-C_{27}); 17: octacosane (*n*-C_{28}); 18: nonacosane (*n*-C_{29}); 19: triacontane (*n*-C_{30}); 20: hentriacontane (*n*-C_{31}); 21: dotriacontane (*n*-C_{32}); e: *n*-alkenes; b: branched hydrocarbons.

n-C_{16}, followed by *n*-C_{14}. These results are in consonance with those of Bernardini et al. (1982) in muscle tissue of pigs, and Lintas et al. (1979) in muscle tissue of cattle. That could be due to the major absortion from the small intestine of short carbon-chain *n*-alkanes than long ones (Mayes et al., 1988). However, in subcutaneous fat from Iberian ham the most abundant *n*-alkanes were those in the *n*-C_{18} to *n*-C_{25} range, of which linear onca hydrocarbons prevailed (Tejeda, Antequera, Ruiz, Cava, Ventanas, & Antequera, 1999). The different tendence of *n*-alkanes to accumulate in animal tissues have been observed by different authors (Bernardini et al., 1982; Di Muccio, Boniforti, Palomba, Bernardini, & Delise, 1979; Lintas et al., 1979). In this sense, the *n*-alkanes are concentrated preferentially in the subcutaneous adipose tissues, following by perirrenal and intramuscular fat (Lintas et al., 1979). Some other peaks were observed in the chromatograms, and identified as unsaturated and branched hydrocarbons. The presence of squalene is not reported here, because by the method utilized, this compound was purposely eliminated during TLC to facilitate the quantitative determination of *n*-C_{28} (octacosane), as both components exhibited the same retention times on GC analysis.

The contents of *n*-alkanes in intramuscular *B. femoris* lipids were not affected by diet or genotype of Iberian pigs ($P < 0.05$); samples of pigs, Iberian and Iberian×Duroc, fed on an extensive system (acorn and pasture) and concentrate feed had a similar content of *n*-alkanes. These results are in contrast with those of the preceding paper (Tejeda et al., 1999), where significant differences ($P < 0.05$) were noticed for the values of some of the *n*-alkanes of subcutaneous ham fat from pigs fed on an extensive system and concentrate feed. That could be due to the higher tendence of subcutaneous fat to deposite *n*-alkanes than intramuscular lipids (Lintas et al., 1979). The origin of *n*-alkanes in animal tissues has not been well elucidated yet. The animal food chain is considered the main source of *n*-alkanes in animal tissues, playing a special role the vegetables. However, hydrocarbons can be also generated, at least in part, during fatty acid oxidation (Loury, 1972; Sahidi, Rubin, & D'Souza, 1986), for example, during meat processing (Gray & Pearson, 1984).

Table 3
n-Alkane content in intramuscular lipids (mg/Kg intramuscular fat) of *Biceps femoris* muscle from Iberian and Iberian×Duroc pigs fed on extensive system (*Montanera*) or concentrate feed (*Pienso*) (mean±standard error)[a]

	Iberian		Iberian×Duroc		Effect	
	Montanera $n=10$	*Pienso* $n=10$	*Montanera* $n=7$	*Pienso* $n=7$	Feeding genotype	
Lipid content[b]	8.10	6.81	7.90	6.00	**	ns[c]
n-*Alkanes*						
n-C_{12}	5.17±1.99	1.72±0.32	1.44±0.71	3.72±0.98	ns	ns
n-C_{13}	0.13±0.04 b	0.45±0.27 a	0.23±0.18 b	1.34±0.60 a	*	ns
n-C_{14}	7.70±1.16 a	2.45±0.71 c	3.92±0.66 b	5.62±2.19 ab	ns	ns
n-C_{15}	0.35±0.05 b	0.31±0.03 b	0.29±0.02 b	0.53±0.06 a	ns	ns
n-C_{16}	3.90±0.35 a	2.07±0.27 b	2.07±0.59 b	2.78±0.78 ab	ns	ns
n-C_{17}	0.59±0.06	0.70±0.21	0.40±0.02	0.62±0.09	ns	ns
n-C_{18}	2.03±0.18	1.54±0.36	1.51±0.11	1.49±0.33	ns	ns
n-C_{19}	0.42±0.03	0.43±0.05	0.34±0.01	0.42±0.03	ns	ns
n-C_{21}	0.73±0.05	0.73±0.05	0.77±0.07	0.81±0.08	ns	ns
n-C_{22}	0.96±0.08	0.85±0.06	0.80±0.03	0.86±0.08	ns	ns
n-C_{23}	0.58±0.06	0.55±0.04	0.46±0.01	0.55±0.05	ns	ns
n-C_{24}	0.67±0.09	0.58±0.08	0.50±0.04	0.58±0.10	ns	ns
n-C_{25}	0.95±0.09	0.80±0.06	0.74±0.06	0.79±0.09	ns	ns
n-C_{26}	0.83±0.11	0.55±0.09	0.41±0.03	0.52±0.11	ns	ns
n-C_{27}	0.54±0.08	0.33±0.07	0.29±0.03	0.32±0.05	ns	ns
n-C_{28}	0.63±0.13	0.36±0.09	0.28±0.04	0.27±0.07	ns	ns
n-C_{29}	0.87±0.16	0.55±0.08	0.57±0.07	0.46±0.05	ns	ns
n-C_{30}	0.57±0.15	0.41±0.07	0.38±0.05	0.35±0.05	ns	ns
n-C_{31}	0.50±0.12	0.34±0.06	0.40±0.05	0.25±0.03	ns	ns
n-C_{32}	0.43±0.12	0.31±0.04	0.32±0.04	0.29±0.04	ns	ns
Total onca *n*-alkanes	5.68±0.69	4.96±0.60	4.52±0.28	6.08±0.46	ns	ns
Total enca *n*-alkanes	22.91±4.08 a	11.26±1.54 b	11.64±1.63 b	16.48±4.53 ab	ns	ns
Totals	28.60±4.48 a	16.22±1.84 b	16.16±1.57 b	22.57±4.41 ab	ns	ns

[a] Within rows, values with different letters differ significantly.
[b] Expressed as g/100 g of muscle.
[c] ns, not significative.
*($P < 0.05$).
**($P < 0.01$).

The results reported in this study will serve to establish baseline values, together with the results reported in the preceding paper (Tejeda et al., 1999), for subcutaneous fat from dry cured ham, to evaluate paraffin hydrocarbon levels in tissues from Iberian pigs. On the other hand, other investigations carried out by authors have reported the presence in muscle and adipose subcutaneous tissues of cattle of various branched hydrocarbons (Bernardini et al., 1982; Lintas et al., 1979), which have been directly associated with ingestion of herbage. In this sense, we are investigating the presence of some of these compounds in lipids of Iberian pig fed on an extensive system, as *n*-alkanes of intramuscular lipids are not a good indicator to differentiate the feeding regime and crossbreeding of Iberian pigs.

References

AOAC. (1984). *Official methods of analysis* (3rd ed.). Washington, DC: Association of Official Analytical Chemists.

Bastic, L. J., Bastic, M., Remberg, G., Skala, D., & Jovanovic, J. (1989). Hydrocarbon content of neutral lipid fractions of different hog and cattle tissues. In Proceedings 35th international congress of meat science and technology (pp. 587–590), 20–25 August 1989, Copenhagen, Denmark.

Berdagué, J. L., & García, C. (1990). Les components volatils du jambon sec. *Viandes et Produits Carnés, 11*, 319–320.

Berdagué, J. L., Denoyer, C., Le Quere, J. L., & Semon, E. (1991). Volatile components of dry-cured ham. *Journal of Agricultural and Food Chemistry, 39*, 1257–1261.

Bernardini, M. P., Boniforti, L., Citti, G., & Mosini, V. (1982). Distribution of hydrocarbons and fatty acids in meats imported into Italy. *Food Chemistry, 8*, 51–60.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Fisiology, 37*, 911–917.

Bories, G. F., & Tulliez, J. E. (1977). A versatile method for the determination of normal paraffins in foods. *Journal of the Science of Food and Agriculture, 28*, 996–999.

Calero, R., Cienfuegos, A., & Gómez, J. M. (1999). Spanish industry of Iberian pig meat. In Ed. Fondo Formación, Proyecto Alimex, *Extremeña meat industry of Iberian pig products* (pp. 25–27). Mérida, Spain.

Cava, R., Ruiz, J., López-Bote, C., Martin, L., García, C., Antequera, T., & Ventanas, J. (1997). Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscles of Iberian pig. *Meat Science, 45*, 263–270.

Cava, R., Ruiz, J., Ventanas, J., & Antequera, T. (1999). Oxidative and lipolytic changes during ripening of Iberian hams as affected by feeding regime: extensive feeding and alpha-tocopheryl acetate supplementation. *Meat Science, 52*, 165–172.

De la Hoz, L., López, M. O., Cambero, M. I., Martín-Alvarez, P. J., Gallardo, E., & Ordóñez, J. A. (1993). Fatty acid of Iberian pig liver as affected by diet. *Archiv fur Lebensmittelhygiene, 44*, 81–104.

De Pedro, E., Garrido, A., Martínez, M. L., Angulo, F., & García Olmo, J. (1997). Near infrared reflectance (NIRS) for the analysis quantitative and qualitative of Iberian pig products. *ITEA, 18*, 661–663.

Di Muccio, A., Boniforti, L., Palomba, A., Bernardini, M. P., & Delise, M. (1979). Idrocarburi saturi negli alimenti. Metodo di analisi e valori riscontrati in alcuni alimenti per uso umano e in campioni di organismi unicellulari. *Annali dell Istituto Superiore di Sanità, 15*, 525–540.

Dove, H., & Mayes, R. W. (1991). The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. *Australian Journal of Agricultural Research, 42*, 913–952.

Dove, H., Mayes, R. W., & Freer, M. (1995). Using cuticular wax alkanes to estimate herbage intake in animals fed supplements. *Annales de Zootechnia, 44*, 237–240.

Flores, J., Biron, C., Izquierdo, L., & Nieto, P. (1988). Characterization of green hams from Iberian pigs by fast analysis of subcutaneous fat. *Meat Science, 23*, 253–262.

García, C., Berdagué, J. J., Antequera, T., López-Bote, C., Córdoba, J. J., & Ventanas, J. (1991). Volatile components of dry cured Iberian ham. *Food Chemistry, 41*, 23–32.

García, C., Ventanas, J., Antequera, T., Ruiz, J., Cava, R., & Alvarez, P. (1996). Measuring sensorial quality of Iberian ham by Rasch model. *Journal of Food Quality, 19*, 397–412.

Gray, J. I., & Pearson, A. M. (1984). Cured meat flavor. *Advances in Food Research, 29*, 1–86.

Loury, M. (1972). Possible mechanisms of autosidative rancidity. *Lipids, 7*, 671–675.

Lintas, C., Balduzzi, A. M., Bernardini, M. P., & Di Muccio, A. (1979). Distribution of hydrocarbons in bovine tissues. *Lipids, 14*, 298–303.

Maffei, M. (1996). Chemotaxonomic significance of leaf wax alkanes in the gramineae. *Biochemical Systematics and Ecology, 24*, 53–64.

Malossini, F., Piasenter, E., & Bovolenta, S. (1990). *n*-Alkane content of some forages. *Journal of the Science of Food and Agriculture, 53*, 405–409.

Mayes, R. W., & Lamb, C. S. (1984). The possible use of *n*-alkanes in herbage as indigestible faecal markers. *Proceedings of the Nutrition Society, 43*, 39A.

Mayes, R. W., Lamb, C. S. & Colgrove, P. M. (1988). Digestion and metabolism of dosed even-chain and herbage odd-chain *n*-alkanes in sheep. In Proceeding 12th General Meeting European. Grasslands Fed. (pp. 159–163), July 1988, Dublin, Ireland.

Ohajuruka, O. A., & Palmquist, D. L. (1991). Evaluation of *n*-alkanes as digesta markers in dairy cows. *Journal of Animal Science, 69*, 1726–1732.

Piasenter, E., Pison, S., & Bovolenta, S. (1989). Impiego degli *n*-alcani studi sulla digeribilità in vivo dei foraggi. *Zootecnia e Nutrizione Animale, 15*, 691–696.

Post-Beittenmiller, D. (1996). Biochemistry and molecular biology of wax production in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 47*, 405–430.

Rembold, H., Wallner, P., Nite, S., Kollmannsberger, H., & Drawert, F. (1989). Volatile components of chickpea (*Cicer arietinun* L.) seed. *Journal of Agricultural and Food Chemistry, 37*, 659–662.

Ruiz, J., Cava, R., Antequera, T., Martín, L., Ventanas, J., & López-Bote, C. J. (1998). Prediction of the feeding backround of Iberian pigs using the fatty acid profile of subcutaneous, muscle and hepatic fat. *Meat Science, 49*, 155–163.

Sahidi, F., Rubin, L. J., & D'Souza, L. A. (1986). Meat flavor volatiles: a review of the composition, technique of analysis and sensory evaluation. *CRC Critical Review in Food Science and Nutrition, 24*, 219–227.

SAS Institute, (1989). *SAS/STAT user's guide*. (version 6, 4th ed.) NC: Cary.

Tan, Y., & Kunton, A. (1993). Gas chromatographic determination of hydrocarbons in crude palm kernel oil. *Journal of AOAC International, 76*, 371–376.

Tejeda, J. F. (1999). A study of influence of crossbreeding and feeding regime on intramuscular lipids from Iberian pigs. PhD thesis, University of Extremadura, Cáceres, Spain.

Tejeda, J. F., Antequera, T., Ruiz, J., Cava, R., Ventanas, J., & García, C. (1999). Unsaponifiable fraction content and *n*-alkane profiles of subcutaneous fat from Iberian hams. *Food Science and Technology International, 5*, 229–233.

Tulliez, J. E., & Bories, G. F. (1975). Métabolisme des hydrocarbures

paraffiniques et naphténiques chez les animaux supérieurs. II. Accumulation et mobilisation chez le rat. *Annales de la Nutrition et de l' Alimentation, 29*, 213–221.

Tulloch, A. P. (1976). Chemistry of waxes of higher plants. In P. E. Kolattukudy, Chemistry and Biochemistry of Natural Waxes (pp. 235–287). Amsterdam: Elsevier.

Van Straten, S. (1977). *Volatile compounds in foods* (4th ed.). Zeist, The Netherlands: CIVO-TNO Publisher.

Vulich, S. A., Hanrahan, J. P., & Crowley, B. A. (1995). Modification of the analytical procedures fot the determination of herbage and faecal *n*-alkanes used in the estimation of herbage intake. *Journal of Agricultural Science, 124*, 71–77.