

Linear hydrocarbons content of intramuscular lipids of dry-cured Iberian ham

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Abstract

This study has been carried out to determine the linear hydrocarbons content (*n*-alkane and *n*-alkene) in intramuscular lipids (*biceps femoris* muscle) of dry-cured Iberian ham considering “feeding system” (*Montanera*: fed on acorns and pasture and *concentrate feed*) and “genotype” (hams from Iberian pure pigs and hams from Iberian crossbreed with Duroc in a 50%). The linear hydrocarbons from *n*-C₁₄ to *n*-C₃₂ range were present in the four studied groups. *n*-Alkenes fraction (60–76 mg/kg of intramuscular fat) was higher than *n*-alkanes fraction (34–38 mg/kg). The most abundant *n*-alkane and *n*-alkene were the shortest chain ones. Feeding and genotype did not influence linear hydrocarbons content (neither *n*-alkanes nor *n*-alkenes).

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1. Introduction

Dry-cured Iberian ham is a typical product of high sensory quality produced in the southwest of Spain. Iberian pigs are produced according to an extensive system, with a feeding regime based, exclusively, on acorns and pasture (this way of feeding is called *montanera*) during the period immediately prior to slaughter (2–3 months). The utilization of the crossbreeding and/or the substitution of acorn and pasture by concentrated diet in Iberian pigs produces different sensory qualities of dry-cured hams, that could be caused by different characteristics of meats (García, Ventanas, Antequera, Ruiz, Cava, & Álvarez, 1996), in particular, by differences in intramuscular fat composition.

There are many studies about intramuscular fat composition in different types of dry-cured Iberian ham, such as total fatty acid composition analysis (Cava et al., 1997), fatty acid composition of triacylglycerols and phospholipids (Cava et al., 1997; Martín, Córdoba,

Ventanas, & Antequera, 1999) and some other fat components like aldehydes (Antequera et al., 1992) or volatile compounds (García, Berdagüé, Antequera, López-Bote, Córdoba & Ventanas, 1991) produced during the processing of Iberian hams. However, the unsaponifiable fraction of the intramuscular lipids from Iberian hams has not been studied yet. This fraction has a high number of compounds, hydrocarbons being one of them.

Hydrocarbons are an important component of vegetable wax (Post-Beittenmiller, 1996; Tulloch, 1976). Several authors have detected the presence of hydrocarbons in animal tissues and these compounds have been related to the vegetables consumed by animals as a part of their diet (Berdagüé & García, 1990; Tejeda, Antequera, Ruiz, Cava, Ventanas, & García, 1999; Tulliez & Bories, 1978). Hydrocarbons are inert in the digestive tract and they are lightly modified during the digestion and metabolism (Mayes, Lamb, & Colgrove, 1998; Rembold, Wallner, Nite, Kollmannsberger, & Drawert, 1989; Van Straten, 1977). In view of this information, the earlier mentioned unsaponifiable fraction in pig meats, could be used to characterize the feeding regime of Iberian pigs.

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Thus, Tejeda, García, Petrón, Andrés, and Antequera, (2001), analysing the *n*-alkane content of the feeds administrated to the pigs, found that the *n*-alkane values were greater in pasture (consumed by animals in *montanera*) than in acorns and concentrated feed. In the same way, some differences have been found between subcutaneous fat of dry-cured Iberian hams from pigs fed in different management systems, such as *montanera* and concentrated feed (Tejeda et al., 1999), but there are no studies about this fraction in the intramuscular lipids from dry-cured ham. However, some compounds belonging to the unsaponifiable fraction, such as linear hydrocarbons, have been found in dry-cured Iberian ham (García et al., 1991) and others aged hams (Babbieri et al., 1992) as a part of the volatile components and the *n*-alkane content was higher in these dry-cured Iberian hams than in those which came from intensively farmed pigs (Berdagué & García, 1990). These results show the possibility of using the *n*-alkane and *n*-alkene profiles to distinguish between feeding regimes.

The objectives of this study were: (1) to identify components of the linear hydrocarbons fraction of dry-cured Iberian hams; and (2) to elucidate the effect of the feeding system (*Montanera* and *Concentrate feed*) and the genetic factors (crossbreeding of Iberian pig with Duroc pig) on the *n*-alkane and *n*-alkene profile of the intramuscular lipids.

2. Material and methods

2.1. Processing and sampling of hams

A total of 34 Iberian dry-cured ham from Iberian pure pigs and Iberian×Duroc (50%) crossbreed pigs were used in this study. The hams 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 (55 days) prior to slaughter: *Montanera* (fed on acorn and pasture), and *Intensive system* (fed with concentrated feed). The hams studied were divided into four groups from Iberian pigs fed on *Montanera* ($n=10$), Iberian pigs fed with concentrated feed ($n=10$), Iberian×Duroc pigs fed on *Montanera* ($n=7$) and Iberian×Duroc pigs fed with concentrated feed ($n=7$).

The hams were processed in a traditional way that includes two defined steps: salting/post-salting and ripening. During the first period (4–6 months), salting and post-salting were combined with low temperature (0–3 °C) and high relative humidity (80–90%) to reduce the risk of bacterial spoilage. Then, the hams were left to mature for 15 months in a cellar at temperatures ranging from 10 to 27 °C and relative humidity of 58–80%.

After processing, the *biceps femoris* muscles were dissected from hams and immediately placed under vacuum in a freezer maintained at –80 °C until the analysis.

2.2. Reagents and standards

The solvents used were of PRS (extra pure) grade supplied by Panreac (Barcelona, Spain). The absorbent for column chromatography was silica gel 60 (Panreac, Barcelona, Spain). For thin layer chromatography (TLC) 20×20 cm plates silica gel SIL G-50 UV₂₅₄ (0.5 mm) (Panreac, Barcelona, Spain) were used.

Standards of *n*-alkanes between pentane (*n*-C₅) and dotriacontane (*n*-C₃₂) (all from Sigma Chemical Co., St. Louis, MO) were used. Eicosane (*n*-C₂₀) (Sigma Chemical Co., St. Louis, MO) was used as internal standard.

2.3. Lipid extraction

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

2.4. Saponification and hydrocarbon fraction extraction

n-Alkanes were extracted and isolated according to a method adapted by Tejeda et al. (2001) from that of Boriez and Tulliez (1977). A total of 8 g of intramuscular lipids sample was saponified by refluxing for 2 h with 280 ml of a 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, and then dried over anhydrous sodium sulphate and concentrated to 2 ml. The extract was transferred to a chromatography column (1.5 cm i.d.) that had been prepared by adding 2 g of silica gel and 8 g of anhydrous sodium sulphate. The hydrocarbons were eluted with 50 ml of hexane. After direct evaporation under vacuum, the residue (1 ml) was applied as a thin band to a TLC plate 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 identify the hydrocarbons band of the sample. This band was scraped from the plate, transferred to a small glass column and eluted with 25 ml of hexane. Then, the solution with the *n*-alkane and *n*-alkene fraction was evaporated to dryness under a nitrogen steam, dissolved in 100 µl of hexane and analysed by gas chromatography.

2.5. Identification and quantitative determination of n-alkanes and n-alkenes

n-Alkanes and *n*-alkenes were analysed by gas chromatography (GC) on a Hewlett Packard HP-5890A chromatograph, equipped 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 µm stationary phase of methyl silicone (HP-1, Cross-linked Methyl Silicone Gum). 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. The injector and detector temperatures were 260 and 270 °C, respectively. The split ratio was 1:25. The inlet pressure was 14 psi, and the sample volume injected was 2 µl.

Peaks of *n*-alkanes and *n*-alkenes were identified by comparison of their retention times with *n*-alkane standards and confirmed by gas chromatography-mass spectrometry (GC-MS), using a Hewlett Packard HP-6890A chromatograph with a Hewlett Packard 5973A 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 identified by Wiley, Hewlett Packard and The National Institute of Standards and Technology libraries.

The quantitative determination was performed by adding a suitable amount of internal standard (12 mg of C₂₀/kg of intramuscular fat) to the final extract. *n*-Eicosane (*n*-C₂₀) was used as the internal standard because the samples showed a negligible quantity of this *n*-alkane. Recoveries of linear hydrocarbons were individually calculated and they were related to the length of their chains. The longer were the chains the higher were the recoveries of hydrocarbons. Recoveries for *n*-alkanes and *n*-alkenes in the C₁₄–C₂₃ rank ranged from 62 to 84% and from 84 to 93% for the rest of the linear hydrocarbons (C₂₄–C₃₂).

2.6. Statistical analysis

The obtained results, were subjected to an analysis of variance (ANOVA) according to the general lineal model (GLM) procedure. The model used included the feeding system (*montanera* or concentrated feed) and the genotype (Iberian pure or crossbreeding) as factors. This statistical procedure was carried out using the SPSS package (SPSS Institute, 1990).

3. Results and discussion

The results of *n*-alkanes quantitative analysis reported in Table 1 shows the presence of these compounds in

intramuscular lipids of examined dry-cured Iberian hams. The even-numbered carbon atoms (ENCA) *n*-alkane concentration was more abundant than the odd-numbered carbon atoms (ONCA) one. The *n*-alkanes composition in these samples is qualitatively the same; *n*-alkanes from *n*-C₁₄ to *n*-C₃₂ range were present in the unsaponifiable fraction of intramuscular lipids in the four groups studied. The *n*-alkane content ranged from 0.51 to 10.82 mg/kg of intramuscular lipids. *n*-Alkanes with a chain shorter than *n*-C₁₄ were not found, because the saponification and solvent evaporation processes led to a loss of the short-chain *n*-alkanes (Tan & Kunton, 1993). Some, ENCA and ONCA *n*-alkanes up to *n*-C₃₂, were not identified. We have observed that hydrocarbons composition was not quantitatively the same in every group. The ENCA short chain *n*-alkanes (*n*-C₁₄ to *n*-C₁₈) were the most abundant in all the samples, with the maximum in *n*-C₁₄, followed by *n*-C₁₆. That could be due to the major absorption of short carbon-chain *n*-alkanes by the small intestine (Mayes et al., 1988). A similar range of *n*-alkanes has been reported in different pig and cattle tissues (Bastic, Bastic, Remberg, Skala, & Jovanovic, 1989; Bernardini, Boniforti, Citti, & Mosini, 1982; Lintas, Balduzzi, Bernardini, & Di Muccio, 1979) but only few studies of this fraction have been carried out in dry-cured products. Tejeda et al. (1999), reported *n*-alkane profiles in subcutaneous fat from dry-cured Iberian hams. In those samples the shortest *n*-alkane found was *n*-C₁₄, and the largest *n*-alkane was *n*-C₂₉. The most abundant *n*-alkanes were those in the *n*-C₁₈ to *n*-C₂₅ range, of which ONCA hydrocarbons prevailed. The different tendency of *n*-alkanes to accumulate in animal tissues have been observed by several authors (Bernardini et al., 1982; Di Muccio, Boniforti, Palomba, Bernardini, & Delise, 1979; Lintas et al., 1979). In this sense, *n*-alkanes are more concentrated in the subcutaneous adipose tissues than in the intramuscular fat (Lintas et al., 1979).

In this work, the *n*-alkane content of intramuscular fat of dry-cured Iberian hams (34–38 mg/kg intramuscular fat of *biceps femoris*) is higher than the *n*-alkane content of the same samples in the fresh hams (16–29 mg/kg intramuscular fat of *biceps femoris*) (Tejeda et al., 2001). The origin of *n*-alkanes in animal tissues has not been well elucidated yet. Vegetables of the diet are considered the main source of *n*-alkanes in animal tissues. However, the hydrocarbons can be also generated during fatty acid oxidation (Loury, 1972; Shahidi, Rubin, & D'Souza, 1986); for example, during meat processing (Gray & Pearson, 1984). In addition, the *n*-alkane content in dry-cured ham can be modified as a consequence of a fatty acid decarboxylation producing *n*-alkanes during meat processing (Bastic et al., 1989). Different authors have indicated a higher content of these components in aged dry-cured hams (García et al.,

Table 1

n-Alkanes content in intramuscular lipids (mg/Kg intramuscular fat) of dry-cured hams (*biceps femoris* muscle) from Iberian and Iberian×Duroc pigs fed on *montanera* or concentrated feed (mean±standard error)

Lipid content ^a	Iberian		Iberian×Duroc		Effect	
					Genotype	Feeding
	Montanera	Concentrate feed	Montanera	Concentrate feed		
	11.3	10.4	10.7	7.5	Ns	*
<i>n</i> -Alkanes						
<i>n</i> -C ₁₄	7.11±1.33	10.24±2.04	8.04±0.89	10.82±0.59	Ns	Ns
<i>n</i> -C ₁₅	1.47±0.18	1.83±0.28	1.44±0.16	1.74±0.25	Ns	Ns
<i>n</i> -C ₁₆	4.71±0.50	4.70±0.60	4.10±0.28	4.93±0.54	Ns	Ns
<i>n</i> -C ₁₇	1.36±0.13	1.47±0.17	1.30±0.09	1.70±0.24	Ns	Ns
<i>n</i> -C ₁₈	2.22±0.27	2.36±0.20	1.68±0.37	2.32±0.45	Ns	Ns
<i>n</i> -C ₁₉	0.65±0.06	0.67±0.05	0.69±0.02	0.72±0.05	Ns	Ns
<i>n</i> -C ₂₁	0.70±0.09	0.51±0.06	0.63±0.10	0.53±0.06	Ns	Ns
<i>n</i> -C ₂₂	1.11±0.16	0.85±0.06	0.97±0.15	0.90±0.13	Ns	Ns
<i>n</i> -C ₂₃	0.90±0.17	0.70±0.05	0.76±0.12	0.75±0.05	Ns	Ns
<i>n</i> -C ₂₄	1.19±0.26	0.86±0.05	0.88±0.09	0.91±0.09	Ns	Ns
<i>n</i> -C ₂₅	1.55±0.24	1.39±0.12	1.38±0.27	1.27±0.07	Ns	Ns
<i>n</i> -C ₂₆	0.95±0.09	1.10±0.08	1.19±0.11	1.13±0.07	Ns	Ns
<i>n</i> -C ₂₇	1.61±0.07	1.69±0.03	1.70±0.07	1.64±0.02	Ns	Ns
<i>n</i> -C ₂₈	1.20±0.24	1.73±0.31	1.66±0.31	1.10±0.09	Ns	Ns
<i>n</i> -C ₂₉	1.89±0.06	2.03±0.07	1.99±0.06	1.90±0.02	Ns	Ns
<i>n</i> -C ₃₀	1.89±0.05	1.95±0.09	1.93±0.07	1.89±0.02	Ns	Ns
<i>n</i> -C ₃₁	1.69±0.05	1.75±0.05	1.73±0.06	1.69±0.02	Ns	Ns
<i>n</i> -C ₃₂	1.88±0.06	1.90±0.05	1.87±0.03	1.83±0.03	Ns	Ns
Total ENCA <i>n</i> -alkanes	22.27±2.55	25.69±3.04	22.70±1.31	25.39±0.67	Ns	Ns
Total ONCA <i>n</i> -alkanes	11.82±0.76	12.04±0.61	11.62±0.70	11.93±0.57	Ns	Ns
Totals	34.09±3.27	37.73±3.50	33.93±1.90	37.75±1.77	Ns	Ns

Ns, not significant.

^a Expressed as g/100 g of ham (*biceps femoris* muscle).

* (*P*<0.05)

1991) than in others cured meats (Berdagué, Denoyer, Le Quere, & Semon, 1991; Gray & Pearson, 1984). Among these components, short chain *n*-alkanes have been identified (Berdagué & García, 1990; García et al., 1991; Timón, Ventanas, Martín, Tejeda, & García, 1998). This fact could explain, in general, the high content of *n*-alkanes and particularly, the high content of short chain *n*-alkanes in our samples.

The content of *n*-alkanes in intramuscular lipids was not affected by diet or genotype of Iberian pigs (*P*<0.05); the samples of hams from Iberian and Iberian×Duroc pigs, fed on *montanera* and concentrated feed had a similar content of *n*-alkanes. These results are in contrast with those of the preceding paper (Tejeda et al., 1999). In this paper, significant differences (*P*<0.05) were noticed for *n*-alkanes values of subcutaneous fat from ham of pigs fed on *montanera* and concentrated feed, showing higher *n*-alkane content the samples of *montanera*. Nevertheless, in the previous work carried out on the same samples in the fresh hams (Tejeda et al., 2001) and following the same procedure, the *n*-alkanes content of the intramuscular lipids was not affected either by crossbreeding or by feeding.

The results of the quantitative analysis of *n*-alkenes are reported in Table 2.

The analysis of linear hydrocarbons in the intramuscular fat revealed higher levels of *n*-alkenes than *n*-alkanes (Fig. 1), but only the ENCA *n*-alkenes were identified. These results are in agreement with those of Timón et al. (1998) in a previous study about the volatile components in *biceps femoris* of dry-cured Iberian ham.

Table 2 shows the *n*-alkenes present in the intramuscular lipids from *biceps femoris* of the examined dry-cured Iberian hams, ENCA *n*-alkenes in *n*-C₁₄ to *n*-C₃₂ range were present in the unsaponifiable fraction of intramuscular lipids in the four groups studied. Quantities ranged from 1.70 to 17.69 mg/kg of intramuscular lipids. The most abundant *n*-alkenes were the shortest chain ones, in the same way we have formerly described regarding *n*-alkanes. Iberian hams from pigs fed on acorns and pasture showed higher *n*-C_{14:1} content than hams from pigs fed with concentrated feed. *n*-C_{32:1} was affected by diet and genotype, so hams from Iberian×Duroc pigs fed with concentrated feed showed a lower *n*-C_{32:1} content than the rest of the groups. However, no data are available in the literature about the *n*-alkenes content in dry-cured products.

In this paper, the linear hydrocarbons composition in intramuscular fat of dry-cured Iberian ham has been

described. The results reported in this study, in addition to the results reported in the preceding papers about subcutaneous fat from dry-cured ham (Tejeda et al., 1999) and intramuscular fat from fresh hams (Tejeda et al., 2001), will contribute to establish baseline values. At

the same time, this experiment was designed to compare hams from different diets and genotypes of Iberian pigs. The linear hydrocarbons content in intramuscular *biceps femoris* lipids was not affected by feeding or genotype of pigs.

Table 2

n-Alkenes content in intramuscular lipids (mg/kg intramuscular fat) of dry-cured hams (*biceps femoris* muscle) from Iberian and Iberian×Duroc pigs fed on *montanera* or concentrated feed (mean±standard error)^a

Lipid content ^b	Iberian		Iberian×Duroc		Effect	
	Montanera	Concentrate feed	Montanera	Concentrate feed	Genotype	Feeding
	11.3	10.4	10.7	7.5	Ns	*
<i>n</i> -Alkenes						
<i>n</i> -C _{14:1}	9.23±1.94a	16.30±2.47ab	14.58±1.85ab	17.35±1.88b	Ns	*
<i>n</i> -C _{16:1}	14.58±1.69	17.69±1.37	15.81±1.27	16.62±1.09	Ns	Ns
<i>n</i> -C _{18:1}	11.84±1.11	13.52±1.07	13.29±1.07	13.02±0.52	Ns	Ns
<i>n</i> -C _{20:1}	8.78±0.69	10.07±0.79	10.38±1.04	9.71±0.33	Ns	Ns
<i>n</i> -C _{22:1}	4.56±0.38	5.23±0.43	5.49±0.60	4.93±0.31	Ns	Ns
<i>n</i> -C _{24:1}	3.03±0.25	3.71±0.29	3.72±0.45	3.34±0.23	Ns	Ns
<i>n</i> -C _{26:1}	2.26±0.20	3.02±0.20	2.97±0.37	2.54±0.20	Ns	Ns
<i>n</i> -C _{28:1}	1.90±0.21	2.63±0.14	2.28±0.28	1.99±0.12	Ns	Ns
<i>n</i> -C _{30:1}	2.00±0.04	2.09±0.02	2.02±0.05	2.03±0.03	Ns	Ns
<i>n</i> -C _{32:1}	1.90±0.03b	1.70±0.01a	1.86±0.03b	1.92±0.02b	**	*
Totals	59.56±5.87	75.95±5.93	72.40±5.50	72.63±2.58	Ns	Ns

Ns, not significant.

^a Within rows, values with different letters differ significantly.

^b Expressed as g/100 g of ham (*biceps femoris* muscle).

* (*P*<0.05).

** (*P*<0.01).

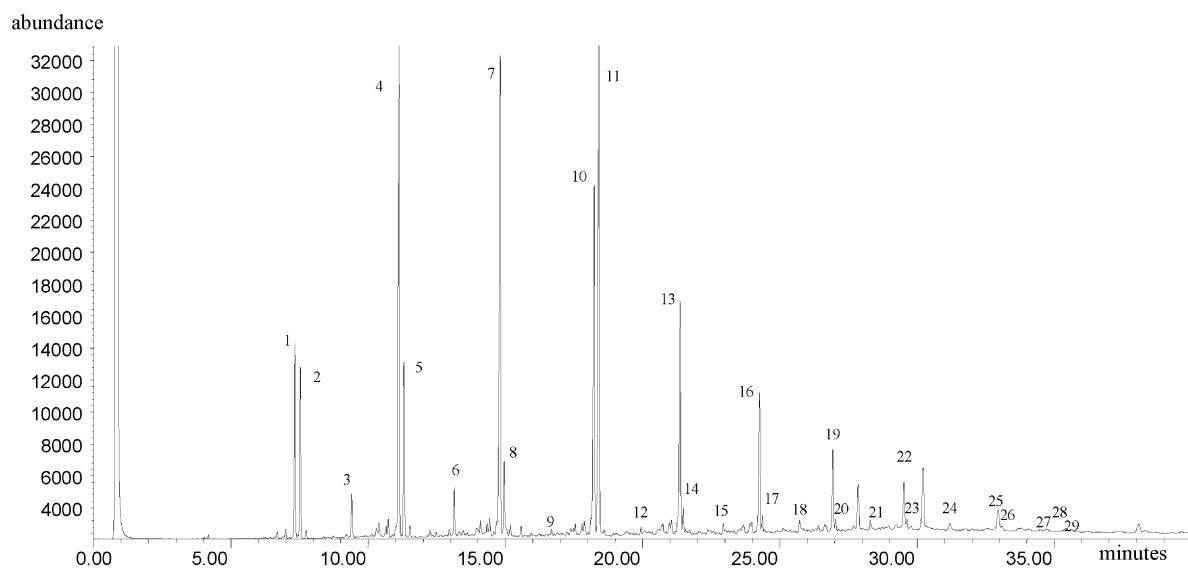


Fig. 1. Chromatogram of *n*-alkanes and *n*-alkenes identified in the unsaponifiable fraction of intramuscular fat of Iberian hams 1: (1-tetradecene (*n*-C_{14:1}); 2: tetradecane (*n*-C₁₄); 3: pentadecane (*n*-C₁₅); 4: 1-hexadecene (*n*-C_{16:1}); 5: hexadecane (*n*-C₁₆); 6: heptadecane (*n*-C₁₇); 7: 1-octadecene (*n*-C_{18:1}); 8: octadecane (*n*-C₁₈); 9: nonadecane (*n*-C₁₉); 10: 1-eicosene (*n*-C_{20:1}); 11: internal standard (eicosane) (*n*-C₂₀); 12: heneicosane (*n*-C₂₁); 13: 1-docosene (*n*-C_{22:1}); 14: docosane (*n*-C₂₂); 15: tricosane (*n*-C₂₃); 16: 1-tetracosene (*n*-C_{24:1}); 17: tetracosane (*n*-C₂₄); 18: pentacosane (*n*-C₂₅); 19: 1-hexacosene (*n*-C_{26:1}); 20: hexacosane (*n*-C₂₆); 21: heptacosane (*n*-C₂₇); 22: 1-octacosene (*n*-C_{28:1}); 23: octacosane (*n*-C₂₈); 24: nonacosane (*n*-C₂₉); 25: 1-triacontene (*n*-C_{30:1}); 26: triacontane (*n*-C₃₀); 27: hentriacontane (*n*-C₃₁); 28: 1-dotriacontene (*n*-C_{32:1}); 29: dotriacontane (*n*-C₃₂).

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