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Identification of branched hydrocarbons in subcutaneous fat from pigs reared outdoors

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SUMMARY – Neophytadiene, a natural branched hydrocarbon from the unsaponifiable lipid fraction of subcutaneous fat from Iberian and Gascon pigs, was analysed. Three groups of pigs were studied according to the management system (Iberian pigs fed outdoors, i.e. *Montanera*, Iberian pigs fed indoors, i.e. *Cebo*, and Gascon pigs fed outdoors, with concentrate feed). Neophytadiene was identified in all samples of subcutaneous fat from Iberian pigs fed on *Montanera*. This natural branched hydrocarbon was identified in some of the samples of subcutaneous fat from Gascon pigs, with lower levels than those in Iberian pigs fed outdoors. Finally, neophytadiene was not found in Iberian pigs fed on concentrate feed and in intensive conditions.

Keywords: Extensive system, pasture, pig, subcutaneous fat, natural hydrocarbons.

RESUME – *"Identification d'hydrocarbures ramifiés dans le gras sous-cutané de porcs élevés en plein air". Le néophytadiène, un hydrocarbure ramifié d'origine naturelle qui fait partie de la fraction insaponifiable de la graisse sous-cutanée des porcs Ibériques et Gascons, a été analysé. Nous avons étudié trois groupes de porcs selon le système de production (porcs Ibériques alimentés en plein air, i.e.* Montanera*, porcs Ibériques alimentés en système intensif, i.e.* Cebo*, et porcs Gascons élevés en plein air avec un aliment composé. Le néophytadiène a été identifié dans tous les échantillons de graisse sous-cutanée des porcs Ibériques de* Montanera*, qui ont présenté les niveaux les plus élevés de ce composé. Cet hydrocarbure ramifié naturel a été identifié dans quelques échantillons de porcs Gascons, à des niveaux inférieurs à ceux des porcs Ibériques alimentés en plein air. Finalement, nous n'avons pas identifié le néophytadiène dans la graisse sous-cutanée des porcs Ibériques alimentés avec des aliments concentrés.*

Mots-clés : *Système plein air, herbe, porc, graisse sous-cutanée, hydrocarbures ramifiés.*

Introduction

Several authors have related specific compounds of the unsaponifiable fraction in animal tissues to the feed, mostly of plant origin, consumed by the animals (Berdagué and García, 1990; Tejeda *et al*., 1999). In a recent paper, Tejeda *et al*. (2001) found a natural branched hydrocarbon, identified as neophytadiene (3-methylene-7,11,15-trimethylhexadecen-1-ene), which was present in intramuscular fat from Iberian pigs fed extensively (with acorn and pasture), whereas it was not in pigs fed on an intensive system with a commercial diet. These differences were due to the presence of neophytadiene in pasture consumed by pigs reared outdoors.

Hydrocarbon compounds are important natural components of vegetables wax (Tulloch, 1976; Post-Beittenmiller, 1996). Natural hydrocarbons are inert in the digestive tract and are little modified during digestion and metabolism (Van Straten, 1977; Mayes, *et al*., 1988; Rembold *et al.,* 1989). The absorption of hydrocarbons in the small intestine decreases with increasing length of the hydrocarbon chain (Mayes and Lamb, 1984). Normal biosynthesis is thought to be of limited importance in determining the presence of hydrocarbons in animal tissues (Bernardini, *et al.,* 1982).

The objective of this work was to isolate and quantify neophytadiene in subcutaneous fat from Iberian and Gascon pigs fed in extensive system, and to compare with Iberian pigs fed on concentrate feed exclusively in intensive system.

Materials and methods

Animals and diets

In the present study, subcutaneous fat from a total of 30 Gascon and Iberian pigs has been analysed. Gascon pigs (n=10) were reared outdoor (with pasture and concentrate feed), while Iberian pigs were divided into two groups, one reared outdoors (with acorn and pasture, system known as *Montanera*) (n=10) and the other reared indoors (with concentrate feed, system known as *Cebo*) (n=10).

Methods

Hydrocarbons were determined following the method described by Bories and Tulliez (1977), modified by Tejeda *et al*. (2001). Samples (4 g) of subcutaneous fat were saponified by refluxing for 2 hours with 140 ml of 15% KOH in ethanol (w/v). The warm solution was transferred into a separating funnel, and 70 ml of distilled water added and the unsaponifiable fraction extracted with 70 ml of hexane. The organic layer was washed three times with 50 ml distilled water, then dried over anhydrous sodium sulphate and concentrated to 2 ml. The extract was then transferred into a chromatography column (1.5 cm i.d.) that had been prepared by adding successively 2 g of silica gel and 8 g of anhydrous sodium sulphate. Hydrocarbons were eluted with 50 ml of hexane. After evaporating to dryness under vacuum, the residue was dissolved in 1 ml of hexane, and 50 µl were taken for gas chromatographic analysis.

Branched hydrocarbons 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 x 0.2 mm i.d.) with a film of 0.33 mm thick stationary phase of methyl silicone. Helium was the carrier gas at a flow rate of 1.49 ml/min. The oven program was from 100 to 270℃ at 6℃/min and 25 min at 270℃. Injector and detector temperatures were 260℃ and 270℃, respectively. The split ratio was 1:25, inlet pressure 14 psi, and the volume injected 2 l. Neophytadiene was identified 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 held at 280℃. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV, a multiplier voltage of 1756 V and collected data at 1 scan/s over a m/z range from 40 to 300. Spectra were compared with those of the standards and spectra from Wiley, Hewlett Packard and NIST (National Institute of Standards and Technology) libraries.

Results were subjected to analysis of variance (ANOVA) according to the General Linear Model (GLM) procedure, using SPSS software v.11.5 (2003).

Results and discussion

Figure 1 shows the natural branched hydrocarbons identified in subcutaneous fat from Iberian and Gascon pigs. The existence of branched hydrocarbons in intramuscular fat from pigs have been reported by different authors. Tejeda *et al*. (2001) found higher levels of these compounds in Iberian pigs fed in extensive system with respect to Iberian pigs fed in intensive system with a concentrate diet. Bastic *et al*. (1989) and Bernardini *et al.* (1982) identified natural branched hydrocarbons in subcutaneous and intramuscular fat from different commercial pigs. A possible source of branched hydrocarbons is fatty acid oxidation occurring in the meat (Loury, 1972; Shahidi *et al*., 1986). Gray and Pearson (1984) found an increase in branched hydrocarbon content in cured meat products during processing. However, Tejeda *et al*. (1999) suggested lipid oxidation is not the origin of most branched hydrocarbons in the adipose tissue of Iberian dry-cured hams, since the hydrocarbon chains are too long to be such oxidation products.

Neophytadiene content significantly differed ($p<0.001$) between groups. Neophytadiene was identified in every sample of subcutaneous fat from Iberian pigs fed extensively (with acorn and pasture), which presented the highest levels of this natural branched hydrocarbon. However, neophytadiene was not present in any of the samples from Iberian pigs fed indoor with concentrate feed. Finally, neophytadiene was identified in some of the samples of subcutaneous fat from Gascon

pigs, with lower levels than those in Iberian pigs fed outdoor (Fig. 2). These results are related with the differences in the levels of branched hydrocarbons in the feeds consumed by pigs during fattening period, and particularly in the case of neophytadiene, which was identified by Tejeda *et al*. (2001) in pasture samples exclusively. Our results are in accordance with those of Tejeda *et al*. (2001) in Iberian pig meat and with those of Petrón *et al*. (2005) in Iberian dry-cured ham. Although, Tejeda *et al.* (2001) reported than the content of neophytadiene in Iberian pig tissue is positively related to the time pigs remain in extensive conditions.

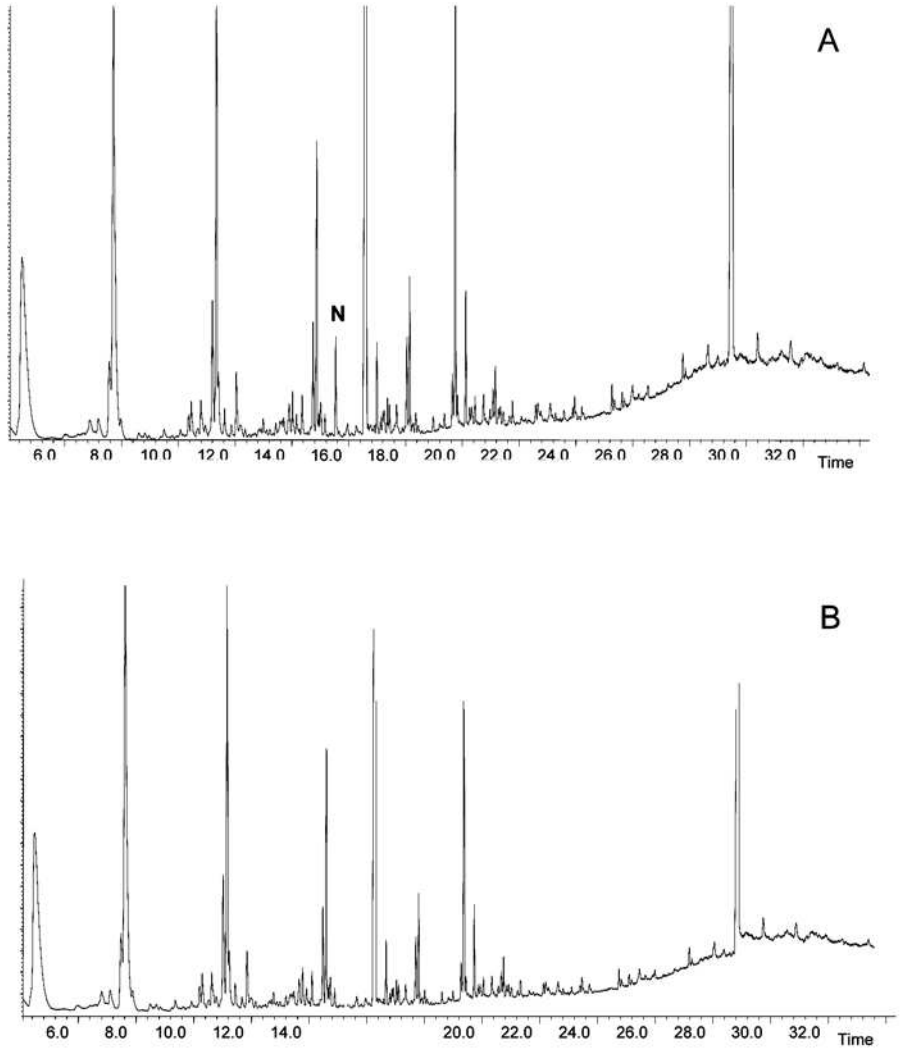

Fig. 1. Chromatograms of branched hydrocarbons identified in the subcutaneous fat from Iberian pigs fed in free-range system (A) and in intensive system (B). Note the presence of the peak corresponding to neophytadiene (N) in the chromatogram from samples of pigs fed extensively (A) and in absence in pigs fed on a concentrate feed in intensive conditions (B).

Conclusions

The results obtained in this paper confirm the presence of different natural compounds, included in the unsaponifiable fraction, such as branched hydrocarbons, which occur in animal tissue and are mainly derived from plant origin. Isolation and quantification of neophytadiene, a natural branched hydrocarbon, in subcutaneous fat from pigs could be used to differentiate between feeding regimes (extensive or in intensive conditions).

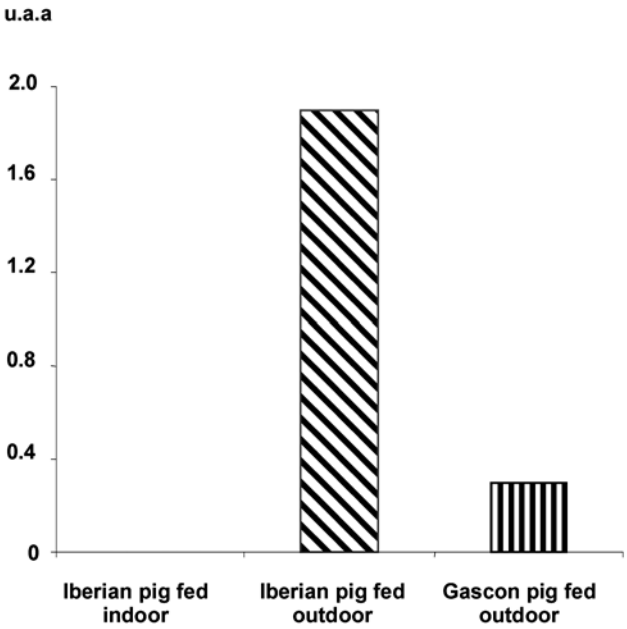

Fig. 2. Neophytadiene content in subcutaneous fat from Iberian and Gascon pigs.

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