# A STUDY OF THE UNSAPONIFIABLE FRACTION OF FAT FROM IBERIAN PIG.

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### BACKGROUND

Unsaponifiable fraction is a component of Iberian pig fat that still remains unstudied. This fraction is composed by different types of chemical classes, such as sterols, hydrocarbons, tocopherols and carotenoids. Some compounds of unsaponifiable fraction, as cholesterol exhibits health implications, due to their close relation with heart diseases (Peng et al., 1992; Morin et al., 1992); tocopherols and carotenoids have technological implication due to their antioxidant activity. Hydrocarbons normally occurs in foods from plant origin mostly, so the study of these compounds could be used to know the feeding system of Iberian pigs. Methods to distinguish between Iberian hams from different feeding systems of pig during fattening period have been carried out by study of fatty acids from different tissues (Flores et al., 1988; Ruiz et al., 1998; Cava et al., 1997). In this sense, due to vegetables are particularly rich in this type of compounds (Tulloch, 1976; Van Straten, 1997; Rembold et al., 1989) and that the unsaponifiable fraction and, more specifically, some components such as the alkanes seem to undergo little changes during digestion and metabolism until being deposed in the fat (Van Straten, 1997), the study of this type of compounds could contribute to differentiate between Iberian pig hams from different fattening systems.

### **OBJECTIVES**

The objective of this study was to analyse the unsaponifiable fraction of subcutaneous adipose tissue of fresh and dry-cured hams, and to identify its n-alkanes profiles.

# MATERIAL AND METHODS

Twenty Iberian x Duroc pigs were divided into two groups according to the traditional types of feeding during the fattening period prior to slaughter, named as *Montanera* (n=10) (fed on acorn and pasture) and *Pienso* (n=10) (fed on a commercial diet). Samples of subcutaneous adipose tissue from fresh and dry-cured hams Iberian were analysed.

Total lipid were extracted according to Bligh and Dyer (1959) method. Saponification process was carried out with potassium hydroxide ethanolic (15 % p/v), and unsaponifiable fraction was determined gravimetrically.

Preparative TLC plates (Silica Gel G) were used to separate unsaponifiable compounds, and the band corresponding n-alkanes was scraped from the plate transferred to a small glass column and eluted with 10 ml of hexane. n-Alkanes were analysed by gas chromatography (Hewlett Packard HP-5890A) equipped with a FID. Peaks of n-alkanes were identified by standard n-alkanes, and by GC/MS.

ANOVA was carried out to determine the differences between groups of hams, Montanera and Pienso, in fresh and final product.

# **RESULTS AND DISCUSSION**

In subcutaneous fat from fresh Iberian hams, similar unsaponifiable fraction percentage were observed in *Montanera* and *Pienso* hams (0.074 versus 0.077 %), however, in subcutaneous fat from dry-cured hams, unsaponifable fraction percentage was higher in *Montanera* than in *Pienso* hams (0.069 versus 0.056 %). These results are slightly lower than unsaponifiable fractions percentages reported in the literature in pig fat (over 0.1%) (Belitz and Grosch, 1988). When analysing unsaponifiable fraction by thin layer chromatography the bands corresponding to hydrocarbons and sterols (mainly cholesterol) were identified using cholesterol and squalene as standards.

Gas chromatography analysis of hydrocarbon TLC band revealed the presence of n-alkanes having a hydrocarbon chain longer that fourteen carbon atoms. n-Alkanes identified were: tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, heneicosane, docosane, tetracosane, pentacosane, hexacosane, heptacosane and nonacosane (Figure 1). In fresh hams, four n-alkanes (tetradecane, pentadecane, hexadecane and eneicosane) showed significant differences between the two groups of feeding pigs, and the values were higher in *Montanera* than in *Pienso* samples (Table 1).

In dry-cured hams the same tendency was observed, but in this case the differences were significant in some other n-alkanes: pentadecane, heptadecane, octadecane, nonadecane, eicosane and pentacosane (Table 2).

The high content of hydrocarbons in pasture (Tulloch, 1976; Van Straten, 1977) could be the cause that explain the higher n-alkanes amount in Montanera than in Pienso hams.

#### CONCLUSION

The study of unsaponifiable fraction of subcutaneous fat from Iberian pig, and particularly its n-alkanes profile could be a new method to differentiate, together with the study of fatty acids, Iberian pigs from different feeding systems.

# PERTINENT LITERATURE

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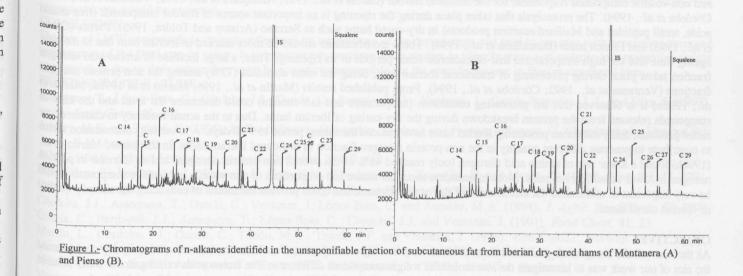
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<u>Table 1.</u>- n-Alkanes identified in the unsaponifiable fraction of fresh ham subcutaneous fat of Iberian pigs fed in two management systems: Montanera (MO) and Pienso (PI), and P values. Means and standards <sup>errors</sup> (x10-4) of the n-alkanes peaks areas.

<u>Table 2.</u> n-Alkanes identified in the unsaponifiable fraction of drycured ham subcutaneous fat of Iberian pigs fed in two management systems: Montanera (MO) and Pienso (PI), and P values. Means and standards errors (x10-4) of the n-alkanes peaks areas.

n-Alkanes	MO	PI	Р	n-Alkanes	MO	PI	Р
C14	$276 \pm 40$	$124 \pm 10$	0.020	C14	55 ± 19	50 ± 12	0.649
C15	$180 \pm 29$	88 ± 13	0.043	C15	$53 \pm 11$	23 ± 7	0.001
C16	$269 \pm 33$	$122 \pm 22$	0.021	C16	$51 \pm 11$	$129 \pm 20$	0.001
C17	$102 \pm 19$	$60 \pm 14$	0.139	C17	89 ± 8	66 ± 8	0.002
C18	$123 \pm 24$	61 ± 3	0.063	C18	$174 \pm 51$	$70 \pm 31$	0.004
C19	$44 \pm 6$	$20 \pm 10$	0.111	C19	133 ± 66	$32 \pm 16$	0.010
C20	56 ± 8	55 ± 7	0.858	C20	56 ± 10	$44 \pm 3$	0.032
C21	192 ± 7	$41 \pm 10$	0.003	C21	$149 \pm 43$	$160 \pm 40$	0.692
C22	97 ± 23	98 ± 19	0.980	C22	30 ± 5	$26 \pm 3$	0.182
C24	$192 \pm 48$	$199 \pm 40$	0.921	C24	53 ± 6	$46 \pm 14$	0.314
C25	$770 \pm 124$	541 ± 53	0.437	C25	159 ± 59	52 ± 15	0.004
C26	193 ± 59	$220 \pm 45$	0.733	C26	59 ± 6	69 ± 37	0.552
C27	171 ± 53	$178 \pm 34$	0.913	C27	79 ± 5	$62 \pm 38$	0.359
C29	$149 \pm 51$	$105 \pm 17$	0.463	C29	51±5	$37 \pm 16$	0.089