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Effect of pasture on chestnut or acorn on fatty acid composition and aromatic profile of fat of Cinta Senese dry-cured ham

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RESUMEN

Efecto del sistema de alimentación a base de castaña o bellota sobre el perfil de ácidos grasos y de los compuestos volátiles del jamón curado de "Cinta Senese".

El objetivo de este estudio fue la evaluación de la composición de los ácidos grasos y de los compuestos volátiles del jamón curado Toscano obtenido de cerdos Cinta Senese alimentados con bellota, castaña o pienso. Quince cerdos de raza Cinta Senese fueron criados en extensivo: 5 fueron criados en bosque de bellota (AC), 5 en bosque de castaña (CH) y 5 en un cercado y alimentados con pienso (MI). El día posterior al sacrificio se curaron los jamones. La composición de los ácidos grasos fue estadísticamente diferente entre los tipos de alimentación. La grasa de AC presentó el mayor porcentaje de AGMI total en comparación con CH y MI (51.3 y 53.9 vs 49.5% respectivamente) gracias al mayor porcentaje de ácido oleico (48.4 y 50.8 vs 46.2%, respectivamente), y la menor de AGPI total (13.8 vs 15.4 y 16.3%, respectivamente). En relación a los compuestos volátiles, la grasa de AC, en comparación con CH y MI, presentó mayores valores de aldehídos (44.1 vs 30.3 y 33.5% respectivamente) y éteres (4.04 vs 1.15 y 1.71% respectivamente).

PALABRAS CLAVE: Ácidos grasos – Bellota – Castaña – Cerdo – Compuestos volátiles – Jamón curado.

SUMMARY

Effect of pasture on chestnut or acorn on fatty acid composition and aromatic profile of fat of Cinta Senese dry-cured ham.

The objective of this study was to evaluate the effect of pasture on oak and chestnut grove on chemical and aromatic traits of Cinta Senese cured ham. Fifteen Cinta Senese barrows (124 kg of l.w. on average) were allotted to three groups: one group (MI) was reared outdoors in confined area and fed commercial feedstuff. The other two groups were raised under free-range conditions on acorn (AC) or chestnut (CH) woods. At slaughtering (148 kg of l.w.) the right thighs were seasoned for 360 days, on average, to obtained cured hams. Results demonstrated that subcutaneous fat of ham of CH and AC groups, respect to CONC group, was the richest in MUFA (51.3 and 53.9 vs 49.5% respectively), because of the higher content of oleic acid (48.4 and 50.8 vs 46.2%, respectively). AC showed a lower percentage of PUFA than CH and CONC (13.8 vs 15.4 and 16.3%, respectively). As regard volatile compounds, AC, respect to CH and MI groups, showed the highest value of aldehydes (44.1 vs 30.3 and 33.5% respectively) and ethers (4.04 vs 1.15 and 1.71% respectively).

KEY-WORDS: Acorn – Aromatic compounds – Chestnut – Dry-cured ham – Fatty acids – Pigs.

1. INTRODUCTION

The Tuscan dry-cured ham is a traditional pork meat product from Central Italy with a Protected Denomination of Origin (PDO) registration. Nowadays an increasing number of Tuscan hams is produced with the local Cinta Senese pig and consumers confer to those hams a consistent added value (Franci, 2004). The farming system used with the Cinta Senese is typical of the extensive pig production in the Mediterranean area. The characterization of meat from the extensive rearing system of the local breed could be exploited to increase the commercial value of the ham, similarly to the Iberian pig experience (Lopez-Bote, 1998). Dry-curing of Cinta Senese hams is a traditional process that leads to a food product with unique flavour. The aroma is due to chemical and biological changes and lipids serve several roles in flavour development (Calkins and Hodgen, 2007) giving rise to a large number of volatile compounds. Several studies on the flavour of raw ham of Mediterranean area have been dealing (Dirinck *et al.*, 1997). Buscailhon *et al.* (1994) and Careri *et al.* (1993) studied the relations between compositional traits and sensory qualities of French and Italian dry-cured ham, while López *et al.* (1992) and Carrapiso *et al.* (2002) described the volatile compounds of Iberian dry-cured ham as affected by rearing system. Compounds coming from feeds contribute to the final flavour of dry-cured meat products. Also the quality of Cinta Senese meat strongly depends on rearing and feeding system (Pugliese and Bozzi, 2004) then, in the future, the commercial value of dry-cured products of this breed could be linked to a particular rearing system. So, the aim of this study was to evaluate the effect of pasture on oak plantation and on chestnut grove on chemical and aromatic traits of dry-cured ham.

2. MATERIAL AND METHODS

2.1. Animals and diets

Fifteen Cinta Senese barrows, weighing about 124 kg, were randomly allotted to three groups (5 pigs per group) according to the type of feeding during the finish-fattening period (last 90 days prior to slaughter). One group (MI) was reared in confinement (paddock of 1 ha) and had free access to appropriate diet (3 kg/pig/d). The other two groups were raised under extensive production system according to the traditional free-range conditions: pasture and acorn (AC) for the group reared in oak plantation (8 ha) *(Quercus cerris* and *Quercus pubescens)*; pasture and chestnut (CH) for the group reared in chestnut grove (8 ha) (*Castanea sativa*). Chemical composition, tocopherol content and fatty acid composition of experimental mixture, acorn and chestnut are shown in table 1. Before the experimental period, animals were reared together, under a confinement system, and fed the same feedstuff. Pigs were slaughtered at the weight of 148 kg, on average.

2.2. Materials and technology

At slaughtering thighs were removed from the right half-carcasses and processed according to the dry-curing method of Tuscan ham (Table 2). At the end of seasoning period, a sample-slice, about 3 cm thick, was taken transversally from the caudal portion of the cured ham in the middle of the area where the femur was removed, immediately vacuum-packed and frozen at −80 °C until the analysis.

Table 1
Composition of diets

	Chestnut	Acorn	Mixture
Proximate analysis (% on DM)			
Protein	7.90	5.47	18.00
Ether extract	2.16	2.96	4.00
N-free extract	80.92	86.80	72.86
Cellulose	4.03	3.44	3.46
Ash	4.97	1.32	0.76
Tocopherol content (mg/kg of DM)			
γ-tocopherol	43.94	41.65	11.07
α-tocopherol	2.21	5.16	34.17
Fatty Acids (% of total fatty acid)			
C16:0	13.90	15.00	16.70
C18:0	1.00	1.70	2.00
C18:1	37.50	46.80	19.70
C18:2	42.30	30.20	56.20
C18:3	3.90	4.00	4.50
SFA	15.40	17.30	19.00
MUFA	38.30	48.30	20.30
PUFA n-3	3.90	4.00	4.50
PUFA n-6	42.30	30.20	56.20

2.3. Chemical analysis

The chemical analysis of food was carried out following the methods of A.O.A.C. (1990) and tocopherol analysis were performed according to the method described by Rey *et al.* (2006).

The following analyses were carried out on the subcutaneous fat: 1) moisture; 2) total lipids extracted according to Folch *et al.* (1957); 3) fatty acid profile of total lipids. Fatty acid methyl esters were prepared by esterification in presence of sulphuric acid (Morrison and Smith, 1964), and were analysed by gas chromatography, using a DANI 86.10 apparatus equipped with a flame ionisation detector (FID). Fatty acids were separated on a capillary column coated with FFAP-TPA stationary phase (30 m length; 0.32 mm internal diameter; 0.25 mm film thickness). Temperature of the column started at 160 °C and reached 220 °C, with 2 °C/min. increase. Temperature of the detector was set at 260 °C. Methyl esters were identified by their retention time and expressed as percentage of total detected methyl esters.

2.4. Volatile compounds analysis

Volatile compounds of seasoned fat were extracted by headspace-SPME (Ruiz *et al.*, 2001). One gram was weighed into a 4 ml vial and screw-capped with a Teflon-silicone disk. A SPME (Supelco, Bellefonte, PA, USA) fibre coated with carboxen/poly (dimethylsiloxane) (75μm thickness) was inserted into the sample vial and exposed to the headspace. Extraction was performed at 37 °C for 30 min in a water bath. Before the analysis the SPME fibre was preconditioned at 280 °C for 50 min. in the gas chromatograph injection port. Volatile compounds analysis was performed using a HP-6890-GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector (HP-5973 Network, Hewlett-Packard). Volatiles were separated using a 5% phenyl-methylsilicone (HP-5) bonded phase fused-silica capillary column (50 m 0.32 mm i.d., film thickness 1.05 μm, Hewlett-Packard). Carrier gas was helium at a flow rate of 1.45 mL min^{-1}. Compounds were identified by comparing their mass spectra with those contained in the NIST and Wiley libraries and by comparison of Kovats indexes with those reported in the literature (Kondjoyan and Berdagué, 1996).

2.5. Statistical analysis

The effect of feeding system was tested by analysis of variance (ANOVA), using GLM procedure (SAS, 2007). Data were analysed with the following linear model: $Y_{ij} = \mu + D_i + b_i{*}X_{ij} + e_{ij}$, where Y_{ij} is the I^{th} observation; μ is the overall mean; D is the i^{th} diet; the b_i term is the regression coefficient on cured ham weight (X), e_{ij} is the residual error. For the proportion of identified volatiles compounds only the effect of feeding system was tested.

Table 2
The dry-curing process of Tuscan ham

Stage	Characteristics
Trimming	Ham is defatted and trimmed, by leaving 10 cm of lean beyond the femur knob. In the hams of Cinta Senese, foot is not removed.
Salting	Ham is covered with salt and a mixture of natural and typical Tuscan aromas, then they were placed in a salting cellar for 18-21 days, at 4 °C and 90% relative humidity. In the rule of Tuscan ham the use of nitrite is admitted, but in this case no preservatives were used.
Resting	Ham is first brushed to remove superficial salt, than it is kept for 40 days in a chamber temperature varied between 2-4 °C, 60% relative humidity.
Greasing	Six month after starting the processing (salting), the lean portions of hams are covered with minced pork fat with the addition of salt and pepper. This treatment avoid that the outer muscular layers are dried out in relation to the internal ones.
Seasoning	Ham is kept in a cellar for 300 days. The conditions in the cellar are highly constant: temperature varies between 17-18° C, 60 % relative humidity.

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition

In table 3 the chemical composition of fat of dry-cured ham is reported. The pigs pastured on acorn showed the highest content of lipid, while no differences were found between the other two groups. Given that moisture was analogous among the groups, this result is probably linked to the high content of N-free extractive of acorn. As reported by Edwards (2004), the high quality of dry-cured ham in Mediterranean silvopastoral system is associated to a relatively high level of intramuscular fat resulting from access of local breeds to acorn which is high in starch. It is likely that in subcutaneous fat the high energy level of acorn lead to higher synthesis of lipids as well.

Fatty acids percentage (Table 3) is significantly affected by feeding system. Pigs fed acorn showed the lowest content of SFA and the highest percentage of MUFA, oleic acid prevalently. Significant enrichment in MUFA in hams from pigs raised extensively with free availability of acorn and pasture has been reported by several Authors (Flores *et al*., 1988; Cava *et al*., 2000; Carrapiso *et al*., 2003). This results can be explained by a ready incorporation of dietary fatty acids that occurs during fattening (Fontanillas *et al*., 1998). In fact, as reported in table 1, acorn showed a very high concentration of oleic acid (nearly 50%), and a more limited concentration of linoleic acid, in relation to the other two feedstuffs. These results are in agreement with previous findings on fresh fat of the same animals (Pugliese *et. al*., 2006).

Table 3
Fatty acid composition of seasoned fat (% total fatty acid)

	DIET			
	CH	**AC**	**MI**	**RSD**
Moisture	1.37	2.23	2.00	0.55
Lipids	77.62^{a}	81.69^{b}	78.80^{a}	1.74
C16:0	21.48^{ab}	21.10^{a}	22.12^{b}	0.69
C16:1	1.65	1.72	1.84	0.18
C18:0	10.24	9.62	10.4	1.03
C18:1	48.42^{a}	50.76^{b}	46.24^{c}	0.90
C18:2	13.64^{a}	12.25^{b}	14.61^{a}	0.90
C18:3	1.0	0.80	0.84	1.17
C20:1	0.95^{a}	1.16^{b}	0.94^{a}	0.10
C20:2	0.61	0.62	0.72	0.08
SFA	33.26^{ab}	32.30^{a}	34.20^{b}	1.33
MUFA	51.35^{a}	53.93^{b}	49.48^{c}	0.96
PUFA	15.38^{a}	13.77^{b}	16.28^{a}	1.01
PUFA N-6	14.37^{a}	12.98^{b}	15.44^{a}	0.98
PUFA N-3	1.01	0.8	0.84	0.17
MDA (mg/Kg)	1.17	1.74	1.65	0.45

$^{a, b}$ within criterion means different ($P < 0.05$).

No differences among groups were found in MDA content that, however, resulted very low. This confirms previous results on cured lard of the same animals (Pugliese *et al*., in press) and the positive effect of pasture on wood on oxidative stability of pig.

3.2. Volatile profile

About 60 volatile compounds were identified and assigned to the respective chemical families. The main compounds are shown in table 4. Most of them

Pigs reared on chestnut showed lower content of MUFA than pigs fed acorn but higher than the ones fed mixture. Also in this case the quality of lipids is strongly related to the feeding conditions. Indeed also chestnut is characterized by higher content of oleic acid respect to concentrate (Table 1). The higher MUFA percentage of CH pigs respect to MI pigs is in general agreement with findings of Sirtori *et al.* (2008) on Cinta Senese reared in confinement and fed chestnut and findings of Coutron-Gambotti *et al.* (1998) on subcutaneous and intramuscular fat of Corsican pigs. As regard PUFA content AC group showed the lowest value while no differences between the other two groups were found. In pigs reared outdoor the variability of PUFA content, mainly depend on grass availability. As reported by Lopez-Bote (2000), the grass is the main source of long chain PUFA (n-3 prevalently) but, in extensive conditions, it is very difficult to quantify the intake of grass, so in this trial it is probable that the pasture on chestnut wood, which was more rich in grass than those of oak wood, leaded to higher accumulation of PUFA n-3. However, the picture relative the PUFA n-6 content appears in good agreement with the content of the same fatty acids in the original feedstuff.

Table 4
Percentage of volatile compounds

		DIET			
COMPOUNDS*	**KI****	**CH**	**AC**	**MI**	**RSD**
ACIDS		18.85	12.09	21.64	9.45
acetic acid	595	1.91	1.97	2.53	3.07
butanoic acid	788	1.38	0.75	1.27	0.81
butanoic acid, 3-methyl	841	0.56	0.40	0.45	0.40
butanoic acid 2-methyl	850	0.21	0.20	0.14	0.22
pentanoic acid	879	0.40	0.39	0.60	0.24
hexanoic acid	974	5.42	5.15	4.08	4.11
octanoic acid	1175	0.72	0.49	0.71	0.33
nonanoic acid	1226	0.04	ND	0.23	0.31
decanoic acid	1279	0.51	0.26	0.50	0.19
dodecanoic acid	1567	0.06	ND	1.32	1.78
tetradecanoic acid	1755	1.74	0.41	3.07	3.11
hexadecanoic acid	1977	5.88	2.06	6.72	4.18
ALCOHOLS		7.90	10.81	9.63	4.03
Ethanol	<500	7.90	10.81	9.63	4.03
ALDEHYDES		33.43[a]	46.54[b]	36.60[ab]	9.00
acetaldehyde	<500	3.87	1.58	2.23	1.83
2-propanal	510	0.75	3.49	1.77	2.72
butanal, 3 methyl	642	0.09	0.19	0.32	0.19
Pentanal	698	1.74	4.33	3.95	2.90
2-heptanal (Z)	957	0.62	0.90	1.21	0.77
Hexanal	800	23.23	29.54	23.87	6.20
Heptanal	900	1.41	3.44	1.66	2.21
Benzaldehyde	965	0.17	1.70	0.24	1.59
Octanal	1000	0.84	0.87	0.66	0.35
Benezeneacetaldehyde	1048	0.06	0.12	0.08	0.11
Nonanale	1142	0.47	0.33	0.54	0.47
Decanal	1186	0.034	ND	0.031	0.06
2,4 decadienal	1262	ND	0.05	0.02	0.05
Tetradecanal	1405	0.13	ND	0.02	0.12
ALIFATIC HYDROCARBONS		15.90	8.49	13.29	10.45
Pentane	500	3.99[ab]	7.18[a]	3.09[b]	2.87
Hexane	600	4.09	0.12	2.32	4.36
heptanes	700	0.35	ND	0.07	0.36
Undecane	1100	0.19	0.55	0.15	0.39
Pentadecane	1500	0.10	0.02	0.01	0.09
3-eicosene (E)	1636	0.90	0.02	0.88	1.02
Heptadecane	1700	2.67	0.23	2.69	3.24
pentadecane 2,6,10,14 tetramethyl	1703	1.97	0.30	2.06	2.47
1-nonadecene	1957	1.64	0.06	2.01	2.17
AROMATIC HYDROCARBONS		0.30	0.60	0.27	0.36
benzene methyl (toluene)	772	0.19	0.40	0.17	0.23
benzene 1,3 dimethyl	873	0.11	0.20	0.09	0.18
CHLORIDE COMPOUNDS		1.06	0.64	0.87	0.45
Chloroform	609	1.06	0.64	0.87	0.45
ESTERS		3.30	4.21	3.96	1.41
Ethylacetate	605	1.25	1.06	1.24	0.95
hexanoic acid ethyl ester	995	1.79	2.74	2.38	0.89
butanoic acid ethyl ester	1091	0.09	0.10	0.09	0.16
octanoic acid ethyl ester	1193	0.16[a]	0.30[b]	0.25[ab]	0.08
FURANS		0.76	1.07	0.67	0.64
furan 2-ethyl	704	0.16	0.19	0.33	0.23
furan 2-pentyl	991	0.59	0.87	0.34	0.61
KETONES		16.81	13.61	10.80	10.88
2-propanone	512	6.80	7.85	4.89	4.84
2-pentanone	684	5.64	3.00	3.37	4.68
2,3- pentanedione	696	3.31	1.66	1.45	2.83
2-butanone 3 hydroxy	710	0.12	ND	ND	0.16
2-hexanone	791	0.27	0.21	0.32	0.24
2-heptanone	890	0.67	0.88	0.77	0.29
NITROGENS COMPOUNDS		0.55	0.46	0.55	0.27
Pyridine	748	0.08	0.05	0.14	0.13
pyrazine 2,6 dimethyl	912	0.47	0.41	0.42	0.23
SULPHUR COMPOUNDS		1.06	1.24	1.57	2.04
carbon disulfide	544	1.06	1.24	1.57	2.04
TERPENS		0.08	0.23	0.12	0.16
Linalool	1097	0.08	0.23	0.12	0.16

[a, b] within criterion means different ($P < 0.05$).
* Expressed as area percentage of total volatile compounds identified.
** KI = Kovats index in agreement with literature.

are derived from the autoxidation of unsaturated fatty acids, although some could also derive from amino acids, having origin from Strecker degradation (Ruiz *et al*., 1999). According to research on Iberian dry-cured ham (Andrés *et al*., 2002), a high number of acids has been detected. Some of these, as acetic acid, are originated from the microbial metabolism of glucides (Kandler, 1983). The other acids could derive from the hydrolysis of triglycerides and phospholipids and from lipid oxidation reactions (Ruiz *et al*., 2002). Among short chain acids, 3-methylbutanoic acid and 2-methylbutanoic acid have been detected. These acids, as reported by Muriel *et al*., (2004), play an important role in aroma development. Aldehydes compounds represent the most important chemical family in Cinta Senese ham, hexanal being the major one. Hexanal derives from the oxidation of n-6 fatty acids, heptanal arises from oleic or linoleic autooxidation and, finally, octanal comes from oleic acid oxidation. Other compounds from lipid oxidation were also aliphatic hydrocarbons (as pentane and hexane), furans (as furan-2-ethyl) and ketones (as 2-pentanone, 2-propanone, 2,3-pentanedione). About aromatic hydrocarbons, methylbenzene (toluene) could derive from cyclation of unsaturated carboxylic chains produced by lipid degradation (Min *et al*., 1977). The major ester found was ethyl acetate, which is formed through esterification reactions between ethanol and carboxilic acids. It leads to fruity notes and it has a low odour threshold (Muriel *et al*., 2004). Other chemical families, such as sulfur compounds (i.e. carbon disulfide) and nitrogen compounds (i.e. 2,6-dimethylpirazine and pyridine), derive from Maillard reaction (Ruiz *et al*., 2001). Pirazine has characteristic walnut, toasted or potato odours (Meynier *et al*., 1999). The main terpen found is linalool that leads to flowery notes. The accumulation of terpens in fat depots has two possible origins: from animal feeding or from spices added during processing (Muriel *et al*., 2004).

As regard the rearing system effect the significance was found only for ethers compounds and for total aldehydes percentage, highest in AC group. The latter results is in agreement with those reported by Muriel *et al.* (2004) on Iberian dry-cured loin and by López *et al.* (1992) on Iberian dry-cured ham. Hexanal is the main compound derived from the oxidation of *n-6* fatty acids, heptanal arises from oleic or linoleic acid while octanal and nonanal comes from oleic acid oxidation. A relationship between these volatile compound and fatty acids percentage seems to be only for heptanal, higher in pigs fed acorn that showed also the highest content of oleic acid. On the other hand hexanal, highest in AC group, is associated to green, fruity and acorn-like odour (Carrapiso *et al*., 2002). For the other compounds, the high variability found among data, didn't allowed to reach the significance threshold, nevertheless the high differences in absolute values. Apart of the significance level it is possible to underline the highest percentages of ketons, as 2-propanone, 2,3-pentanedione and aliphatic hydrocarbons, showed by pigs fed chestnut.

4. CONCLUSIONS

The pasturing on wood, especially on acorn wood, affected the fatty acid composition of cured subcutaneous fat. In particular the highest level of oleic acid found in ham from pigs fed acorn could be an important item of characterization of Cinta Senese dry-cured ham. These differences in acidic composition seem to be related to the same differences in volatile compounds, even if only for aldehydes and ethers compounds statistic differences among groups were achieved.

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