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Food Chemistry 87 (2004) 367–376
Food Chemistry
www.elsevier.com/locate/foodchem

Lipolytic and oxidative changes during refrigeration of cooked loin chops from three lines of free-range-reared Iberian pigs slaughtered at 90 kg live weight and industrial genotype pigs

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Received 17 June 2003; received in revised form 8 December 2003; accepted 8 December 2003

Abstract

Lipolytic and oxidative changes in cooked and refrigerated loin chops from Iberian and industrial genotype pigs were studied. Iberian pigs were free-range-reared and fed with access to pasture and a concentrate feed based on cereals. *M. longissimus dorsi* from industrial genotype pigs (Large White-Landrace × Large White) were obtained from a local slaughterhouse. Cooked loins from Iberian pigs showed higher intramuscular fat (IMF) ($p < 0.05$) and lower polar lipid (PL) contents than the industrial genotype pig loins (g PL/g fat, $p < 0.05$). The increase of TBA numbers (mg MDA/kg defatted dry basis) after cooking and refrigerated storage was significantly higher ($p < 0.05$) in the industrial genotype pig loins than in the Iberian ones. After cooking and refrigerated storage, percentages of polyunsaturated fatty acids (PUFA) from intramuscular fat and the polar lipid fraction decreased while saturated and monounsaturated fatty acid percentages increased. Linoleic, arachidonic and total PUFA proportions in the polar lipid fraction, decreased in the four groups but these were only statistically significant in the cooked loins from the industrial genotype pig breed.

Keywords: Cooked meat; Refrigerated storage; Lipolysis; Oxidation; Free range rearing; Meat quality

1. Introduction

Cooking is the commonest technology in meat preparation. High temperatures during cooking lead to a dramatic increase in lipid oxidation in muscle (Kingston, Monahan, Buckley, & Lynch, 1998). This being one of the most important factors in deterioration of refrigerated cooked meat (Pearson, Love, & Shorland, 1977). The acceleration of lipid oxidation, following cooking, has been attributed to heat-induced changes in muscle components, including disruption of cellular compartmentalization and exposure of membrane lipids to a prooxidative environment, release of catalytic free iron from myoglobin (Kristensen & Andersen, 1997; Kristensen & Purslow, 2001), and thermal inactivation of antioxidant enzymes (Lee, Mei, & Decker, 1996). So, lipid oxidation processes are largely determined by the level of intrinsic prooxidative factors, such as heme and lipid content, fatty acid composition (mainly polyunsaturated fatty acids) and their distribution in lipid fractions (neutral and polar lipids) (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Additionally, during refrigerated storage, lipolytic processes liberate free fatty acids which are the substrates for subsequent oxidative reactions (Nawar, 1996) which lead to the development of rancidity, loss of nutritional value and formation of undesirable flavours (e.g. WOF; Warm-Over-Flavour) in cooked meat (Morrissey et al., 1998).

Recently, a growing interest in meat obtained from traditional farm animal rearing systems has arisen in Europe. The strong economy and quality of life, achieved in Europe, makes consumers demand a high quality in meat products. The organic production

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doi:10.1016/j.foodchem.2003.12.007

system (and the free-range rearing of pigs) modifies the fatty acid profiles of intramuscular lipids and increases the amounts of natural antioxidants that increase stability against oxidation (Cava, Ventanas, Tejeda, Ruiz, & Antequera, 2000; Nilzén, Babol, Dutta, Lundeheim, Enfält, & Lundström, 2001). Additionally, it increases meat juiciness, tenderness and overall acceptability (Enfält, Lundström, Hansson, Lundeheim, & Nyström, 1997) and improves animal welfare (Sundrum, 2001). In the southwest of Spain the Iberian pig is traditionally reared under extensive systems in which animals are fed on natural resources. Genetic differences and the nature of the rearing system for the Iberian pigs result in muscles from Iberian pigs containing higher amounts of intramuscular fat and heme pigments than muscles from industrial genotypes reared under intensive systems (Cava, Estévez, Ruiz, & Morcuende, 2003; Estévez, Morcuende, & Cava, 2003a).

The aim of the present work was to investigate lipolytic and oxidative changes in cooked loin chops from three lines of free-range-reared Iberian pigs and industrial genotype pigs under refrigeration, in order to assess the stability of the samples after culinary and storage processes.

2. Materials and methods

2.1. Animals and sampling

Twenty one Iberian pigs from three different lines 'Lampiño' (LAM; $n = 7$), 'Retinto' (RET; $n = 7$) and 'Torbiscal' (TOR; $n = 7$) were reared outdoors with access to pasture grazing and slaughtered at 90 kg live weight. Industrial genotype pigs (IND) (Large White-Landrace × Large White) ($n = 5$) were reared in an intensive system. Samples from the *m. longissimus dorsi* (LD) muscle were taken from the carcasses.

Whole samples of the muscles (about 20 g) were placed in plastic bags and cooked in a hot water bath to an internal temperature of 80 °C for 10 min. Internal temperature in loin chops was determined using a thermocouple placed in the core of the chop. They were then rapidly chilled to 15–20 °C with a cold water shower for 10 min and dried on the surface with a paper towel. Samples were weighed before and after cooking. Total weight loss in chops after cooking was expressed as cooking loss.

After cooling, cooked samples were placed on Styrofoam meat trays, over-wrapped in oxygen permeable PVC films, and stored at +4 °C for 10 days under fluorescent light.

Samples from raw meat and cooked and refrigerated meat were vacuum-packaged and stored at −80 °C before chemical analysis.

2.2. Analytical methods

2.2.1. Samples

Samples from Iberian and industrial genotype pigs (both raw and cooked and refrigerated ones) were analysed according to the following methods.

2.2.2. Moisture analysis

Moisture was determined by using an AOAC method (AOAC, 2000).

2.2.3. Lipid extraction

Lipids were extracted from 5 g of muscle with chloroform/methanol (2:1), according to the method described by Bligh and Dyer (1959). The dry lipid extract was weighed to estimate the lipid content.

2.2.4. Lipid fractions content

Determination of phosphorus content was carried out with the method described by Barlett (1959). Polar lipid content was calculated by multiplying phosphorus content by 25. Neutral lipid fraction content was calculated by difference between total lipids and polar lipid fraction.

2.2.5. Lipid extract fractionation

Total lipid extracts were fractionated into neutral lipids, polar lipids and free fatty acids on aminopropyl cartridges, following the procedure described by García-Regueiro, Gilbert, and Díaz (1994).

2.2.6. Fatty acid profiles analysis

Fatty acid methyl esters from neutral, polar and free fatty acid fractions were prepared by acidic esterification in the presence of sulphuric acid, following the method of Cava et al. (1997). Fatty acid methyl esters (FAME) were analysed using a Hewlett Packard HP-5890A gas chromatograph, equipped with a flame ionisation detector (FID). The derivatives were separated on a capillary column (Hewlett Packard FFAP–TPA fused-silica column, 30 m long, 0.53 mm internal diameter and 1.0 μm film thickness). The injector and the detector temperature were held at 230 °C. Column oven temperature was maintained at 220 °C. The flow rate of the carrier gas (N_2) was set at 1.8 ml/min. Identification of FAME was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percent of total fatty acid methyl esters.

2.2.7. Free fatty acids quantification

Assessment of lipolytic processes was by quantification of free fatty acids. Free fatty acids were quantified

in the FFA fraction after methylation, by GC analysis using C13:0 acid as internal standard.

2.2.8. Thiobarbituric acid-reactive substances number

Thiobarbituric acid-reactive substances (TBARS) were evaluated using the method of Salih, Smith, Price, and Dawson (1987).

2.3. Statistical analysis

Data were analysed using the analysis of variance (ANOVA) of SPSS 10.0 statistical package (SPSS, 1997). The effect of cooking and refrigerated storage on oxidation was analysed using a Student's test for dependent variables. When a significant P was detected ($P < 0.05$), means were compared using Tukey' s test.

Table 1
Cook loss and intramuscular fat, neutral and polar lipid contents of m Longissimus dorsi from three lines of free-range-reared Iberian pigs and industrial genotype pig

	Raw meat (day 0)						Refrigerated cooked meat (day 10)					
	Iberian pig lines						Iberian pig lines					
	LAM	RET	TOR	IND	sem	P	LAM	RET	TOR	IND	sem	P
% Moisture	72.28	73.37	73.65	73.78	0.00	ns						
% Cooking loss							37.04	38.93	36.90	37.72	0.07	ns
% IMF[1]	3.34[a]	3.17[a]	2.51[a]	1.41[b]	0.02	**	4.48[a]	3.88[ab]	2.52[bc]	1.56[c]	0.03	***
g NL[2]/g total lipid	0.84[a]	0.82[a]	0.79[a]	0.52[b]	0.01	**	0.80[a]	0.84[a]	0.73[a]	0.46[b]	0.01	***
g NL/100 g muscle	2.79[a]	2.72[a]	1.98[a]	0.85[b]	0.03	***	3.65[a]	3.26[a]	1.85[b]	0.85[b]	0.03	***
g PL[3]/g total lipid	0.16[b]	0.18[b]	0.21[b]	0.41[a]	0.00	*	0.20[b]	0.17[b]	0.27[b]	0.54[a]	0.00	***
g PL/100 g muscle	0.54[ab]	0.45[b]	0.52[ab]	0.57[a]	0.00	***	0.84	0.82	0.68	0.72	0.00	ns
mg FFA/100 g total lipid	4.43[b]	4.24[b]	5.26[b]	12.0[a]	0.18	***	10.1	12.9	12.2	12.8	0.28	ns
mg FFA/100 g muscle	0.15[ab]	0.12[b]	0.13[b]	0.17[a]	0.04	**	0.45[b]	0.56[a]	0.29[c]	0.31[c]	0.08	***

In the same line, means with different superscripts, are significantly differed in ANOVA test. Pairs of means on days 0 and 10 underlined, significantly differed in a Student test for dependant variables. [1,2,3]IMF: Intramuscular fat; NL, Neutral lipids; PL: Polar lipids.

Table 2
Fatty acid profile (% methyl esters analysed) of intramuscular fat (total lipids) of raw and cooked and refrigerated loins from three lines of free-range-reared Iberian pigs and industrial genotype pigs, after 10 days at 4 °C

	Raw meat (day 0)						Refrigerated cooked meat (day 10)					
	Iberian pig line						Iberian pig line					
	LAM	RET	TOR	IND	sem	P	LAM	RET	TOR	IND	sem	P
C12	0.12[b]	0.12[b]	0.13[b]	0.15[a]	0.00	**	0.08	0.05	0.07	0.08	0.01	ns
C14	1.62[a]	1.55[a]	1.61[a]	1.35[b]	0.01	**	1.55[a]	1.58[a]	1.47[ab]	1.29[b]	0.11	*
C16	25.8[a]	26.3[a]	26.3[a]	24.3[b]	0.04	***	22.0	26.4	25.7	24.3	0.01	ns
C17	0.19[b]	0.29[a]	0.30[a]	0.36[a]	0.00	***	0.17c	0.32[ab]	0.31[b]	0.44[a]	0.04	***
C18	12.5	12.7	12.6	12.2	0.00	ns	13.2	13.4	13.03	13.85	0.00	ns
C20	0.24[a]	0.22[ab]	0.21[b]	0.18c	0.03	***	0.27[a]	0.23[b]	0.23[b]	0.25[ab]	0.02	*
ΣSFA	40.5[ab]	41.2[a]	41.1[a]	38.6[b]	0.07	*	37.2	42.0	40.7	40.1	0.01	ns
C16:1	4.21[a]	4.07[a]	3.97[a]	2.88[b]	0.02	***	7.55	4.21	4.34	3.41	0.18	ns
C17:1	0.20[b]	0.28[a]	0.29[a]	0.29[a]	0.00	***	0.20[b]	0.29[a]	0.31[a]	0.35[a]	0.00	***
C18:1	45.4[a]	43.5[a]	42.9[a]	36.4[b]	0.14	***	46.9[a]	44.6[a]	44.35[a]	39.69[b]	0.13	***
C20:1	0.75	0.78	0.83	0.68	0.00	ns	0.94	0.96	0.87	0.93	0.00	ns
ΣMUFA	50.6[a]	48.7[a]	48.0[a]	40.3[b]	0.06	***	55.6[a]	50.1[ab]	49.88[ab]	44.37[b]	0.24	**
C18:2	6.76[a]	7.52[a]	8.29[a]	16.1[b]	0.16	***	6.11[b]	6.71[b]	7.76[b]	17.44[a]	0.22	***
C18:3	0.64[a]	0.80[ab]	0.77[ab]	1.11[b]	0.01	*	0.32[b]	0.47[ab]	0.44[ab]	0.67[a]	0.01	**
C20:2	0.19[a]	0.23[a]	0.22[a]	0.53[b]	0.01	***	0.14[b]	0.14[b]	0.17[b]	0.48[a]	0.01	***
C20:4	1.33[a]	1.62[a]	1.62[a]	3.44[b]	0.04	***	0.65[b]	0.63[b]	1.06[b]	3.11[a]	0.05	***
ΣPUFA	8.92[a]	10.2[a]	10.9[a]	21.2[b]	0.20	***	7.21[b]	7.95[b]	9.42[b]	21.69[a]	0.28	***
ΣMUFA/ΣSFA	1.25[a]	1.18[a]	1.17[a]	1.05[b]	0.00	***	1.76	1.15	1.22	1.11	0.00	ns
ΣPUFA/ΣSFA	0.22[b]	0.25[b]	0.26[b]	0.55[a]	0.01	***	0.22[b]	0.19[b]	0.23[b]	0.52[a]	0.02	***

Results expressed as percentage of total methyl esters analysed. [a,b,c]: Means with different superscripts, differed significantly in an ANOVA test. Pairs of underlined means on day 0 and 10 differed in a Tukey's test for dependent variables. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ns: non-significant.

*: $P < 0.05$.
**: $P < 0.01$.
***: $P < 0.001$.

3. Results

3.1. Physico-chemical analysis and fatty acid profile of raw m. longissimus dorsi

Fat and moisture contents of fresh *m. longissimus dorsi* are given in Table 1.

Moisture percentage was inversely related to fat content and followed the increasing order: 'Lampiño'< 'Retinto'<'Torbiscal'<industrial genotype pig ($P > 0.05$). Fat content showed values from 1.4% to 3.4%, being significantly higher ($P < 0.05$) in Iberian pig's *m. longissimus dorsi* (2.5%–3.3%) than in industrial genotype pigs' *m. longissimus dorsi* (1.4%).

Neutral (NL) and polar lipid (PL) contents (Table 1) depended on the breed studied. Neutral lipid content was significantly higher in LD from Iberian lines ('Lampiño': 0.84 g/g fat; 'Retinto': 0.82 g/g fat; 'Torbiscal': 0.79 g/g fat; $P < 0.05$) than in LD from the industrial genotype pigs (0.57 g/g fat).

Polar lipid content showed opposite behaviour to neutral lipids, being higher in industrial genotype pig's *longissimus dorsi* (0.43 g/g fat; $P < 0.05$) than in those from Iberian pigs ('Lampiño': 0.16 g/g fat; 'Retinto': 0.18 g/g fat; 'Torbiscal': 0.21 g/g fat).

The fatty acid profiles of intramuscular fat of LD from Iberian and industrial genotype pigs are shown in Table 2. Fatty acid profile of total lipid content depended on the group studied. *Longissimus dorsi* from Iberian pigs showed significantly higher proportions of saturated fatty acids (SFA) ($p < 0.05$) and monounsaturated fatty acids (MUFA) ($p < 0.001$), and lower proportions of polyunsaturated fatty acids (PUFA) ($p < 0.001$) than LD from the industrial genotype pig. The neutral lipid (NL) profile (Table 3) showed a great resemblance to total fat, due to the abundant NL in intramuscular fat of these muscles.

The fatty acid profile of the FFA fraction is shown in Table 4. Oleic, palmitic, stearic and linoleic acids constitute around 90% of the total fatty acids analysed.

In polar lipids (Table 5), samples from industrial genotype pigs showed higher percentages of PUFA and lower of MUFA than those from Iberian pigs, but differences were not statistically significant. These results are extensively discussed in a previous paper (Estévez et al., 2003a).

Table 3
Fatty acid profile (% methyl esters analysed) of neutral lipids of raw and cooked and refrigerated loins from three lines of free-range-reared Iberian pigs and industrial genotype pigs, after 10 days at 4 °C

	Raw meat (day 0)						Refrigerated cooked meat (day 10)					
	Iberian pig line						Iberian pig line					
	LAM	RET	TOR	IND	sem	*P*	LAM	RET	TOR	IND	sem	*P*
C12	0.14^{b}	0.12^{b}	0.13^{b}	0.17^{a}	0.00	***	0.08	0.05	0.07	0.08	0.00	ns
C14	1.71	1.58	1.61	1.53	0.01	ns	1.36^{ab}	1.37^{ab}	1.60^{a}	1.27^{b}	0.01	*
C16	26.03	26.4	26.3	24.9	0.04	ns	26.1^{a}	27.0^{a}	25.9^{a}	23.0^{b}	0.07	***
C17	0.19^{b}	0.28^{a}	0.29^{a}	0.35^{a}	0.00	***	0.15^{b}	0.32^{a}	0.29^{a}	0.38^{a}	0.03	***
C18	12.4	12.6	12.6	12.0	0.03	ns	13.4	13.2	13.0	13.2	0.00	ns
C20	0.25^{a}	0.23^{ab}	0.23^{ab}	0.20^{b}	0.00	**	0.28	0.24	0.29	0.29	0.00	ns
ΣSFA	40.74	41.16	41.09	39.19	0.07	ns	41.2^{a}	42.1^{a}	41.0^{ab}	38.6^{b}	0.03	*
C16:1	4.37^{a}	4.15^{a}	4.06^{a}	3.20^{b}	0.02	***	4.29^{a}	4.42^{a}	4.51^{a}	3.18^{b}	0.03	***
C17:1	0.20^{ab}	0.27^{a}	0.28^{a}	0.30^{a}	0.00	***	0.21^{b}	0.30^{b}	0.30^{b}	0.40^{a}	0.00	***
C18:1	46.4^{a}	44.5^{a}	44.7^{a}	39.0^{b}	0.13	***	48.2^{a}	46.9^{a}	47.1^{a}	40.4^{b}	0.16	***
C20:1	0.82	0.82	0.89	0.89	0.00	ns	1.02	0.99	0.93	1.16	0.01	ns
ΣMUFA	51.7^{a}	49.7^{a}	50.0^{a}	43.4^{b}	0.15	***	53.7^{a}	52.6^{a}	52.8^{a}	45.1^{b}	0.19	***
C18:2	6.11^{b}	7.11^{b}	7.23^{b}	14.23^{a}	0.14	***	5.77^{b}	5.85^{b}	6.23^{b}	14.5^{a}	0.17	***
C18:3	0.36^{b}	0.45^{ab}	0.52^{ab}	0.66^{a}	0.01	*	0.35	0.47	0.44	0.44	0.01	ns
C20:2	0.15^{b}	0.20^{b}	0.16^{b}	0.39^{a}	0.00	***	0.09^{b}	0.13^{b}	0.10^{b}	0.34^{a}	0.00	***
C20:4	0.89^{b}	1.39^{b}	1.03^{b}	2.27^{a}	0.02	***	0.32^{b}	0.29^{b}	0.34^{b}	1.88^{a}	0.03	***
ΣPUFA	7.51^{b}	9.15^{b}	8.95^{b}	17.6^{a}	0.17	***	6.53^{b}	6.74^{b}	7.12^{b}	17.1^{a}	0.21	***
ΣMUFA/ΣSFA	1.27^{a}	1.21^{ab}	1.22^{ab}	1.11^{b}	0.00	*	1.31	1.25	1.29	1.17	0.00	ns
ΣPUFA/ΣSFA	0.19^{b}	0.22^{b}	0.22^{b}	0.45^{a}	0.00	***	0.15^{b}	0.16^{b}	0.17^{b}	0.44^{a}	0.00	***

Results expressed as percentage of total methyl esters analysed; ns: non-significant; ^{a,b,c}: Means with different superscripts, differed significantly in an ANOVA test; Pairs of underlined means on day 0 and 10 differed in a Tukey's test for dependent variables; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

*: $P < 0.05$.

**: $P < 0.01$.

***: $P < 0.001$.

Table 4
Fatty acid profile (% methyl esters analysed) of FFA lipid fraction of raw and cooked and refrigerated loins from three lines of free-range-reared Iberian pigs and industrial genotype pigs after 10 days at 4 °C

	Raw meat (day 0)						Refrigerated cooked meat (day 10)					
	Iberian pig lines						Iberian pig lines					
	LAM	RET	TOR	IND	sem	*P*	LAM	RET	TOR	IND	sem	*P*
C12	0.31	0.25	0.34	0.28	0.00	ns	0.10	0.09	0.11	0.12	0.02	ns
C14	1.58^{a}	2.14^{a}	1.44^{a}	1.39^{a}	0.02	*	2.10	1.90	2.13	1.74	0.12	ns
C16	22.9^{ab}	22.0^{b}	20.9^{b}	25.98^{a}	0.12	*	25.6	26.4	20.7	23.6	0.02	ns
C17	0.49	0.70	0.41	0.48	0.01	ns	0.98	0.91	1.07	0.91	0.11	ns
C18	12.6^{ab}	13.3^{a}	11.6^{ab}	10.81^{b}	0.07	*	16.2^{b}	18.5^{a}	20.2^{a}	14.0^{b}	0.01	***
C20	0.56	0.40	0.30	0.30	0.01	ns	0.54	0.57	0.63	0.47	0.14	ns
ΣSFA	38.5^{ab}	38.8^{ab}	34.9^{b}	39.2^{a}	0.12	*	45.4^{ab}	48.3^{a}	44.7^{ab}	40.8^{b}	0.02	*
C16:1	2.48	2.53	2.30	2.14	0.01	ns	3.42	3.43	2.78	2.79	0.19	ns
C17:1	0.54^{b}	1.31^{a}	0.44^{b}	0.48^{b}	0.02	***	0.46	0.62	0.95	0.53	0.01	ns
C18:1	39.4	40.2	36.3	37.1	0.19	ns	37.6^{a}	36.2^{ab}	30.9^{b}	32.1^{ab}	0.18	*
C20:1	3.41^{a}	1.94^{ab}	1.26^{b}	2.34^{ab}	0.06	*	1.13	1.21	1.10	0.94	0.01	ns
ΣMUFA	45.9	46.0	40.3	42.1	0.20	ns	42.57	41.5	40.0	36.3	0.25	ns
C18:2	11.1^{ab}	11.7^{ab}	9.40^{b}	14.7^{a}	0.14	*	9.31^{b}	7.76^{b}	8.91^{b}	17.6^{a}	0.19	***
C18:3	1.07^{ab}	0.65^{b}	1.99^{a}	0.84^{b}	0.03	*	0.67	0.82	0.80	0.94	0.01	ns
C20:2	0.50	0.43	0.35	0.47	0.01	ns	0.36	0.53	1.34	0.68	0.01	ns
C20:4	3.09	2.45	3.18	2.87	0.05	ns	1.65	1.12	4.22	3.76	0.01	ns
ΣPUFA	15.8	15.3	13.9	18.9	0.17	ns	12.0^{b}	10.2^{b}	13.1^{b}	22.9^{a}	0.04	***
ΣMUFA/ΣSFA	1.19	1.19	1.15	1.08	0.00	ns	0.94	0.86	1.00	0.89	0.01	ns
ΣPUFA/ΣSFA	0.41	0.40	0.40	0.49	0.00	ns	0.26^{b}	0.21^{b}	0.32^{b}	0.56^{a}	0.02	***

Results expressed as percentage of total methyl esters analysed; ns: non-significant; ^{a,b,c}: Means with different superscripts, differed significantly in an ANOVA test; Pairs of underlined means on day 0 and 10 differed in a Tukey's test for dependant variables; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
*: $P < 0.05$.
**: $P < 0.01$.
***: $P < 0.001$.

3.2. Physico-chemical analysis of cooked loins after refrigerated storage

Means of cook losses, IMF, polar lipid and neutral lipid contents of cooked and refrigerated loins are shown in Table 1.

Cook losses values were between 36.9% and 38.9%. There were no differences between groups ($P > 0.05$).

IMF content after cooking was statistically different in the four groups ($P < 0.001$). Cooked loins from 'Lampiño' Iberian line showed the highest fat content (4.5%) and the industrial genotype pigs one the lowest (1.6%). These results agree with those previously obtained in fresh meat. Cooking and refrigerated storage had a significant effect on fat content. Samples from 'Lampiño' and 'Retinto' Iberian pig lines presented significant increases ($P < 0.05$) (from 3.34% to 4.49% and from 3.17% to 3.89% respectively), while those from 'Torbiscal' and the industrial genotype pigs did not changed significantly (from 2.51% to 2.52% and from 1.41% to 1.57% respectively; $P > 0.05$).

Cooked and refrigerated loin chops from Iberian pigs showed higher amounts of neutral lipids (0.73–0.84 g/g fat, $P < 0.05$) than the industrial genotype pig ones (0.46 g/g fat). Intramuscular fat from samples from industrial genotype pigs contained higher amounts of polar lipids than the Iberian ones (0.64 vs. 0.17–0.27 g/g fat; Table 1), being in agreement with results obtained from the analysis of the raw meat.

3.3. Fatty acid profile changes in loin chops after cooking and refrigerated storage

Changes in fatty acid profile of total lipids were not remarkable (Table 2). In general, there was an increase in the proportion of MUFA and a reduction in percentages of PUFA, but these changes were not statistically significant in any group.

In neutral lipids (Table 3), there was an increase in the SFA percentages with a significant increment of stearic acid percentages ($P < 0.05$) in LD from 'Lampiño' and industrial genotype pigs. As described for fatty acid composition of intramuscular fat, there was an increase in the proportion of MUFA and a decrease in eicosadienoic acid (C20:2) and arachidonic acid (C20:4) percentages in all groups, but these changes were only statistically significant in the three Iberian pig lines.

The content of FFA significantly increased in cooked samples stored at 4 °C for 10 days. The initial amounts were significantly elevated from 0.15, 0.12, 0.13 and 0.17

Table 5
Fatty acid profile (% methyl esters analysed) of PL of raw and cooked and refrigerated loins from three lines of free-range-reared Iberian pigs and industrial genotype pigs, after 10 days at 4 °C

	Raw meat (day 0)						Refrigerated cooked meat (day 10)					
	Iberian pig line						Iberian pig line					
	LAM	RET	TOR	IND	sem	*P*	LAM	RET	TOR	IND	sem	*P*
C12	0.13	0.14	0.13	0.06	0.00	ns	0.10	0.11	0.09	0.08	0.00	ns
C14	0.43	0.58	0.38	0.32	0.01	ns	0.45	0.59	0.43	0.59	0.01	ns
C16	19.8	22.4	23.9	21.2	0.09	ns	20.7	22.6	21.4	22.6	0.06	ns
C17	0.37	0.63	0.53	0.48	0.01	ns	0.35^{c}	0.51^{ab}	0.54^{a}	0.42^{bc}	0.01	***
C18	8.84	9.05	8.99	9.65	0.05	ns	11.5^{bc}	12.6^{ab}	11.1^{c}	13.40^{a}	0.05	**
C20	1.03	0.17	0.15	0.10	0.01	ns	0.12	0.11	0.09	0.07	0.00	ns
ΣSFA	30.6	32.9	34.0	31.8	0.13	ns	34.9	35.0	34.7	35.1	0.05	ns
C16:1	1.33	1.76	1.51	1.45	0.02	ns	1.86	2.06	2.04	2.31	0.01	ns
C17:1	0.34	0.53	0.30	0.34	0.01	ns	0.46	0.51	0.46	0.58	0.00	ns
C18:1	24.3	25.2	23.0	20.5	0.21	ns	26.9	29.2	25.8	27.7	0.15	ns
C20:1	2.13	0.64	0.83	0.56	0.02	ns	0.62	0.66	0.56	0.46	0.01	ns
ΣMUFA	28.1	28.1	25.6	22.8	0.23	ns	29.8	32.5	28.9	31.0	0.17	ns
C18:2	23.2^{b}	26.8^{ab}	33.4^{a}	31.3^{a}	0.23	**	25.2	24.4	27.7	24.2	0.13	ns
C18:3	1.08	0.87	1.04	0.78	0.01	ns	0.87	0.85	0.83	1.18	0.01	ns
C20:2	2.48	1.13	1.45	1.43	0.01	ns	1.16^{a}	0.85^{b}	0.91^{ab}	1.02^{ab}	0.01	*
C20:4	14.5	10.0	12.4	11.84	0.09	ns	7.99^{a}	6.38^{b}	7.05^{ab}	7.72^{a}	0.04	*
Σ PUFA	41.3	38.9	48.2	45.35	0.29	ns	35.2	32.5	36.5	34.1	0.15	ns
ΣMUFA/ΣSFA	0.92^{a}	0.85^{a}	0.75^{a}	0.72^{b}	0.01	*	0.85	0.92	0.83	0.89	0.00	ns
ΣPUFA/ΣSFA	1.39	1.20	1.42	1.42	0.07	ns	1.01	0.92	1.05	0.96	0.00	ns

Results expressed as percentage of total methyl esters analysed; ns: non-significant; [a,b,c]: Means with different superscripts, differed significantly in an ANOVA test; Pairs of underlined means in day 0 and 10 differed in a Tukey's test for dependent variables; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
*: $P < 0.05$.
**: $P < 0.01$.
***: $P < 0.001$.

mg/100 g muscle to 0.45, 0.56, 0.29 and 0.31 mg/100 g muscle in samples from 'Lampiño', 'Retinto', 'Torbiscal' and industrial genotype pigs, respectively (Table 1).

As well as FFA contents, the relative proportions of fatty acids of this fraction showed noticeable changes (Table 4). Stearic and total SFA percentages significantly increased ($P > 0.05$) in all groups after 10 days of refrigerated storage. In contrast, MUFA percentages decreased at the end of the storage in all groups, being only statistically significant in 'Retinto' and 'Torbiscal' lines.The fatty acid profile from the polar lipid fraction was most affected by the cooking and refrigerated storage (Table 5).

Stearic acid percentage increased significantly ($P < 0.05$), causing an increase in the proportion of SFA in all groups, this only being statistically significant in samples from the 'Lampiño' Iberian line and in the industrial genotype pig. The proportions of oleic acid and MUFA also increased in samples from the four groups, but significantly only in those from the industrial genotype ($P < 0.05$). The changes registered for linoleic fatty acid depended on the group studied. Samples from 'Lampiño' showed an increment around 8%, while samples from 'Retinto', 'Torbiscal' and the industrial genotype pig suffered a reduction in linoleic acid percentages (9%, 17% and 23%, respectively), only being statistically significant for samples from the industrial genotype ($P < 0.05$). Arachidonic fatty acid percentages decreased after cooking and refrigeration in the four groups ($P < 005$). Consistently, PUFA proportions were reduced with a different intensity depending on the Iberian pig line and breed (14.6% in 'Lampiño', $P > 0.05$, 16.3% in 'Retinto', $P > 0.05$, 24.3% in 'Torbiscal', $P < 0.05$, and 24.8% in the industrial genotype pig, $P < 0.05$).

3.4. Fatty acid profile in lipid fractions after refrigerated storage

Fatty acid profiles of IMF from the samples analysed are given in Table 2. The differences noted in fresh meat are smoothed after cooking and refrigeration but, even then, cooked and refrigerated loins from Iberian pigs presented a higher proportion of MUFA and lower of PUFA ($P < 0.05$) than those from industrial genotype pigs.

Fatty acid profile of the neutral lipid fraction was similar to that reported for total lipids, and the differences between groups were the same (Table 3).

Table 5 shows the fatty acid composition of the polar lipids. SFA (34.6–35.1%), MUFA (28.8–32.5%) and PUFA (32.5–36.2%) presented; among them there are similar proportions in cooked and refrigerated meat.

These results are clearly different from those described in fresh meat or refrigerated meat (Estévez et al., 2003a, 2003b). In those studies, PUFA were the most abundant fatty acids in polar lipids.

3.5. Oxidative changes in loins after cooking and refrigerated storage

TBARS numbers of raw (day 0) and cooked and refrigerated meat (day 10) are given in Fig. 1(a) . Cooked samples suffered great oxidative changes after storage. Oxidation values increased 24- 26- 24- and 48-times the initial values in samples from 'Lampiño' (from 0.3 to 7.28 mg MDA/ kg muscle, $P < 0.05$), 'Retinto' (from 0.25 to 6.45 mg MDA/kg muscle, $P < 0.05$), 'Torbiscal' (from 0.30 to 7.09 mg MDA/kg muscle, $P < 0.05$) and industrial genotype pigs (from 0.15 to 7.13 mg MDA/kg muscle, $P < 0.05$), respectively. The increment in TBA numbers in samples from industrial genotype pigs was twofold higher than that obtained in samples from the Iberian pig lines. In spite of that, TBA values (mg MDA/kg muscle) are not statistically different between breeds after cooking and 10 days of refrigerated storage ($P > 0.05$) (Fig. 1(a)).

Differences between breeds appeared clearly significant ($P < 0.001$) when fat content is considered as an influential factor and TBARS values are expressed as mg MDA/kg muscle on a defatted dry basis (d.d.b.) (Fig. 1(b)). Like this, cooked and refrigerated loin chops from the industrial genotype pig breed showed a higher content of TBARS (28.7 mg MDA/ kg muscle d.d.b.) than those found in samples from the Iberian pig lines: 'Torbiscal', 'Lampiño' and 'Retinto' (14.7, 9.84 and 9.00 mg MDA/kg muscle d.d.b. respectively).

4. Discussion

4.1. Physico-chemical analysis of cooked loins after refrigerated storage

Meat composition after cooking is of great importance, due to its influence on the eating quality of the meat. Intramuscular fat is known to affect meat palatability attributes, by improving the tenderness sensation,

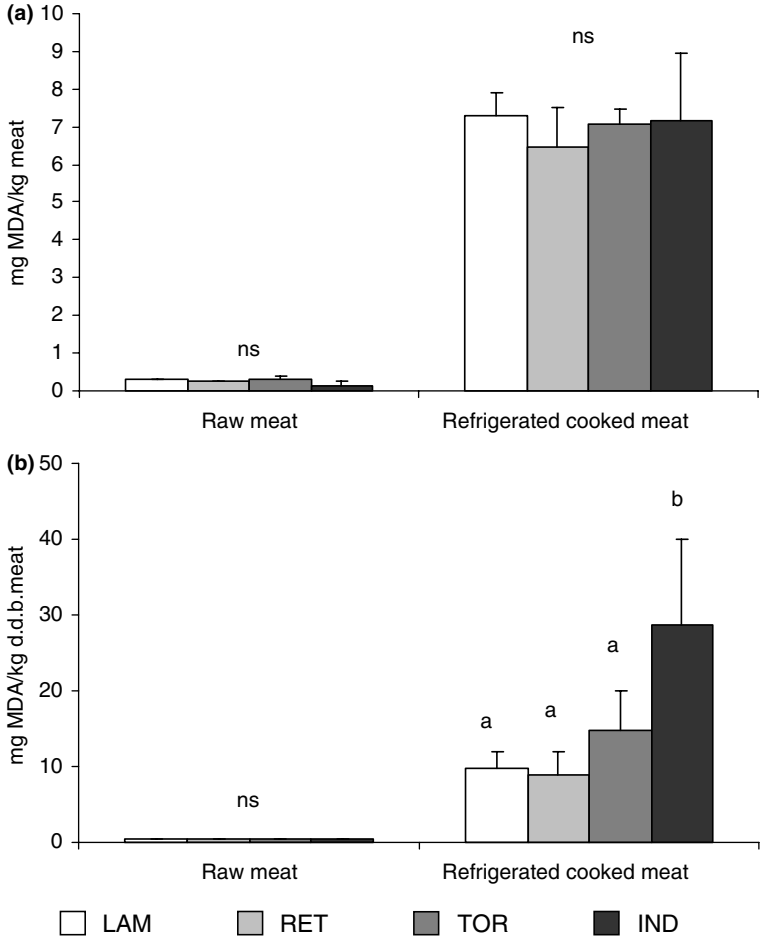

Fig. 1. Oxidative changes in cooked loin chops from three lines of free-range-reared Iberian pigs and industrial genotype pig under refrigerated storage. TBA numbers expressed as mg MDA/kg meat (a) and mg MDA/kg meat defatted dry basis (b).

reducing shear force during chewing and making easier muscle fibre separation (Wood, 1993). Intramuscular fat also regulates water loss during cooking, but this effect was not observed in the present work. Thus, intramuscular fat promotes salivation during meat chewing by increasing juiciness (Lawrie, 1998); that is reduced after cooking due to water loss. Therefore, cooked loins from Iberian pigs, should present higher palatability attributes than industrial genotype loins as a result of a higher content of intramuscular fat.

On the other hand, a slightly higher content of polar lipids in commercial loins, together with a higher susceptibility to oxidation, due to a higher proportion of PUFA in neutral and polar lipid fractions, could influence the generation of off-flavours and undesirable odours in these samples. During refrigerated storage of cooked meat, abnormal flavours develop, named as WOF (warmed-over-flavour), because of the oxidative processes that mainly involve PUFA from PL (St. Angelo et al., 1987).

4.2. Fatty acid profile changes in loin chops after cooking and refrigerated storage

Cooking and refrigeration had clear effects on fatty acid profiles of LD. In some previous studies of changes in lipids from meat during its refrigerated storage (Alasnier, David-Brian, & Gandemer, 2000a, 2000b, Estévez, Morcuende, Ventanas, & Cava, 2002), the changes in the amounts of free fatty acids and in the fatty acid profiles were mainly due to lipolytic processes mediated by active muscle enzymes (Alasnier & Gandemer, 1998).

Results from the present work showed that oxidative deterioration mainly caused the modifications and differences in fatty acid profiles between groups.

The ratio SFA/PUFA increased after storage of cooked meat in polar lipids through a reduction of PUFA percentage, the latter being highly susceptible to oxidative degradation (Fig. 2(a)). These results disagree with changes described in previous papers concerning lipid composition of raw muscles from pigs during refrigerated storage (Estévez et al., 2002; Morcuende, Estévez, Ruiz, & Cava, 2002). In those, important decreases of palmitic acid and SFA proportions in polar lipids are described (Estévez et al., 2002; Fig. 2(b)).

Lipolysis, like lipid oxidation, is one of the most important degradation phenomena during refrigeration of meat (Alasnier & Gandemer, 1998; Morrissey et al., 1998), but oxidation seems to be more relevant during refrigerated storage of cooked meat (Rhee, Ziprin, & Calhoun, 2001). The cooking process leads to thermal

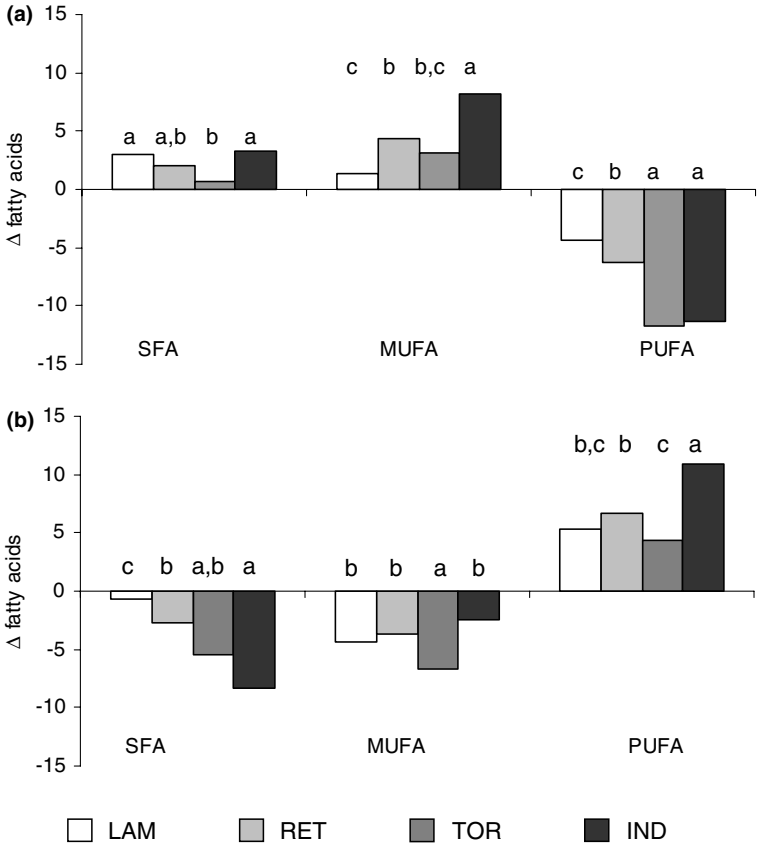

Fig. 2. Increments of SFA, MUFA and PUFA in polar lipids from raw m. *Longissimus dorsi* after cooking (80 °C/10′) and refrigerated storage (4 °C/10 days) (present work) (a) and after refrigerated storage (4 °C/10 days) without previous cooking (Estévez et al., 2002) (b).

denaturation of muscle enzymes, such as lipolytic enzymes, and this inactivation is believed to contribute to the reduction of the release of free fatty acids in cooked meats. This fact is supported by the results of the present work. After 10 days of refrigerated storage the measurement of lipolytic changes showed an increase in FFA contents, although this was less intense than in refrigerated meat (see Estévez et al., 2002). Also, the effect of high temperatures on lipolytic enzymes supports the hypothesis proposed in previous work (Estévez et al., 2002), in which we attributed the decrease of SFA percentages in polar lipids to the activity of phospholipases on these fatty acids. The present work complements those results by showing that a previous cooking process (Fig. 2(a) vs. Fig. 2(b)) reduces lipolytic activity during refrigeration of meat. Therefore, differences in the evolution of fatty acid profiles between groups are mainly due to different developments of oxidative processes that lead to the degradation of PUFA. The decrease of linoleic acid in PL was significantly greater in the industrial genotype pig loins ($\Delta + 2.0\%$, $\Delta - 2.4\%$, $\Delta - 5.7\%$ and $\Delta - 7.1\%$, for 'Lampiño', 'Retinto', 'Torbiscal' and industrial genotype pigs, respectively). Moreover, the reduction of the proportion of PUFA in PL from the industrial genotype pig loins (24.8%) was more intense ($P < 0.05$) than in the Iberian pig ones ('Lampiño': 14.6%; 'Retinto': 16.3%). These data predict the development of warmed-over-flavour in cooked meat during refrigerated storage since the intensities of decrease of PUFA in polar lipids are positively and significantly correlated with WOF development in cooked meat (Byrne, Bredie, Bak, Bertelsen, Martens, & Martens M, 2001). Based on the data obtained, this onset of rancidity would be more intense in cooked and refrigerated meat from industrial pigs, than that from Iberian pigs. These results agree with those obtained in a previous work in which the development of WOF, as analysed by the generation of lipid-derived volatiles, was studied (Estévez, Morcuende, Ventanas, & Cava, 2003).

4.3. Oxidative changes in loins after cooking and refrigerated storage

Cooking and refrigeration of pig meat greatly increased the oxidation index in the four groups studied. These results agree with those found by other authors in cooked pork (Kingston et al., 1998; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001). Kanner (1994) showed that high temperatures reduce activation energy for the development of lipid oxidation and favour the breakdown of preformed hydroperoxides in meat. Substances reactive to thiobarbituric acid proceed from lipid oxidation and, therefore, loins from Iberian pigs offer many more substrates for lipid oxidation than industrial genotype pig loins. In this sense, Iberian pig loins with higher intramuscular fat (2.9 g/100 g vs. 1.4 g/100 g) and heme iron contents (6.74 mg/kg vs. 402 mg/kg) than industrial pig loins (Estévez et al., 2003a) may be considered more susceptible to oxidative processes during cooking and subsequent storage. Nevertheless, TBARS numbers (mg MDA/kg meat) found in this work suggest that cooked and refrigerated Iberian loins are less prone to oxidative deterioration. Furthermore, the lower TBARS numbers (expressed as mg MDA/kg meat, d.d.b.) are in agreement with the lower PUFA degradation in cooked and refrigerated meat from Iberian pigs. The high percentage of MUFA in lipids from Iberian loins, in contrast to the high proportion of PUFA in samples from industrial genotype pigs, could be responsible for the lower lipid oxidation products found in the former. Moreover, several authors have described the positive effect of pasture grazing of animals on tocopherol content in meat – not determined in the present work – and the protective effect of these micronutrients in preventing lipid oxidation (Cava et al., 2000; Kingston et al., 1998; Monahan, Gray, Morrisey, Asghar, Hanrahan, & Lynch, 1990; Nilzén et al., 2001).

5. Conclusions

Cooking leads to a loss of fluid and lipid and hence an increase in the concentration of residual components. Despite lipolytic processes, lipid oxidation seems to be the main cause of deterioration of cooked meat during refrigerated storage, causing a decrease in PUFA percentages and an increase in MUFA and SFA percentages. The higher proportion of PUFA in LD of industrial genotype pigs increases the TBARS numbers in contrast to Iberian pig loins, even when the latter presented a higher content of prooxidant factors, such as total lipids and heme iron. This contradictory behaviour suggests the presence of additional factors, related to the free-range rearing system, that could act as protective agents against lipid oxidation. Traditional rearing systems, in comparison to industrial rearing systems, have potential advantages for the oxidative stability of muscle lipids in cooked and refrigerated meat, increasing the levels of monounsaturated fatty acids and presumably the levels of natural antioxidants from grass.

Acknowledgements

The authors would like to thank the Ministry of Food, Agriculture and Fishery and the Junta de Extremadura for granting this work from the project: "*Establecimiento de parámetros predictores de la calidad de carne fresca de cerdo Ibérico y alentejano destinada a la elaboración de productos cárnicos curados y al consumo en fresco* " (IPR99D001). The authors are grateful to

Mrs. Elena Diéguez and Mr. Pedro Cañuelo (AECER-IBER), for providing us with the animals. The authors are also grateful to Miss Inmaculada Linares Gómez for her excellent technical assistance. Mario Estévez would like to thank the Spanish Ministry of Education and Culture and the Junta of Extremadura for the grants received during the development of this work.

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