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LIPID COMPOSITION OF PDO-BEEF FROM DIFFERENT PORTUGUESE BREEDS

Costa, P[1]; Roseiro, L.C.[1]; Alves, V[2]; Santos, C[1]

[1]*Instituto Nacional de Engenharia, Tecnologia e Inovação, DTIA, Estrada do Paço do Lumiar, 1649-038 Lisboa*
[2]*Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados-Apartado 202, 5001 Vila Real Paulo.Costa@mail2.ineti.pt*

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Introduction

Maronesa and Barrosã are autochthonous bovine breeds reared in the North of Portugal according to a traditional semi-extensive grazing system using local agricultural resources. Maronesa and Barrosã calves are reared close to their mothers, suckling milk and eating either grass until slaughter, between 6-9 months old.

Mertolenga, another portuguese cattle breed, is produced in the South of the territory, in a semi-extensive system where animals are mainly fed on pasture and forage. Depending on corporal condition and food availability, Mertolenga young bulls are usually finished on concentrate and slaughtered from 15 to 30 months of age.

When obtained according to the required specifications, the meat of these breeds is commercialized under Protected Denomination of Origin (PDO) having an important impact on local economy and sustainability of the environment and providing a raise of the producers income. PDO certification of products is under European Union regulation (Council Regulation nº 2081/92 of 14/07, EEC) and imposes the utilization of traditional rearing methods. PDO meat is supposed to present unique characteristics, especially associated with the properties of its lipid fraction. Cholesterol level and lipid content and composition of the diet have become issues of great concern to consumers due to their possible negative effect on human health (Chizzolini *et al*. 1999). Atherosclerotic lesions appear to be related to an elevated total cholesterol, a lower HDL-cholesterol/LDL-cholesterol ratio in plasma and excess fat consumption. Plasma lipid levels are not only influenced by the amount of fat consumed but by its nature as well (Steinberg & Witztum, 1990). The P/S (polyunsaturated fatty acids/saturated fatty acids) and *n-6*/*n-3* ratios are also thought to be important nutritional parameters of foods. Health organizations recommend reductions in total and saturated fat intake and at the same time the increasing in consumption of *n-3* PUFA (Polyunsaturated fatty acids) (Department of Health, 1994). High intake of *n-6* PUFA with low negligible intake of *n-3* PUFA, may increase cardiovascular disease risk because of the proinflammatory and prothrombotic effects of *n-6* PUFA (Cunnane, 2003). Recent research in this domain has focused also on the nutritional relevance of conjugated linoleic acid (CLA) in the human diet. Some CLA isomers are considered beneficial to human health, due to anticarcinogenic, antiatherogenic and immune-modulating properties (Mulvihill, 2001).

A number of different factors including anatomical location, production system, sex, breed, age and diet have been reported to influence fat content and intramuscular fatty acid composition in meat (Calkins *et al.*, 1981; Zembayashi *et al.*, 1995; Gandemer, 1998; Deland *et al.*, 2001; Varela *et al.*, 2004). Therefore, it is expectable that differences in intramuscular fat content and composition between Portuguese breeds could exist.

Objectives

The aim of this work was to evaluate and compare the intramuscular lipid composition of PDO beef from Maronesa, Barrosã and Mertolenga breeds.

Methodology

One day after slaughter, about 200 grams of *Longissimus dorsi* muscle (L4-L6) from Maronesa, Barrosã and Mertolenga were excised and stored at –20ºC until analysis.

Intramuscular total lipids (ITL) were extracted from duplicate 20 g samples of muscle, trimmed of visible adipose and connective tissues as described by Folch *et al.* (1957). Separation into neutral lipids (NL) and phospholipids (PL) was performed according to the procedure of Juaneda & Rocquelin (1985). Lipid extracts were esterified with KOH (2N) in methanol (ISO 5509, 2000) and resulting fatty acid (FA) methyl esters were analysed by gas-liquid chromatography, using a HRGC 5160, Mega series from Carlo Erba instruments, equipped with a flame ionisation detector and a 60 m long DB 23 capillary column. The oven temperature was raised from 70-195ºC at 5ºC/min for LN analysis and from 70-195 ºC (10 min) to 220 ºC (60 min) at a rate of 5 ºC/min for LP analysis. Injector and detector temperatures were 220 ºC and 280 ºC, respectively.

Identification of FA was based on comparison with standard FA mixtures (Supelco and Nuchek GLC reference standard FAME mixture). FA were expressed as weight percentage.

Cholesterol (mg/g) was quantified according to Roseiro *et al.* (2002), using a HPLC with a Spectra-Physics Model Spectra 100 equipped with variable wavelength UV detector set at 206 nm and a Spherisorb S5W silica cartridge (Waters PSS 845549). The mobile phase was hexane/isopropanol (97:3) at a flow rate of 1.0 mL/min.

Data were analysed using one-way analysis of variance (ANOVA). Analysis of means was performed by the LSD test for 95% of probability (Statistica 6.0-StatSoft Inc., 2001).

Results & Discussion

Intramuscular lipids and cholesterol levels were affected by breed (Table 1). Barrosã presented higher ITL ($P<0.01$) and NL ($P<0.001$) than Maronesa and Mertolenga breeds. This is in agreement with the results obtained by Wagenhoffer & Szabo (2004), whom also reported differences on ITL content among several breeds. Barrosã and Maronesa showed higher PL (polar lipids) ($P<0.01$) and cholesterol ($P<0.001$) contents than Mertolenga. The values found for cholesterol in Barrosã and Mertolenga, respectively 0.52 and 0.44 (mg/g) are coincident to those reported by Quaresma *et al.* (2004) for *Longissimus lumborum*.

Regarding NL, with the exception of SFA (saturated fatty acids), *n-3* PUFA and hypocholesterolaemic/hypercholesterolaemic ratio (h/H), all the other fatty acid proportions in Ld muscle were affected by breed (Table 2). Barrosã and Maronesa presented higher MUFA (monounsaturated fatty acids) proportion than Mertolenga ($P<0.01$). On the other hand, Mertolenga showed higher PUFA ($P<0.001$), P/S ($P<0.01$), *n-6* PUFA ($P<0.01$) and *n-6/n-3* ($P<0.001$) contents in NL fraction than Barrosã and Maronesa. The higher CLA content was found in Barrosã-PDO veal ($P<0.001$). CLA results from ruminal production and by endogenous synthesis of C18:1 *trans-11* by Δ^9-desaturase enzyme in adipose tissue and is preferentially deposited in NL than in PL. The increase of pasture in diet resulted in higher level of CLA in intramuscular fat (French *et al.*, 2000).

Webb *et al.*, (1998) reported for *Longissimus thoracis* from Belgian Blue breed, lower SFA (45.46%) and PUFA (3.07%) in triacylglycerol fraction than the mean values obtained in Portuguese breeds. These differences could be attributed to breed and diet effects. In contrast, the MUFA content observed by those authors (46.46%) was similar to that found for Barrosã and Maronesa but higher than that detected in Mertolenga breed.

Zembayashi *et al.* (1995) also obtained significant differences on MUFA content in NL among steers from different breeds. According to those authors, some breeds have a genetic predisposition for synthesis and deposition of MUFA in that lipid fraction.

No differences on SFA were observed in PL among breeds, following the same trend observed for NL, which corroborates the results obtained by Zembayashi *et al.* (1995). The SFA content, P/S and h/H indices were also not different among breeds.

Unlike most individual SFA, MUFA have neutral effects on human cholesterol levels (Scientific Review Committee, 1990). Barrosã showed the highest MUFA content among the 3 breeds ($P<0.001$). Deland *et al.* (2001) also reported differences among breeds on MUFA content in PL of Ld muscle. The breeds studied by those authors presented higher MUFA and SFA proportions and lower PUFA content than those used on this work. Laborde *et al.* (2001) studying Simmental and Red Angus finishing steers referred that PL fatty acid profile of Ld muscle included approximately 34% SFA, 26% MUFA, 31% *n-6* PUFA and 8% *n-3* PUFA. Except for MUFA (23.30%) and *n-3* PUFA (9.50%) the proportions referred by Laborde *et al.* (2001) are similar to those found in Maronesa-PDO veal. In addiction, those authors reported significant differences among breeds for *n-3* PUFA and *n-6/n-3* ratio but not for SFA, MUFA, PUFA, *n-6* PUFA and P/S ratio in PL. In agreement with these results, differences were found between breeds on *n-6* PUFA proportion and *n-6/n-3* ratio ($P<0.001$) in PL, with Mertolenga young bulls presenting the highest values. In contrast, Mertolenga showed lower *n-3* PUFA than Barrosã and Maronesa ($P<0.001$) and higher PUFA content than Barrosã ($P<0.01$). These differences could be attributed to the diet, age at slaughter, genetic potential and type of finishing among the studied breeds.

According to nutritional recommendations *n-6/n-3* and P/S ratios in diet should not exceed 4.0 and 0.45, respectively (Department of Health, 1994). The *n-6/n-3* ratio hardly exceeded the recommended value in Mertolenga-PDO meat (10.64) but was accomplished in Barrosã and Maronesa-PDO veal (respectively, 3.88 and 3.96). P/S ratio was lower than 0.45 in Barrosã (approximately 0.33) but not in Maronesa and Mertolenga-PDO meat (approximately 0.60 for both). Low P/S values are considered unfavourable since they could induce hypercholesteroleaemia. The h/H ratio is at the

present considered a better approach to the nutritional evaluation of fat because is based on individual effects of FA on cholesterol metabolism (Williams, 2000; Santos-Silva *et al.*, 2002). The values obtained in Portuguese breeds ranged from 1.8 to 2.2 and were similar to those referred by Santos-Silva (2002) for light lambs.

Conclusions

Barrosa-PDO veal presented higher ITL and NL than Maronesa and Mertolenga-PDO meat. In respect to PL and cholesterol contents, Barrosã and Maronesa-PDO veal showed the higher values.

In general, the NL and PL FA compositions of Mertolenga-PDO meat were significant different from Barrosã and Maronesa-PDO meat. Mertolenga-PDO meat was the only with an unfavourable *n-6/n-3* ratio whereas Barrosa-PDO veal showed a P/S ratio below the recommended value which was accomplish in Mertolenga and Maronesa-PDO meat. The h/H ratio was similar among breeds. The results suggest that from a nutritional point of view, Mertolenga finished stage based on concentrate could induce unfavourable modifications on FA profile.

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Tables and Figures

Table 1. Age (months±STDEV) and weight (Kg±STDEV) at slaughter and means of intramuscular total lipids (ITL, g/100g), neutral lipids (NL, g/100g) polar lipids (PL, g/100g) and cholesterol (mg/g) contents of Barrosã, Maronesa and Mertolenga PDO-meat.

	Breed			Rsd	P
	Barrosã	Maronesa	Mertolenga		
Carcass characteristics					
n	92	10	44	-	-
Age	7.4±0.9	9.4±3.2	22.0±4.2	-	-
Weight	99.4±16.1	102.0±30.8	249.1±32.4	-	-
Intramuscular lipids and cholesterol					
ITL	2.98[a]	1.40[b]	1.58[b]	0.91	***
NL	2.44[a]	0.71[b]	0.99[b]	0.92	***
PL	0.66[a]	0.66[a]	0.60[b]	0.09	**
Cholesterol	0.52[a]	0.49[a]	0.44[b]	0.08	***

* = P<0.05; ** = P<0.01; *** = P<0.001

Rsd = residual standard deviation of the analysis of variance

Table 2. Fatty acid composition (% w/w) of Barrosã, Maronesa and Mertolenga PDO-meat

	Breed			Rsd	P
	Barrosã	Maronesa	Mertolenga		
n	40	10	20		
Fatty acid composition of NL					
SFA	47.46	48.35	49.02	3.18	ns
MUFA	46.69^{a}	46.84^{a}	43.21^{b}	3.33	**
PUFA	4.77^{b}	3.61^{b}	6.80^{a}	2.13	***
CLA	0.84^{a}	0.51^{b}	0.36^{b}	0.16	***
P/S	0.10^{b}	0.08^{b}	0.14^{a}	0.05	**
n-3 PUFA	0.79	0.69	0.62	0.40	ns
n-6 PUFA	3.13^{b}	2.96^{b}	5.92^{a}	1.81	***
n-6/n-3	4.16^{b}	4.35^{b}	13.16^{a}	3.79	***
h/H	1.50	1.41	1.54	0.22	ns
Fatty acid composition of PL					
SFA	32.69	34.94	32.45	2.97	ns
MUFA	27.23^{a}	23.30^{b}	22.92^{b}	3.67	***
PUFA	38.50^{b}	41.34^{ab}	43.55^{a}	5.73	**
CLA	0.34^{a}	0.31^{ab}	0.26^{b}	0.10	*
P/S	1.20	1.19	1.36	0.26	ns
n-3 PUFA	10.28^{a}	9.50^{a}	5.36^{b}	2.03	***
n-6 PUFA	27.90^{b}	31.81^{b}	37.94^{a}	5.64	***
n-6/n-3	2.85^{b}	3.55^{b}	7.54^{a}	1.61	***
h/H	2.94	2.80	3.26	0.47	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n-3* + C18:4 *n-3* + C20:3 *n-3* + C20:5 *n-3* + C22:5 *n-3* + C22:6 *n-3*

n-6 PUFA = C18:2 *n-6* + C18:3 *n-6* + C20:2 *n-6* + C20:3 *n-6* + C20:4 *n-6* + C22:2 *n-6* + C22:4 *n-6*

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis-9*, C18:2 *n-6*, C18:3 *n-6*, C18:3 *n-3*, C20:3 *n-3*, C20:4 *n-6*, C20:5 *n-3*, C22:4 *n-6*, C22:5 *n-3* and C22:6 *n-3*)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Rsd = residual standard deviation of the analysis of variance

Figure 1. Geographical distribution of Barrosã, Maronesa and Mertolenga Breeds.