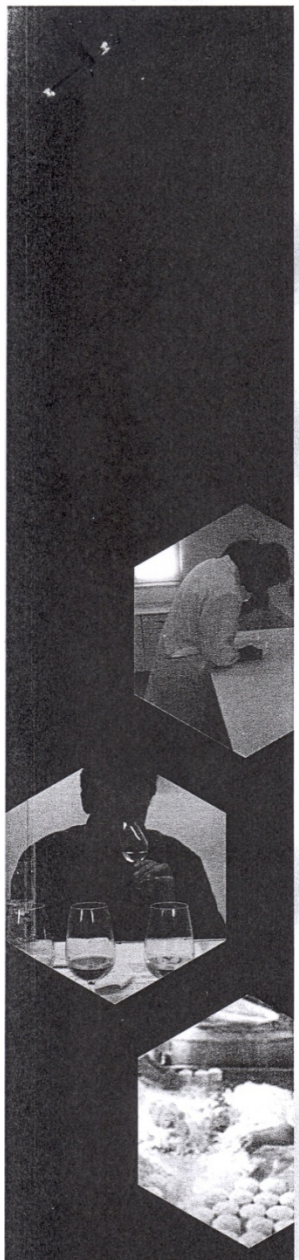
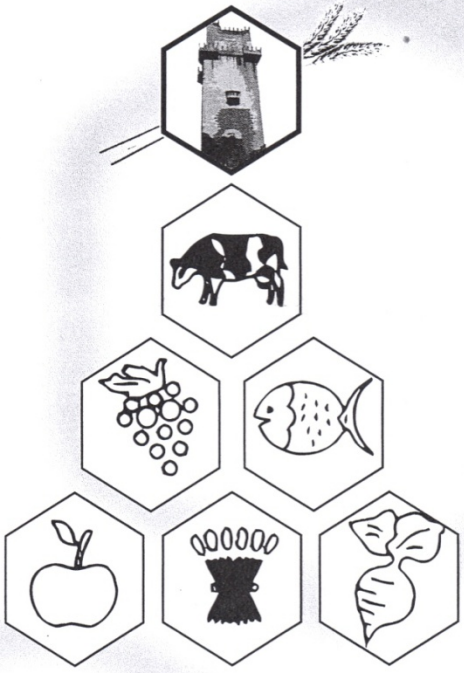


ACTAS DO 8º ENCONTRO DE QUÍMICA DOS ALIMENTOS



Alimentos Tradicionais, Alimentos Saudáveis e Rastreabilidade

Beja, Março de 2007

Instituto Politécnico de Beja
Escola Superior Agrária de Beja
Sociedade Portuguesa de Química

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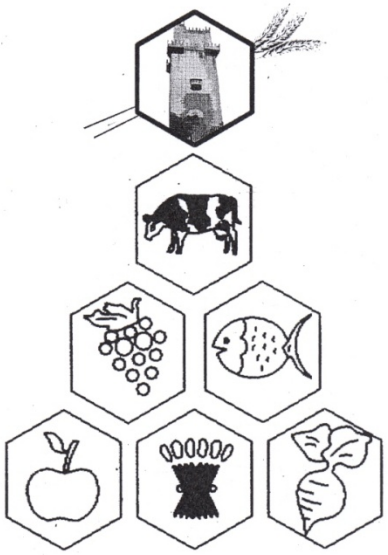

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LIPID COMPOSITION AND NUTRITIONAL VALUE OF SOME MEATS WITH PROTECTED DESIGNATION OF ORIGIN (PDO)

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Palavras-chave: meat-PDO; fatty acids; CLA isomers; total lipids, cholesterol.

Resumo: The aim of this work was to characterise and compare the lipid composition (lipid and cholesterol contents, fatty acid composition and conjugated linoleic acid isomers) and nutritional value (PUFA/SFA and n-6/n-3 ratios) of four Portuguese meats with Protected Designation of Origin (PDO; Carnalentejana-PDO beef, Mertolenga-PDO beef, Barrosã-PDO veal and Arouquesa-PDO veal), in two distinct slaughter seasons (early autumn and late spring). The purebred bovines were maintained according to the traditional production systems following the rules established in the meat-PDO product specifications. In addition, we also compared the nutritional quality of intramuscular fat in these meats-PDO with the meat obtained from Alentejana×Charolais crossbred young bulls produced in a typical intensive concentrate-based system. The results obtained for Carnalentejana-PDO beef (Meat Science, 2006, 72, 425-436), Mertolenga-PDO beef (Journal of the Science of Food and Agriculture, 2006, 86, 2196-2205), Barrosã-PDO veal (Food Chemistry, 2006, 94, 469-477; Meat Science, 2007, 75, 44-52) and Arouquesa-PDO veal (Meat Science, submitted), indicate that intramuscular fats of meats-PDO, as a result of the beneficial grass effects on the characteristics of meat lipids, are of greater nutritional quality than intensively produced beef from crossbred young bulls throughout the year. However, the data suggest that beef-PDO (Carnalentejana-PDO and Mertolenga-PDO) intramuscular fat, relative to that from veal-PDO (Barrosã-PDO veal and Arouquesa-PDO veal), depicts a low nutritional quality throughout the year. These differences may be explained by the finishing period of Alentejana and Mertolenga purebred young bulls on concentrate, which attenuates the beneficial effects on the characteristics of meat fat associated with grass intake.

1. INTRODUCTION

Fatty acid composition and cholesterol levels in meat have received an increased attention in view of their implications for human health and product quality [1]. The ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and n-6/n-3 PUFA are widely used to evaluate the nutritional value of fat. Low ratios of PUFA/SFA and high levels of cholesterol in typical Western diets have been considered as major risk factors of

cardiovascular diseases, which are among the most important causes of human mortality in developed countries. Moreover, typical Western diets display a very high n-6/n-3 ratio (15-17/1), which not only favours the development of cardiovascular diseases, but also cancer, inflammatory and autoimmune diseases. In addition, fats presenting low PUFA/SFA ratio are considered unfavourable, because they may induce an increase in cholesterolaemia. It is well established that lower PUFA/SFA and higher n-6/n-3 ratios of most meats are a major cause of the imbalance in the fatty acid intake of today's consumers. Finally, meat also provides from one third to one half of the maximum daily-recommended intake of cholesterol (300 mg).

Conjugated linoleic acids (CLA) is a generic term used to describe the positional and geometric isomers of linoleic acid (18:2n-6) in which the double bonds are conjugated. The major CLA isomer, *cis*-9,*trans*-11 18:2 (18:2c9,t11), is produced in the rumen during the microbial biohydrogenation of dietary 18:2n-6 and in the tissues (endogenously) through delta9 desaturation of 18:1t11 [2]. It is now accepted that the major contribution to 18:2c9,t11 present in milk and in ruminant meats is the endogenous synthesis. Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat. In animal models, some CLA isomers exhibit anticarcinogenic, antithrombotic, antiatherogenic and immune modulator properties. Specific physiological effects have been linked with individual CLA isomers. The t10,c12 isomer may play an important role in lipid metabolism, while the c9,t11 and the t10,c12 isomers seem to be equally effective in anticarcinogenesis and antiatherogenesis. Since individual CLA isomers have different biological activities, the determination of the CLA isomeric profiles in meat is required.

Fatty acid composition of beef is influenced by dietary factors and, to a lesser extent, by non-dietary environmental and genetic factors [3]. Dietary factors are often linked with a particular production system. Meat from grazing ruminants is expected to reflect the variability of pasture biomass. In fact, the nutritive value of pasture is highly dependent on cultural practices, season and geographical factors. In addition, breed differences in fatty acid composition in several farm animal species have also been reported. Meats with Protected Designation of Origin (PDO), derived from local production systems and animal breeds, are certified by European Union legislation and are expected to present unique quality and organoleptic characteristics, especially associated with specific properties of its lipid fraction (Council Regulation nº2081/92 of 14/7, EEC).

In spite of being four important commercial Portuguese meats-PDO, there are no detailed reports on the composition of the Carnalentejana-PDO beef, Mertolenga-PDO beef, Barrosã-PDO veal and Arouquesa-PDO veal. Therefore, the goal of this work was to characterise the lipid composition and nutritional value of these meats-PDO in two distinct and more important slaughter seasons (early autumn and late spring).

2. MATERIALS AND METHODS

The purebred bovines (Alentejana, Mertolenga, Barrosã and Arouquesa) were maintained according to the traditional production systems following the rules established in the meats-PDO product specifications. The Alentejana and Mertolenga young bulls, and the Barrosã and Arouquesa calves, were slaughtered in October 2002 (early autumn sampling) or June 2003 (late spring sampling). Meat samples were taken from the ribeye portion (T1-T3) of *longissimus dorsi* (LD) and distal region of *semitendinosus* (ST) muscles of young bulls. Comparing with ST muscle, LD muscle is relatively red and differently involved in the physical activity imposed by grazing. All meat samples were collected 2-3 days after

slaughter (+1 °C), ground using a food processor (3 × 5 s), vacuum packed and stored at -80 °C until required for analysis.

Meat samples were lyophilised (-60 °C and 2.0 hPa) until constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), maintained exsiccated at room temperature, and analysed within two weeks. Lyophilised meat samples were weighed (approximately 250 mg), in triplicate (two tubes for total lipid determination and the other tube for both FAME and CLA methyl ester profiles), into screw teflon-lined cap tubes. Intramuscular fat was extracted as described by Alfaia *et al.* [4].

Gas chromatography analyses of FAME were performed with an Agilent 6890 Series II gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a flame ionization detector (FID). The FAME were separated, on a SP^{TM}-2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), identified and quantified as described by Alfaia *et al.* [4].

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an high performance liquid chromatography (HPLC) system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector (DAD) adjusted to 233 nm. The separation conditions, as well as the identification and quantification of CLA isomers were described previously [4].

Total cholesterol was extracted from lyophilised meat, after direct saponification with saturated methanolic KOH solution, according to the procedure of Prates *et al.* [6]. Cholesterol was analysed by normal-phase HPLC (column Zorbax Rx-Sil, 250 mm × 4.6 mm i.d., 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and DAD set at 206 nm, as described in Prates *et al.* [6].

The data were analysed using the MIXED procedure of SAS, considering the animal within slaughter season group as subject and the muscle type as repeated measures. The model considers as fixed effects the slaughter season (meat-PDO from early autumn and that from late spring), the muscle type (LD and ST) and the interaction between animal group and muscle type.

3. RESULTS AND DISCUSSION

Table 1 depicts the results concerning the total lipids, total cholesterol, CLA isomers and nutritional ratios of fatty acids in LD and ST muscles of intensively produced beef and meats-PDO from early autumn and late spring. Detailed information on the nutritional value of intensively produced beef and Carnalentejana-PDO beef was published in Meat Science (2006, 72, 425-436) [4], Mertolenga-PDO beef in Journal of the Science of Food and Agriculture (2006, 86, 2196-2205) [5], Barrosã-PDO veal in Food Chemistry (2006, 94, 469-477) [6] and Meat Science (2007, 75, 44-52) [7] and Arouquesa-PDO veal submitted to Meat Science [8]. The ratios of n-6/n-3 and PUFA/SFA (as defined in Table 1) are nutritional indexes widely used to evaluate the nutritional value of fat for human diet. Current nutritional recommendations are that the PUFA/SFA ratio in human diet should be above 0.45 and, within the PUFA, the n-6/n-3 ratio should not exceed 4.0 (British Department of Health, 1994).

Meat from Alentejana Portuguese purebred young bulls raised according to Carnalentejana-PDO specifications showed no important seasonal differences in the levels of

fatty acids, CLA isomers and total cholesterol. In contrast, there were some different intramuscular fat characteristics when beef-PDO was compared with meat from Alentejana×Charolais crossbred young bulls fed intensively with concentrate. This observation might reflect the semi-extensive production system used for raising the Alentejana purebred animals. However, the findings suggest that the finishing period of Alentejana purebred young bulls on concentrate attenuates most of the typical effects on the characteristics of meat fat associated with grass intake. Nevertheless, from a nutritional point of view, Carnalentejana-PDO beef seems to be more healthful than meat obtained from the conventional intensively-fed young bulls because of its lower n-6/n-3 ratio, although this ratio is always above the recommended values for human diet, and higher proportions of c9,t11 CLA isomers. Taken together, the data indicate that Carnalentejana-PDO beef is of greater nutritional quality than intensively produced beef from crossbred young bulls throughout the year.

Mertolenga-PDO beef showed seasonal differences in the levels of several fatty acids, some CLA isomers and total cholesterol. The data indicate that beef-PDO has intermediate values (between meat from grain- and pasture-fed cattle) in the content of several fatty acids, total lipids, n-6/n-3 ratio, total CLA and some CLA isomers. These data may be explained by the semi-extensive production system of Mertolengo young bulls. However, beef from June, relative to that from October, seems to have a less attenuated grass effect, possibly due to the shortest finishing period of young bulls on concentrate feeds. From a nutritional point of view, meat-PDO from June seems to be more healthful than that from October because of its lower n-6/n-3 ratio, although this ratio is always above the recommended values for the human diet. Overall, the data indicate that, although the finishing period of Mertolengo young bulls with cereal-rich concentrate attenuates most of the beneficial grass effects on the characteristics of meat fat throughout the year, beef-PDO from late spring is of greater nutritional quality than that from early autumn.

Barrosã-PDO veal only showed seasonal differences in the levels of some minor fatty acids and CLA isomers, as well as in the PUFA/SFA ratio. The data indicate that Barrosã-PDO veal has values of pasture-fed cattle, in both slaughter seasons, concerning the content of several fatty acids, some partial sums of fatty acids, n-6/n-3 ratio, total and specific CLA contents, and some individual CLA isomers (grass intake indicators). This observation might reflect similar final effects of the different suckling and grazing periods between autumn-slaughtered and spring-slaughtered calves. From a human nutrition point of view, veal-PDO originated in both slaughter seasons presents health related parameters because the CLA contents and the percentages of c9,t11 isomer are relatively high, and the n-6/n-3 ratios are inside the recommended values for the human diet. Overall, the data indicate that Barrosã-PDO veal intramuscular fat, as a result of the beneficial grass effects on the characteristics of meat lipids, depicts a high nutritional quality throughout the year.

Arouquesa-PDO veal only showed seasonal differences in the levels of some minor fatty acids and CLA isomers. The data indicate that Arouquesa-PDO has values of pasture-fed cattle, in both slaughter seasons, concerning the content of several fatty acids, some partial sums of fatty acids, n-6/n-3 ratio, total and specific CLA contents, and some individual CLA isomers (grass intake indicators). This observation might reflect similar final effects of the different suckling and grazing periods between autumn-slaughtered and spring-slaughtered calves. From a human nutrition point of view, veal-PDO originated in both slaughter seasons presents good health related parameters because the CLA contents and the percentages of c9,t11 CLA isomer are relatively high, and the n-6/n-3 ratios are inside the recommended values for the human diet. Overall, the data indicate that Arouquesa-PDO veal intramuscular fat, as a result of the beneficial grass effects, depicts a high nutritional quality throughout the

year.

4. CONCLUSIONS

In conclusion, the data indicate that intramuscular fat of meats-PDO, as a result of the beneficial grass effects on the characteristics of meat lipids, is of greater nutritional quality than that of intensively produced beef from crossbred young bulls throughout the year. However, the results suggest that beef-PDO (Carnalentejana-PDO and Mertolenga-PDO) intramuscular fat, relative to that from veal-PDO (Barrosã-PDO veal and Arouquesa-PDO veal), depicts a low nutritional quality throughout the year. This difference may be explained by the finishing period of Alentejana and Mertolenga purebred young bulls on concentrate, which attenuates most of the typical effects on the characteristics of meat fat associated with grass intake. These differences clearly result from the benefits of grass-fed on ruminant meat, which should be brought to the attention of the public, nutritionists, the medical profession, producers and consumers.

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Table 1. Total lipids (mg/g), total cholesterol (mg/g), total (mg/g) and specific (mg/g fat) contents of CLA isomers, and nutritional ratios of fatty acids in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of intensively produced beef and meats-PDO from early autumn and late spring. Extracted from the detailed information on the lipid composition and nutritional value reported by our group for meats-PDO [ref. 4,5,6,7,8].

	Autumn		Spring			Significance levels		
	LD	ST	LD	ST	SEM	Season	Muscle	S×M
Intensively produced beef (n=15)								
Total lipids	15.2	8.61	-	-	1.351	-	***	-
Total cholesterol	0.37	0.35	-	-	0.010	-	ns	-
CLA								
Total	0.064	0.024	-	-	0.010	-	***	-
Specific	4.45	3.88	-	-	0.563	-	ns	-
Ratios of FA								
n-6/n-3	20.2	16.7	-	-	1.322	-	**	-
PUFA/SFA	0.40	0.84	-	-	0.070	-	***	-
Carnalentejana-PDO beef (n=30)								
Total lipids	21.9	13.1	14.8	11.2	1.351	**	***	ns
Total cholesterol	0.48	0.42	0.49	0.43	0.010	ns	***	ns
CLA								
Total	0.100	0.042	0.066	0.054	0.010	ns	***	ns
Specific	5.07	3.82	4.92	5.06	0.563	ns	ns	ns
Ratios of FA								
n-6/n-3	11.4	10.0	13.7	11.5	1.322	ns	**	ns
PUFA/SFA	0.32^{a}	0.63^{b}	0.42^{a}	0.56^{b}	0.070	ns	***	*
Mertolenga-PDO beef (n=30)								
Total lipids	17.4	12.0	18.0	11.8	0.934	ns	***	ns
Total cholesterol	0.44	0.40	0.50	0.44	0.011	***	***	ns
CLA								
Total	0.062	0.039	0.066	0.031	0.006	ns	***	ns
Specific	3.65	3.39	3.51	2.63	0.350	ns	ns	ns
Ratios of FA								
n-6/n-3	14.9	13.6	7.11	6.87	1.012	***	*	ns
PUFA/SFA	0.41	0.61	0.36	0.68	0.047	ns	***	ns
Barrosã-PDO veal (n=27)								
Total lipids	23.2^{a}	19.6^{b}	22.9^{a}	15.6^{c}	1.136	ns	***	*
Total cholesterol	0.56	0.50	0.45	0.42	0.010	***	*	ns
CLA								
Total	0.199	0.148	0.172	0.111	0.022	ns	***	ns
Specific	8.52	7.59	7.42	7.01	0.795	ns	ns	ns
Ratios of FA								
n-6/n-3	3.10	2.99	3.05	2.92	0.232	ns	ns	ns
PUFA/SFA	0.25	0.29	0.19	0.24	0.023	*	*	ns
Arouquesa-PDO veal (n=31)								
Total lipids	26.8^{a}	24.6^{a}	30.3^{a}	16.9^{b}	2.563	ns	**	*
Total cholesterol	0.53	0.52	0.52	0.49	0.010	ns	ns	ns
CLA								
Total	0.208^{a}	0.222^{a}	0.257^{a}	0.117^{b}	0.026	ns	**	**
Specific	8.85	8.86	8.74	6.82	0.623	ns	ns	ns
Ratios of FA								
n-6/n-3	2.28	2.19	1.95	1.99	0.157	ns	ns	ns
PUFA/SFA	0.21^{a}	0.21^{a}	0.17^{a}	0.29^{b}	0.017	ns	***	***

Significance: ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001; means in the same row with different superscripts are significantly different (P<0.05); SEM, standard error of mean. S×M means interaction between slaughter season (S) and muscle type (M).

n-6/n-3 = n-6/n-3 ratio [(sum of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6 and 22:4n-6)/(sum of 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3)].

PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2n-6, 18:3n-3, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3 and 22:6n-3)/(sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].