Meat Science 84 (2010) 769–777

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat

Cristina M.M. Alfaia [a], Susana P. Alves [b], Anabela F. Lopes [a], Maria J.E. Fernandes [a], Ana S.H. Costa [a], Carlos M.G.A. Fontes [a], Matilde L.F. Castro [c], Rui J.B. Bessa [a,b], José A.M. Prates [a,*]

[a] CIISA, Faculdade de Medicina Veterinária, Avenida da Universidade Técnica, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal
[b] REQUIMTE, Unidade de Produção Animal, L-INIA, INRB, I.P., Fonte Boa, 2005-048 Vale de Santarém, Portugal
[c] iMed.UL, Faculdade de Farmácia, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 12 March 2009
Received in revised form 3 November 2009
Accepted 18 November 2009

Keywords:
Fatty acid composition
CLA isomers
Cooking methods
Beef

ABSTRACT

The effect of boiling, microwaving and grilling on the composition and nutritional quality of beef intramuscular fat from cattle fed with two diets was investigated. *Longissimus lumborum* muscle from 15 Alentejano young bulls fed on concentrate or pasture was analyzed. Cooking losses and, consequently, total lipids, increased directly with the cooking time and internal temperature reached by meat (microwaving > boiling > grilling). The major changes in fatty acid composition, which implicated 16 out of 34 fatty acids, resulted in higher percentages in cooked beef of SFA and MUFA and lower proportions of PUFA, relative to raw meat, while conjugated linoleic acid (CLA) isomers revealed a great stability to thermal processes. Heating decreased the PUFA/SFA ratio of meat but did not change its *n*−6/*n*−3 index. Thermal procedures induced only slight oxidative changes in meat immediately after treatment but hardly affected the true retention values of its individual fatty acids (72–168%), including CLA isomers (81–128%).

1. Introduction

Fat content and fatty acid composition of meat are of major importance for consumers due to their importance for meat quality and nutritional value (Wood et al., 2004). Saturated fatty acids (SFA) and *trans* fats have been recognized by the international dietary authorities as primary targets for diet reduction (WHO, 2003). Marked reductions in these specific nutrients, as well as an increase in the polyunsaturated fatty acids (PUFA) content, especially *n*−3 PUFA at the expense of *n*−6 PUFA, may have a noticeable knock-on effect on public health improvement (British Department of Health, 1994). Ruminant meat provides a valuable amount of PUFA, namely *n*−3 fatty acids, for the human diet (Scollan et al., 2001). In contrast, it is well known that the low PUFA/SFA and high *n*−6/*n*−3 ratios of some meats contribute to the imbalance in the fatty acid intake of today's consumers (Wood et al., 2004).

Meat from ruminants represents the major source of natural conjugated linoleic acid (CLA) isomers of the human diet. Twenty-four CLA isomers occur naturally in foods, especially in meat, milk and dairy products (Sehat et al., 1998). Complete information about CLA isomeric composition is of utmost importance in nutritional/biochemical studies since some CLA isomers, at least the *cis*9,-*trans*11-18:2 (*c*9,*t*11) and *t*10,*c*12, have been shown to have several biological effects (Park & Pariza, 2007). Animal and *in vitro* studies allowed identification of CLA as an important dietary component, with a multitude of potential health benefits on cancer, cardiovascular diseases, diabetes, body composition, the immune system or modulation of bone growth (reviewed by Wahle, Heys, & Rotondo, 2004). Ruminant fats are the richest natural dietary source of *c*9,*t*11, which is the major CLA isomer, commonly known as rumenic acid (Kramer et al., 1998). This isomer, is mainly produced by *de novo* endogenous synthesis in different tissues via Δ9-desaturation of *trans*-11 octadecenoic acid (vaccenic acid, 18:1*t*11) (Griinari & Bauman, 1999). The origin of all other CLA isomers is supposed to arise from ruminal biohydrogenation of dietary unsaturated C18 PUFA (Bessa et al., 2007).

As a result of the fatty acid imbalance in human diets, dietary strategies have been used to improve the nutritional and health values of cattle intramuscular fat. Thus, manipulation of fatty acid composition in ruminant meat, to reduce SFA content and *n*−6/

Abbreviations: CLA, conjugated linoleic acid; FA, fatty acids; FAME, fatty acid methyl esters; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TBARS, 2-thiobarbituric acid reactive substances; TFA, *trans* fatty acids; TR, true retention.

* Corresponding author. Tel.: +351 213652890; fax: +351 213652895.
E-mail address: japrates@fmv.utl.pt (J.A.M. Prates).

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.11.014

$n-3$ ratio and, simultaneously, increase the PUFA and CLA content, is of major importance in meat research. It has been suggested that in ruminants, grazing has potential beneficial effects on PUFA/SFA and $n-6/n-3$ ratios, increasing the PUFA and CLA contents while decreasing the SFA concentration in beef (French et al., 2000). Although several factors may influence fatty acid composition and CLA content of beef (*e.g.* seasonal variation, animal genetics and production practices), diet plays the most important role (Schmid, Collomb, Sieber, & Bee, 2006). In addition, dietary factors are often linked with particular production systems (Geay, Bauchart, Hocquette, & Culioli, 2001).

Meat composition, as well as its physicochemical properties, undergoes significant changes during heat treatment. It is well known that meat composition, especially its fat content, combined with a specific cooking methodology are among the factors that mostly affect the final quality of meat products (Serrano, Librelotto, Cofrades, Sánchez-Muniz, & Jiménez-Colmenero, 2007). Several authors pointed out that the cooking process can affect the lipid composition of meat, especially the fatty acid content, by changing the nutritional value of cooked products in relation to raw samples (Badiani et al., 2002). Moreover, Rodriguez-Estrada, Penazzi, Caboni, Bertacco, and Lercker (1997) reported that heat treatment can lead to undesirable changes, such as loss of essential fatty acids (FA), reducing the nutritional value of meat, mainly due to lipid oxidation. It has been shown that a higher unsaturation index in meat may affect its oxidative stability, since the unsaturated FA are more prone to oxidation (Bou et al., 2001). However, there is a great variability in changes concerning individual FA in response to different cooking methods (Badiani et al., 2002; Harris, Harberson, Savell, Cross, & Smith, 1992).

Despite the various studies focusing on the effect of cooking on fatty acid composition (see *e.g.* Badiani et al., 2004; Maranesi et al., 2005; Sarriés, Murray, Moloney, Troy, & Beriain, 2009), as far as we know, no data has been reported regarding the effect of cooking on CLA isomeric distribution in beef fat, which is only achieved by HPLC using three silver-ion columns in series (Prates & Bessa, 2009). Moreover, it is not well known if CLA enriched meat is more susceptible to the thermal processes of household cooking methods. Thus, the aim of this work was to investigate the effect of common culinary practices (boiling, microwaving and grilling) on meat fatty acid composition, with special emphasis on the detailed isomeric distribution of CLA, in meat from cattle allocated to two feeding regimens (concentrate or pasture), in order to obtain beef with distinct levels of PUFA and CLA. In addition, true retention (TR) values of these food components for the studied cooking processes were calculated and compared. Finally, the influence of these household cooking methods on the oxidative stability and nutritional value of beef intramuscular fat was also assessed.

2. Materials and methods

2.1. Reagents

Analytical and liquid chromatographic grade chemicals were from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma–Aldrich Ltd. (St. Louis, MO, USA).

Fatty acid methyl ester (FAME) standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis*,*trans*, *trans*,*cis* and *trans*,*trans* from 7,9 to 12,14) of CLA isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003).

2.2. Animals and feeding

The animal experiment was conducted at Estação Zootécnica Nacional (EZN) facilities (Vale de Santarém, Portugal). Sixteen young bulls from Alentejano purebred EZN herd, born between March and July of 2003, were used. After weaning (with ages from 7 to 12 months and live body weights of 279 ± 50 kg, mean ± standard deviation), in February 2004, 8 young bulls were transferred to a feedlot and fed at 2.75% live body weights, on a diet consisting of 70% of concentrate feed and 30% of molasses-fibrous cubes. The ingredient, proximate and fatty acid compositions of the concentrate feed were reported previously by Alfaia et al. (2009). The animals were slaughtered at approximately 600 kg of live body weight (September and October 2004). One of the animals failed to grow at normal rates and was removed from the experiment. The other eight bulls were maintained for 15 months on extensive management on pasture lands (alluvium land nearby Tagus river (39°07′N; 8°43′W) grazing on spontaneous pastures and summer triticale and maize stubbles) until slaughtered in June 2005.

Animals were slaughtered at the EZN experimental abattoir by exsanguination after stunning with a cartridge-fired captive bolt stunner. Carcasses were suspended from the Achilles tendon and chilled at 10 °C for 24 h and kept refrigerated at 2 °C for 5 days. *Longissimus lumborum* muscle samples (*ca.* 200 g) were collected, vacuum packed and stored frozen at −70 °C until analysis.

2.3. Cooking treatments and preparation of samples

Frozen muscle samples were thawed overnight at 4 ± 2 °C, trimmed of connective and adipose tissues, and sliced into square cuts of 5 cm with 2.5 cm of thickness. The cuts were subjected to each of the cooking treatments used (boiling, microwaving and grilling), while the raw cuts were sampled directly as the uncooked control. Internal temperatures were monitored continuously using thermocouples (type K), inserted into the approximate geometric centre of each cut, and connected to a digital temperature recorder (Lufft C100 Series Digital Instruments, USA). Preliminary cooking trials were conducted to determine cooking times and temperatures required to achieve a constant degree of doneness (medium or well done) for the various cooking methods. Boiling was conducted at 80 °C during 60 min in a water bath (well done). For microwave cooking, the samples were placed on a ceramic container in a Pyrex® pan in the centre of the carousel of a microwave oven (2450 MHz, 900 W variable power, Mod. AVM 559, Whirpool®, USA), set at 750 W. Two heating cycles of 1 min 45 s were used and the cut was turned over between cycles (well done). Grilling was performed at 225 °C, using a large preheated electric grill. The cuts were placed on a turnable rack, arranged approximately 4 cm above the heating elements and turned every 2 min during 30 min (medium done). Final internal temperature was the maximum temperature reached by each cut upon removal from the heat source (71.2, 79.0 and 92.1 °C for grilling, boiling and microwaving, respectively) (Table 1). After cooking and cooling (30 min at 20–22 °C), the cuts were manually wiped with a paper towel to remove visible exudates. All cuts were weighed before and after cooking in order to determine the percentage of cooking loss.

2.4. Analysis of fatty acids and CLA isomers

2.4.1. Lipid extraction and methylation of fatty acids

Meat was lyophilized (−60 °C and 2.0 hPa) until constant weight using an Edwards Modulyo lyophilizer (Edwards High

Table 1
Effect of cooking methods on heat-processing parameters, cooking loss and selected nutrients of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Final internal temperature (°C)	–	79.0[a]	92.1[b]	71.2[c]	0.33	***
Cooking loss (%)	–	39.91[a]	42.71[b]	32.64[c]	0.551	***
Selected nutrients						
Moisture (%)	74.78[a]	61.22[b]	54.52[c]	62.28[b]	0.461	***
Total lipids (%)	1.25[a]	2.17[b]	2.61[c]	2.21[b]	0.137	***

[A] Statistical probability of treatment: ***, $P < 0.001$; means in the same row with different superscripts are significantly different ($P < 0.05$); SEM, standard error of mean.

Vacuum International, UK). Intramuscular fat of lyophilized meat samples (*ca.* 250 mg) was extracted with methylene chloride–methanol (4:1 v/v; 3×) and *n*-hexane (1×) for total lipid determination according to Fritsche et al. (2000). Total lipids were measured gravimetrically, in duplicate, by weighting the fatty residue obtained after solvent evaporation.

FAME were directly extracted and methylated from meat samples by a one-step procedure (adapted from Christie, Sébédio, & Juanéda, 2001). Fatty acids (FA) were converted to FAME by a combined transterification procedure with NaOH in anhydrous methanol (0.5 M) followed by HCl/methanol (1/1 v/v) at 50 °C during 30 and 10 min, respectively, according to Raes, de Smet, and Demeyer (2001).

2.4.2. Determination of fatty acid composition

FAME were analyzed using a HP6890A gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a flame-ionization detector (GC-FID) and a fused-silica capillary column (CP-Sil 88; 100 m × 0.25 mm i.d., 0.2 μm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA), as described by Bessa et al. (2007). Identification was accomplished by comparison of sample peak retention times with those of FAME standard mixtures and with values published in the literature (Fritsche et al., 2000). The FAME identification of some unknown peaks was conducted by gas chromatography–tandem mass spectrometry (GC–MS/MS), using a Varian Saturn 2000 system (Varian Inc., Walnut Creek, CA, USA) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness). Quantification of muscle lipids FAME was done using nonadecanoic acid (19:0) as internal standard. Helium was used as the carrier gas and the injector split ratio was 1:50. After injection (1 μl), the initial column temperature of 100 °C was held for 15 min, increased to 150 °C at 10 °C/min and held for 5 min. Then, was increased to 158 °C at 1 °C/min, held 30 min, and finally increased to 200 °C at a rate of 1 °C/min, and maintained for 60 min. The injector and detector temperatures were 250 and 280 °C, respectively. FA were expressed as a percentage of the sum of detected FAME (g/100 g FAME), assuming direct proportionality between peak area and FAME weight, or in gravimetric contents (mg/g muscle), using the conversion factor for lean beef (0.920) for calculation of total FA from total lipids (Weihrauch, Posati, Anderson, & Exler, 1977).

2.4.3. Determination of CLA isomers

The methyl esters of CLA isomers were individually separated by triple column silver-ion in series (ChromSpher 5 Lipids analytical, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector adjusted to 233 nm, according to the procedure reported previously by Alfaia et al. (2006). The mobile phase was 0.1% acetonitrile in *n*-hexane maintained at a flow rate of 1 ml/min and injection volumes of 20 μl were used. Identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature (Fritsche et al., 2000). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000). CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

2.5. Nutrient retention values

TR values for nutrients were calculated using the following formula (Murphy, Criner, & Gray, 1975): TR (%) = [(nutrient content per g of cooked food × g of food after cooking)/(nutrient content per g of raw food × g of food before cooking)] × 100.

2.6. Lipid oxidation

Lipid stability of both raw and cooked cuts was immediately evaluated after cooling (30 min at 20–22 °C), by measuring the 2-thiobarbituric acid reactive substances (TBARS), based on the method of Grau, Guardiola, Boatella, Barroeta, and Codony (2000), with the resulting colour measured at 532 nm in a UV/VIS Spectrophotometer (Pharmacia LKB Biochrom Ltd., UK). A standard curve was constructed with 1,1,3,3-tetraethoxypropane (Fluka Neu Ulm, Germany). The results were expressed as mg of malondialdehyde (MDA) equivalents per kg of meat.

2.7. Statistical analysis

The data were analyzed using the MIXED procedure of Statistical Analysis Systems Institute (SAS, 2004). The model included the fixed effects of animal diet and cooking treatment, as well as the interaction between diet and treatment. Because no significant diet × treatment interactions ($P > 0.05$) were observed, it was removed from the model. Treatment effect was evaluated as repeated measure on the animal within diet. Least squares means were determined using the LSMEANS option and compared, when significant ($P < 0.05$), using the probability difference procedure (PDIFF option).

3. Results and discussion

Detailed data on the level of intramuscular fat and fatty acid composition, including CLA isomeric profile, in *L. lumborum* muscle of Alentejano purebred bulls fed on different diets are reported in Alfaia et al. (2009). Since no significant interactions ($P > 0.05$) between animal diets and cooking treatments were found, only the cooking methods effects (boiling, microwaving and grilling) on beef properties are presented and discussed here.

The absence of interactions indicates that meat from bulls fed on concentrate was not more susceptible to changes induced by heating than that from animals grazing on pasture. Thus, the meat samples used in this study, from both concentrate (higher content of monounsaturated fatty acids (MUFA), mainly oleic acid – 18:1*c*9) and pasture (higher contents of *n*−3 PUFA, mainly α-linolenic acid – 18:3*n*−3, and CLA, on a mg/g fat basis) fed cattle (Alfaia et al., 2009), were analyzed together. However, it is interesting to note that the PUFA enriched meat is not more sensitive to thermal processes when compared with that enriched in MUFA. It is possible that either the high contents of CLA isomers (Yu, Adams, & Gabel, 2002) or the high levels of α-tocopherol (data not shown), or both, provide antioxidant protection for PUFA in these meats.

3.1. Heat-processing parameters, cooking loss and selected nutrients

The three different meat cooking methods used in this study differed in the processing parameters used (cooking time and temperature). These conditions were chosen in order to enable the meat to attain a medium (grilling) to a well (boiling and microwaving, respectively) degree of doneness (Table 1). Lorenzen et al. (1999) observed that consumers preferred medium and well done meat to rare meat. Similar observations were made by Savell et al. (1999), who also reported that consumers most frequently cook steaks to the well done stage. In our study, the final internal temperatures reached were significantly different ($P < 0.05$) among the cooking processes. In addition, the cooking losses were also affected ($P < 0.05$) by the cooking method used. It is well known that losses depend on the mass transfer process during thermal treatment, which is directly related to the cooking procedure (i.e. heating rate, final cooking temperature, time, etc.) and to the properties of raw meat (i.e. moisture, fat, and protein composition, size, pH, etc.) (Gerber, Scheeder, & Wenk, 2009; Serrano et al., 2007). García-Segovia, Andrés-Bello, and Martínez-Monzó (2007) observed that changes in cooking losses tended to be linear with time, with an increase with temperature. Indeed, we observed that increasing final internal temperature (grilling < boiling < microwaving) resulted in greater cooking losses, as more moisture had been lost by evaporation, during processing. In addition to final internal temperature, it has been suggested that water losses tend to be higher after microwave heating and lower after grilling due to the absence of crust formation during microwave cooking (Serrano et al., 2007). The mean values obtained here for cooking losses are in agreement with those reported in similar studies (range 15–40%) (Sheard, Nute, & Chappell, 1998).

The percentages of moisture and total lipids for raw and cooked *L. lumborum* muscle of Alentejano purebred bulls are shown in Table 1. Compared with the raw meat control, cooking led to a significant loss of moisture and, consequently, to a significantly higher intramuscular fat content, with significant differences ($P < 0.05$) among treatments (microwaving > boiling = grilling). Badiani et al. (2002) reported that most nutrients increased their concentration as a consequence of moisture loss through cooking. Significant losses were more evident in microwave heating, as explained above, which induced a notable decrease in moisture level (20.3%). The values of total lipids obtained in this study (1.2% for raw meat and 2.2–2.6% for cooked meat) were generally lower than those reported in the literature for trimmed beef (Wahrmund-Wyle, Harris, & Savell, 2000). However, our results support the fact generally accepted that total moisture content decreases as fat content increases (Woolsey & Paul, 1969). Finally, according to the Food Advisory Committee (1990) criteria (<5% fat), both raw and cooked beef are considered lean meat.

Table 2
Effect of cooking methods on fatty acid composition (g/100 g FAME) of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

Fatty acids	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
10:0	0.10[a]	0.08[b]	0.08[b]	0.08[b]	0.006	**
12:0	0.05[ab]	0.05[b]	0.06[a]	0.06[a]	0.004	*
14:0	1.52[a]	1.82[b]	1.81[b]	1.93[b]	0.132	**
14:1*c*9	0.15	0.17	0.16	0.19	0.024	ns
15:0	0.37	0.42	0.41	0.42	0.022	ns
16:0	19.52[a]	20.96[b]	20.70[b]	21.35[b]	0.603	**
16:1*c*9	2.01[a]	2.20[ab]	2.09[a]	2.36[b]	0.177	*
17:0	0.94[a]	1.07[b]	1.08[b]	1.08[b]	0.042	*
17:1*c*9	0.67	0.63	0.64	0.67	0.044	ns
18:0	16.66[a]	18.20[b]	18.69[b]	17.65[b]	0.645	**
18:1*t*6 + *t*8	0.12	0.13	0.14	0.14	0.013	ns
18:1*t*9	0.17	0.18	0.18	0.19	0.013	ns
18:1*t*10	0.62	0.60	0.66	0.68	0.169	ns
18:1*t*11	1.08[a]	1.29[b]	1.33[b]	1.37[b]	0.099	*
18:1*t*12	0.30	0.31	0.32	0.31	0.020	ns
18:1*c*9	23.38[a]	25.78[b]	25.37[b]	26.44[b]	1.281	**
18:1*c*11	2.19	2.08	2.10	2.10	0.104	ns
18:1*c*12	0.40	0.38	0.37	0.38	0.022	ns
18:1*c*13	0.16	0.17	0.16	0.18	0.020	ns
18:1*c*15	0.07	0.08	0.08	0.08	0.012	ns
18:2*t*,*t* + *c*/*t*[B]	0.36	0.30	0.31	0.32	0.023	ns
18:2*t*11,*c*15	0.19	0.25	0.25	0.28	0.056	ns
18:2*n*−6	13.25[a]	10.36[b]	10.24[b]	9.50[b]	1.009	**
18:3*n*−6	0.09[a]	0.07[b]	0.07[b]	0.07[b]	0.007	*
18:3*n*−3	3.14	2.34	2.54	2.42	0.596	ns
20:0	0.08	0.08	0.09	0.08	0.005	ns
20:1*c*11	0.14[a]	0.15[b]	0.16[b]	0.15[ab]	0.011	*
20:2*n*−6	0.13[a]	0.10[b]	0.10[b]	0.09[b]	0.010	**
20:3*n*−6	0.94[a]	0.73[b]	0.70[b]	0.63[b]	0.086	**
20:4*n*−6	3.94[a]	2.94[b]	2.86[b]	2.65[b]	0.378	**
20:5*n*−3	1.17	0.76	0.83	0.83	0.204	ns
22:4*n*−6	0.26	0.23	0.22	0.19	0.034	ns
22:5*n*−3	1.66[a]	1.13[b]	1.18[b]	1.12[b]	0.215	*
22:6*n*−3	0.12	0.09	0.10	0.09	0.012	ns

[A] Statistical probability of treatment: 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.
[B] This peak may include several 18:2 *t*,*t*, *c*,*t* and *t*,*c* isomers.

3.2. Intramuscular fatty acid composition

Fatty acid composition (% of total FA) in raw and cooked samples of *L. lumborum* muscle from Alentejano purebred bulls is listed in Table 2. In decreasing order of percentage, the major FAs in intramuscular fat of raw and cooked meat, were oleic (18:1*c*9, 23–26%), palmitic (16:0, 20–21%), stearic (18:0, 17–19%) and linoleic (18:2*n*−6, 10–13%) acids. All the cooking methods had a moderate impact on the fatty acid profile of beef, with the content of 16 of the 34 FA analyzed affected ($P < 0.05$) by the thermal treatments. In addition, no novel fatty acid residues or other artefacts due to cooking were detected. Some SFA, namely 14:0, 16:0, 17:0 and 18:0, as well as MUFA, 18:1*c*9, were significantly higher ($P < 0.05$) in cooked meat samples than in the uncooked meat control. In contrast, the percentages of 18:2*n*−6 and almost all long chain *n*−6 FA decreased significantly ($P < 0.05$) in cooked beef compared to raw meat. Within the *n*−3 PUFA, no apparent cooking effects were observed, except for docosapentaenoic (22:5*n*−3, DPA), which had a lower content in cooked beef relative to raw meat. Variations in the fatty acid composition of raw and cooked samples have already been reported by Echarte, Ansorena, and Astiasarán (2003), who observed significant differences in the fatty acid profile of both chicken and beef patties. According to these authors, the contents of only 8 out of 18 FA did not change after microwave heating, including 18:1*c*9 and 20:5*n*−3, while 18:2*n*−6, 18:3*n*−3 and 22:6*n*−3 decreased. Moreover, Scheeder et al. (2001) found

slight changes in fatty acid composition during grilling of beef patties. In contrast, minor variations induced by heating in fatty acid composition of beef lipids were reported, among others, by Harris et al. (1992). However, Duckett and Wagner (1998) reported great differences in fatty acid composition between lipid fractions (neutral and polar lipids), with changes most evident in the polar lipid fraction, where PUFA are primarily located. Several mechanisms that occur during cooking, such as water loss and lipid oxidation, diffusion and exchange, can lead to relative changes in some FA (Dal Bosco, Castellini, & Bernardini, 2001; Rodriguez-Estrada et al., 1997). However, in our experimental conditions, the changes obtained in FA are likely due to the higher susceptibility of PUFA to oxidative degradation, relative to the other FA, since our meat was relatively lean (1.2% of total lipids) and the cooking conditions were relatively high (medium-well done). Finally, the percentages of individual *trans* fatty acids (TFA) remained unaffected ($P > 0.05$) by the cooking treatment, except for 18:1*t*11, which was higher ($P < 0.05$) in cooked beef than in raw meat. *trans* octadecenoic acids are the major intermediates formed during rumen biohydrogenation of C18 PUFA (Bessa, Santos-Silva, Ribeiro, & Portugal, 2000). In this study, the most abundant *trans* octadecenoic acid found was 18:1*t*11, followed by the *trans*-10 octadecenoic acid (18:1*t*10). Recent studies on rabbits suggested that butter enriched with 18:1*t*10, but not with 18:1*t*11, had deleterious effects on plasma lipids and lipoprotein metabolism (Roy et al., 2007).

Data on partial sums (wt%) of intramuscular FA, in both raw and cooked samples, are shown in Table 3. The patterns reflect the values described above for the major individual FA of each group. A significant increase ($P < 0.05$) in the relative proportion of SFA (+3.4–3.7%), as well as of MUFA (+1.9–3.4%), occurred after cooking, which mainly results from an increase in 16:0, 18:0 and 18:1*c*9, respectively. Cooked beef had lower concentrations of PUFA (−5.9% to 7.1%) than raw meat, due to a significant loss ($P < 0.05$) of some *n−6* and *n−3* PUFA. This is consistent with the results of Maranesi et al. (2005) that compared cooked and uncooked lamb rib-loins. The changes observed in the partial sums of FA in the work reported here are, as explained above for individual FA, likely due to the higher susceptibility of PUFA to oxidative degradation, relative to the other FA.

From a nutritional perspective, the quantitative fatty acid composition (expressed as mg/100 g muscle) was determined in both raw and cooked beef (data not shown). As expected, cooking produced significant increases ($P < 0.05$) in FA contents, with the exception of 20:5*n*−3. In general, the microwave cooking provided higher contents of FA than boiling or grilling beef, which likely resulted from the higher moisture loss. The mean content of the health promoting *n*−3 PUFA in cooked cuts was 84.5 mg/100 g muscle (77.5, 77.7 and 98.4 mg/100 g muscle for boiling, grilling and microwave, respectively), which represents a valuable contribution (from 38.8% to 49.2%) to the coverage of daily requirements in *n−3* FA (200 mg, for both sexes) according to the British Department of Health (1994). The ratios of PUFA/SFA and *n−6*/*n−3*, which are indices widely used to evaluate the nutritional value of fat for human consumption, are presented in Table 3. According to some nutritional recommendations (British Department of Health, 1994), the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the *n−6*/*n−3* ratio should not exceed 4.0. In the present experiment, cooked samples showed significantly lower ($P < 0.05$) PUFA/SFA ratios, with values close to the lower recommended limit. In addition, the cooking method did not change the values of the *n−6*/*n−3* ratio in meat. The value for the *n−6*/*n−3* ratio reported here is, in fact, the average for meat from concentrate-fed (11.6) and pasture-fed (1.89) animals.

In order to evaluate the correct increase or loss/degradation of food components during cooking, the TR values of nutrients were calculated (Table 4). The mean value for TR figures of moisture increased ($P < 0.05$) from microwaving (41.8%) to boiling (49.2%) and grilling (56.1%). In contrast to moisture, most of the intramuscular fat was retained after cooking. The TR values of total lipids were higher ($P < 0.05$) for both microwave and grilled meat when compared with boiled meat. As acknowledge by several authors, TR values of beef lipids can vary widely, namely from 91% to 160% for boiling and 71% to 125% for roasting (Bragagnolo & Rodriguez-Amaya, 2003; Harris et al., 1992). This variability has been explained by the presence of unpredictable levels of subcutaneous and intermuscular fats, which liquefy during cooking and absorption by the lean tissue leads to TR values higher than 100% (Bragagnolo & Rodriguez-Amaya, 2003; Smith, Savell, Smith, & Cross, 1989). However, when only intramuscular fat is present, which was the case in this study, a 100% TR is expected, unless fat is partially degraded or lost to the cooking medium (TR values lower than 100%). In contrast, lipid TR values higher than 100% suggest a higher extractability of lipids bound to lipoproteins from the cooked tissues, since heating can denature the muscle lipoproteins and release the bound lipids (Woolsey & Paul, 1969). Moderate differences in TR values for individual (in 12 out of 34 FA) and partial sums (SFA and MUFA) of FA were observed in the different cooking procedures. Moreover, the mean TR values found for individual FA, which varied widely from 72.2% to 168.0%, tended to be lower in boiling than in grilling or microwaving. Since microwave cooking and grilling proceed in the absence of water, these cooking methods probably allowed for a high retention of FA. The TR values obtained for the general individual SFA, MUFA and TFA, as well as for their partial sums, were higher than 100%, whereas for individual and for the sum of PUFA, the values were found to be generally lower (from 72.2% to 109.2%). As explained above, the higher values of TR for SFA, MUFA and TFA are likely due to the great extractability

Table 3
Effect of cooking methods on partial sums of fatty acids (g/100 g FAME), nutritional fatty acid ratios and values of TBARS (mg malonaldehyde equivalents/kg meat) of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Partial sums						
∑ SFA	39.30[a]	42.76[b]	43.00[b]	42.73[b]	1.018	**
∑ MUFA	29.11[a]	31.57[b]	31.05[b]	32.48[b]	1.569	**
∑ TFA	2.85	3.08	3.20	3.30	0.195	ns
∑ PUFA	24.69[a]	18.77[b]	18.84[b]	17.60[b]	1.981	**
∑ *n*−6 PUFA	18.60[a]	14.43[b]	14.19[b]	13.13[b]	1.486	**
∑ *n*−3 PUFA	6.09[a]	4.34[b]	4.65[b]	4.47[b]	0.994	*
∑ unidentified	4.04[a]	3.82[b]	3.91[ab]	3.89[ab]	0.277	*
Ratios						
PUFA/SFA	0.65[a]	0.46[b]	0.45[b]	0.42[b]	0.060	**
n−6/*n*−3	6.41	6.47	6.14	5.91	1.320	ns
Lipid oxidation						
TBARS	0.06[a]	0.10[b]	0.07[a]	0.06[a]	0.010	*

Σ SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0,18:0 and 20:0.
Σ MUFA = sum of *cis*-MUFA = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11, 18:1*c*12, 18:1*c*13, 18:1*c*15 and 20:1*c*11.
Σ TFA = sum of 18:1*t*6 + *t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12, 18:2*t,t* + *c/t* and 18:2*t*11,*c*15.
Σ PUFA = sum of 18:2*n*−6, 18:3*n−6*, 18:3*n*−3, 20:2*n*−6, 20:3*n*−6, 20:4*n*−6, 20:5*n*−3, 22:4*n*−6, 22:5*n*−3 and 22:6*n*−3.
Σ *n*−6 PUFA = sum of 18:2*n*−6, 18:3*n−6*, 20:2*n*−6, 20:3*n*−6, 20:4*n*−6 and 22:4*n*−6.
Σ *n*−3 PUFA = sum of 18:3*n*−3, 20:5*n*−3, 22:5*n*−3 and 22:6*n*−3.
PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n*−6, 18:3*n−6*, 18:3*n*−3, 20:2*n*−6, 20:3*n*−6, 20:4*n*−6, 20:5*n*−3, 22:4*n*−6, 22:5*n*−3 and 22:6*n*−3)/(sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].
n−6/*n−3* = *n−6*/*n−3* ratio [(sum of 18:2*n*−6, 18:3*n−6*, 20:2*n*−6, 20:3*n*−6, 20:4*n*−6 and 22:4*n*−6)/(sum of 18:3*n*−3, 20:5*n*−3, 22:5*n*−3 and 22:6*n*−3)].
[A] Statistical probability of treatment: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; means in the same row with different superscripts are significantly different ($P < 0.05$); SEM, standard error of mean.

Table 4
True selected nutrients and fatty acid retention values (%) for cooked samples of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments			SEM	P^A
	Boiling	Microwaving	Grilling		
Selected nutrients					
Moisture	49.21[a]	41.79[b]	56.13[c]	0.706	***
Total lipids	106.60[a]	121.69[b]	120.89[b]	3.987	***
Fatty acids					
10:0	93.54[a]	108.38[b]	99.60[ab]	6.193	**
12:0	95.88[a]	133.85[b]	136.28[b]	10.764	***
14:0	129.33	150.87	157.46	14.116	ns
14:1*c*9	125.50	141.36	167.99	18.814	ns
15:0	124.06	144.74	146.27	12.658	ns
16:0	116.15[a]	133.36[b]	135.22[b]	6.940	*
16:1*c*9	124.95	143.59	152.33	13.348	ns
17:0	128.35	151.97	148.73	11.887	ns
17:1*c*9	101.31[a]	113.14[b]	114.90[b]	5.784	*
18:0	118.10[a]	140.34[b]	129.91[ab]	7.428	**
18:1*t*6 + *t*8	118.91	143.06	140.77	12.284	ns
18:1*t*9	114.79	137.76	135.16	12.392	ns
18:1*t*10	126.82	146.44	151.75	15.088	ns
18:1*t*11	124.36	147.04	144.92	11.910	ns
18:1*t*12	109.64	131.48	127.76	9.776	ns
18:1*c*9	121.50[a]	140.02[b]	142.71[b]	9.076	*
18:1*c*11	102.65[a]	99.91[a]	115.92[b]	6.640	**
18:1*c*12	106.37[a]	124.30[b]	124.41[b]	10.185	*
18:1*c*13	120.33	139.95	150.49	18.535	ns
18:1*c*15	111.98	140.53	132.60	18.215	ns
18:2*t*,*t* + *c*/*t*[B]	91.68	106.63	107.86	8.910	ns
18:2*t*11,*c*15	132.87	162.48	149.11	18.352	ns
18:2*n*−6	85.71	99.41	90.48	6.746	ns
18:3*n*−6	87.14[a]	109.25[b]	94.61[ab]	7.878	*
18:3*n*−3	89.83[a]	109.13[b]	99.90[ab]	4.908	**
20:0	117.61[a]	139.77[b]	128.73[ab]	7.858	*
20:1*c*11	105.28[a]	125.96[b]	120.64[b]	7.623	**
20:2*n*−6	86.53	97.76	90.07	7.078	ns
20:3*n*−6	84.38	91.38	85.37	8.964	ns
20:4*n*−6	85.19	90.24	83.39	9.731	ns
20:5*n*−3	72.24	95.00	98.29	10.606	ns
22:4*n*−6	92.73	108.62	95.36	10.132	ns
22:5*n*−3	78.83	90.89	88.09	9.174	ns
22:6*n*−3	90.76	104.55	94.77	9.688	ns
Partial sums					
∑ SFA	117.90[a]	137.69[b]	134.38[b]	7.347	*
∑ MUFA	119.26[a]	137.39[b]	140.50[b]	8.707	*
∑ TFA	118.52	141.03	142.68	11.307	ns
∑ PUFA	86.04	99.71	90.61	7.605	ns
∑ *n*−6 PUFA	85.33	95.17	89.57	7.286	ns
∑ *n*−3 PUFA	86.80	102.46	95.89	7.794	ns

[A] Statistical probability of treatment: 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.
[B] This peak may include several 18:2 *t*,*t*, *c*,*t* and *t*,*c* isomers.

of lipids from the cooked tissues. Meanwhile, within some individual *n*−6 and *n*−3 PUFA, the TR values were below or around 100%, suggesting a little loss or degradation of these FA during cooking. Gandemer (1992), quoted by Maranesi et al. (2005), also reported TR values lower than 100% for C20 and C22 PUFA, which was explained by the oxidative degradation of FA.

3.3. Intramuscular CLA isomeric profile

The effects of cooking on the CLA contents and its isomeric distribution in intramuscular fat of beef are displayed in Table 5. Total CLA content (mg/g muscle) was significantly higher ($P < 0.001$) in cooked beef than in raw cuts as a result of moisture loss. In fact, the mean values of total CLA in raw beef mounted to 0.05 mg/g muscle. This value was increased after cooking up to 0.08 mg/g for grilling or 0.09 mg/g for boiling and microwave heating. Previous studies also reported higher values of CLA in cooked beef when compared with uncooked ground beef (Shanta, Crum, & Decker, 1994). Regardless of the cooking method employed, the heating methods with higher internal temperatures had the highest CLA concentrations, probably due to the higher cooking losses. However, if CLA content is expressed on a basis of mg/g fat, no significant differences ($P > 0.05$) were observed between raw and cooked meats.

The CLA isomeric profile showed a clear predominance of the bioactive *c*9,*t*11 isomer in all treatments (67.2–67.4% of total CLA), followed by the *t*11,*t*13 (7.5–8.5%) and *t*7,*c*9 (6.3–6.9%) isomers. The *t*11,*t*13 isomer was the second most predominant CLA isomer, instead of the *t*11,*c*13 isomer in meat from pasture-fed animals, or the *t*7,*c*9 isomer in meat from concentrate-fed bulls (Dannenberger et al., 2005). This CLA isomeric pattern may result from the fact that a heterogeneous group of animals was used in this trial. We showed before that pasture feeding increases the proportion of the *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 CLA isomers and decreases the percentage of the *t*7,*c*9 isomer in beef lipids, when compared with concentrate feeding (Alfaia et al., 2009). Although CLA has been described to be more sensitive than 18:2*n*−6 to oxidation

Table 5
Effect of cooking methods on total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Total CLA	0.05[a]	0.09[b]	0.09[b]	0.08[c]	0.007	***
Specific CLA	3.59	3.95	3.53	3.45	0.254	ns
CLA isomers						
*t*12,*t*14	2.90	3.36	3.58	3.53	0.775	ns
*t*11,*t*13	7.45	8.19	8.48	8.03	1.694	ns
*t*10,*t*12	0.63	0.61	0.59	0.64	0.074	ns
*t*9,*t*11	4.31[a]	3.78[b]	3.44[b]	3.73[b]	0.213	*
*t*8,*t*10	0.26	0.26	0.27	0.25	0.019	ns
*t*7,*t*9	0.92[a]	0.73[b]	0.66[b]	0.71[b]	0.044	***
*t*6,*t*8	0.21	0.19	0.23	0.19	0.022	ns
total *t*,*t*	16.69	17.11	17.25	17.08	2.454	ns
c/*t*12,14	0.94	0.90	0.97	0.97	0.099	ns
*t*11,*c*13	4.48	4.90	4.87	4.51	0.836	ns
*c*11,*t*13	0.75[a]	0.52[b]	0.55[b]	0.52[b]	0.130	*
*t*10,*c*12	1.12	1.08	1.19	1.13	0.154	ns
*c*9,*t*11	67.35	67.34	67.22	67.23	2.359	ns
*t*8,*c*10	1.18	1.47	1.46	1.18	0.135	ns
*t*7,*c*9	6.91	6.26	6.29	6.88	1.003	ns
total *c*/*t*	82.72	82.38	82.55	82.49	2.406	ns
total *c*,*c* (*c*9,*c*11)	0.42	0.49	0.45	0.47	0.059	ns

[A] Statistical probability of treatment: ns, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$; means in the same row with different superscripts are significantly different ($P < 0.05$); SEM, standard error of mean.

and even to isomerization during heat treatments (Yang, Leung, Huang, & Chen, 2000), minor changes in the CLA isomeric profile of beef were observed as a result of cooking. In fact, only 3 (*t*7,*t*9, $P < 0.001$; *t*9,*t*11 and *c*11,*t*13, $P < 0.05$) of the 15 CLA isomers identified in beef were changed ($P < 0.05$), when subjected to the three cooking methods. No changes were identified after cooking in the relative proportions of the bioactive *c*9,*t*11 CLA isomer, in contrast to its precursor in the muscle, the 18:1*t*11. Likewise, the other known bioactive CLA isomer, *t*10,*c*12, was not influenced by heating treatments ($P > 0.05$). The percentage of this isomer in meat, which was residual in all treatments, ranged between 1.1% and 1.2% of total CLA. In addition, no significant effect ($P > 0.05$) in total c/*t* (*c*,*t* and *t*,*c*), total *c*,*c*, as well as in total *t*,*t* CLA isomers, were apparent.

After boiling, the CLA isomeric distribution expressed as mg/g fat (data not shown) varied significantly ($P < 0.05$) for total *t*,*t*, *c*/*t and c*,*c* isomers. The individual percentages of *t*12,*t*14 and *c*9,*t*11 CLA isomers increased significantly ($P < 0.05$) after boiling but had no changes with microwaving or grilling. For *t*7,*t*9 and *c*11,*t*13 CLA isomers, the content decreased from raw to cooked cuts, independently to the treatment used. Moreover, the *t*9,*t*11 CLA isomer amounts also decreased significantly ($P < 0.05$) after microwaving and grilling. From a nutritional point of view, CLA content, expressed as mg/100 g muscle, has the greatest importance. As expected, significant increases ($P < 0.05$) in all CLA isomers were observed, thanks to the higher average lipid contents of cooked meat. In order to discriminate if these increases are due to a simple concentration effect, the TR values for the CLA isomers were calculated (Table 6). The TR values obtained for total CLA in this study, which were in the range 112.1–117.5%, are in line with the TR values obtained for total lipids (see Table 4). This observation is confirmed by the similar specific CLA contents, expressed as mg/g fat, observed between raw and cooked meats. Regarding the individual CLA isomers, no significant differences in TR values were observed among treatments, with the exception of *c*9,*c*11 isomer, which represented the only *c*,*c* CLA isomer detected. The *c*9,*c*11 TR values obtained with microwave cooking, with higher internal temperature, was lower ($P < 0.05$) when compared with the values observed for boiling and grilling, that presented lower internal temperatures. The differences in TR values found for this isomer could be attributed to the distinct oxidative stability of the various geometric groups of CLA isomers. It was previously shown that the stability of CLA isomers is determined by their *cis* or *trans* configuration but not by the position of their double bonds (Yang et al., 2000). According to the same authors, *c*,*c* CLA isomers are relatively more susceptible to oxidative degradation, than *c*/*t* or *t*,*t* isomers, as a result of the higher vulnerability to oxygen attack of the *cis* double bond relative to the *trans* configuration.

3.4. Lipid oxidation

The oxidation of lipids is one of the most important changes during food storage and processing. It depends on the PUFA content, as well on the balance between anti- and pro-oxidant compounds (Nuernberg et al., 2006). The determination of TBARS is

Table 6
True CLA retention values (%) for cooked samples of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments			SEM	P^A
	Boiling	Microwaving	Grilling		
Total CLA	116.86	112.11	117.48	4.255	ns
CLA isomers					
*t*12,*t*14	119.15	126.59	124.15	7.845	ns
*t*11,*t*13	121.67	122.01	119.34	5.506	ns
*t*10,*t*12	114.98	109.27	117.77	7.594	ns
*t*9,*t*11	101.95	89.77	102.46	5.395	ns
*t*8,*t*10	109.14	106.35	103.74	7.016	ns
*t*7,*t*9	92.74	81.13	85.37	6.808	ns
*t*6,*t*8	92.92	106.19	88.86	9.508	ns
Total *t*,*t*	113.88	109.18	114.55	4.942	ns
c/*t*12,14	112.25	116.26	122.77	7.194	ns
*t*11,*c*13	120.80	122.44	111.42	7.918	ns
*c*11,*t*13	105.35	90.84	87.47	7.004	ns
*t*10,*c*12	102.14	113.13	115.44	7.891	ns
*c*9,*t*11	116.75	112.01	117.50	5.598	ns
*t*8,*c*10	124.37	116.41	114.72	6.759	ns
*t*7,*c*9	118.19	110.48	120.07	7.267	ns
Total *c*/*t*	115.86	111.12	116.65	4.781	ns
Total *c*,*c* (*c*9,*c*11)	128.36[a]	115.73[b]	126.65[a]	7.003	*

[A] Statistical probability of treatment: ns, $P > 0.05$; *, $P < 0.05$; means in the same row with different superscripts are significantly different ($P < 0.05$); SEM, standard error of mean.

widely used as an index of lipid oxidation. The effect of the cooking methods used in this study on lipid oxidation is also showed in Table 3. TBARS in raw and cooked beef cuts immediately after treatments were very low, ranging from 0.06 to 0.10 mg MDA/kg meat, which is much below than 1 mg/kg meat, the threshold for off-flavour development (Tims & Watts, 1958, quoted by Jahan, Paterson, & Spickett, 2004). In addition, the increase of TBARS in beef was only significant ($P < 0.05$) for boiling, when compared with the other cooking methods and raw meat. Similar results were found by Dal Bosco et al. (2001), who reported higher TBARS for boiled rabbit meat relative to fried and roasted samples. Although cooking meat causes the disorganization of cell structures, leading to PUFA and pro-oxidant interactions with the development of lipid oxidation (Rhee, 1988), the results presented here showed that the cooking methods used induce only slight oxidative changes. Comparison with literature data is difficult due to the paucity of trials conducted under the same experimental conditions. However, our results agree with the TBARS (0 min after stimulation) in raw and grilled pork, found by Nuernberg et al. (2006), also with high levels of PUFA. Guillevic, Kouba, and Mourot (2009) reported similar values of TBARS at 0 min (<0.10 mg MDA/kg of chop) in chops of pigs fed a control diet and with linseed diet (with high content of PUFA). It is well known that factors associated with thermal treatment (cooking method, rate and final temperature), as well as with meat composition, such as the amount and type of lipids or antioxidants, should be taking into account for the cooking effects on the rate and extent of lipid oxidation (Dal Bosco et al., 2001). In addition, it is also well established that the dietary fat source affects fatty acid composition and influences the oxidative stability of meat (Bou et al., 2001).

4. Conclusion

Taken together the results presented here indicate that beef from pasture-fed bulls, enriched with PUFA and CLA, is not more susceptible to cooking induced changes of FA than that from concentrate-fed bulls. Cooking losses seem to increase directly with the cooking time and internal temperature reached by meat, with microwave processing having the higher moisture loss and, consequently, a greater intramuscular fat content. However, all the household cooking methods studied (boiling, microwaving and grilling) seem to increase the percentages of SFA (mainly 16:0 and 18:0) and MUFA (mainly 18:1*c*9), and decrease the relative proportions of PUFA (due to some $n-6$ and $n-3$ PUFA) in meat, in a similar way (16 of 34 FA seem to be affected). Regarding the nutritional fatty acid ratios, the data suggest that heating decreases the beef PUFA/SFA ratio but does not change its $n-6/n-3$ ratio, relative to raw meat. Total CLA content seems to be higher in cooked beef than in raw meat, as a result of the moisture loss and, thus, fat increase. However, minor changes in CLA profile of beef seem to occur as a result of cooking (only 3 of 15 isomers seem to be affected), with no variation of the relative proportions of the bioactive *c*9,*t*11 and *t*10,*c*12 isomers. In addition, the data also suggest that the TR values of individual FA (72.2–168.0%), including CLA isomers (81.1–128.4%), vary widely with all the cooking procedures studied. Finally, the data indicate that no significant oxidative changes occur immediately after treatment for microwaved and grilled meat, but a slight oxidative increase occurs for boiled meat.

Acknowledgments

Financial support (Grants AGRO/2003/512, POCTI/CVT/2002/44750, PTDC/CVT/2006/66114) and individual fellowships to Susana P. Alves (SFRH/BD/2007/37793) and Anabela F. Lopes (SFRH/BD/2007/35976) are acknowledged.

References

Alfaia, C. P. M., Ribeiro, V. S., Lourenço, M. A., Quaresma, M. A., Martins, S. I., Portugal, A. P., et al. (2006). Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbred bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications. *Meat Science, 72*, 425–436.

Alfaia, C. P. M., Alves, S. P., Martins, S. I. V., Fontes, C. M. G. A., Lemos, J. P. C., Bessa, R. J. B., et al. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry, 114*, 939–946.

AOAC 963.22. (2000). Methyl esters of fatty acids in oils and fats 963.22. In *Official methods of analysis* (17th ed., pp. 24–26). Gaithersburg, MA, USA: Association of Official Analytical Chemists International.

Badiani, A., Stipa, S., Bitossi, F., Gatta, P. P., Vignola, G., & Chizzolini, R. (2002). Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. *Meat Science, 60*, 169–186.

Badiani, A., Montellato, L., Bochicchio, D., Anfossi, P., Zanardi, E., & Maranesi, M. (2004). Selected nutrient contents, fatty acid composition, including conjugated linoleic acid, and retention values in separable lean from lamb rib loins as affected by external fat and cooking method. *Journal of Agricultural and Food Chemistry, 52*, 5187–5194.

Bessa, R. J., Santos-Silva, J., Ribeiro, J. M., & Portugal, A. V. (2000). Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livestock Production Science, 63*, 201–211.

Bessa, R. J. B., Alves, S. P., Jerónimo, E., Alfaia, C. M., Prates, J. A. M., & Santos-Silva, J. (2007). Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acid in lambs. *European Journal of Lipid Science and Technology, 109*(8), 868–878.

Bou, R., Guardiola, F., Grau, A., Grimpa, S., Manich, A., Barroeta, A., et al. (2001). Influence of dietary fat source, α-tocopherol, and ascorbic acid supplementation on sensory quality of dark chicken meat. *Poultry Science, 80*, 800–807.

Bragagnolo, N., & Rodriguez-Amaya, D. B. (2003). New data on the total lipid, cholesterol and fatty acid composition of raw and grilled beef *longissimus dorsi*. *Archivos Latinoamericanos de Nutrición, 53*(3), 312–319.

British Department of Health. (1994). Nutritional aspects of cardiovascular disease. *Report on health and social subjects No. 46*. London: HMSO.

Christie, W. W., Sébédio, J. L., & Juanéda, P. (2001). A practical guide to the analysis of conjugated linoleic acid (CLA). *INFORM, 12*, 147–152.

Dal Bosco, A., Castellini, C., & Bernardini, M. (2001). Nutritional quality of rabbit meat as affected by cooking procedure and dietary vitamin E. *Journal of Food Science, 66*, 1047–1051.

Dannenberger, D., Nuernberg, K., Nuernberg, G., Scollan, N., Steinhart, H., & Ender, K. (2005). Effect of pasture vs. concentrate diets on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids, 40*, 589–598.

Destaillats, F., & Angers, P. (2003). Directed sequential synthesis of conjugated linoleic acid isomers from d7, 9 to d12, 14. *European Journal of Lipid Science and Technology, 105*, 3–8.

Duckett, S. K., & Wagner, D. G. (1998). Effect of cooking on the fatty acid composition of beef intramuscular lipid. *Journal of Food Composition and Analysis, 11*, 357–362.

Echarte, M., Ansorena, D., & Astiasarán, I. (2003). Consequences of microwave heating and frying on the lipid fraction of chicken and beef patties. *Journal of Agricultural and Food Chemistry, 51*, 5941–5945.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., et al. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science, 78*, 2849–2855.

Fritsche, J., Fritsche, S., Solomon, M. B., Mossoba, M. M., Yurawecz, M. P., Morehouse, K., et al. (2000). Quantitative determination of conjugated linoleic acid isomers in beef fat. *European Journal of Lipid Science and Technology, 102*, 667–672.

García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (*M. pectoralis*). *Journal of Food Engineering, 80*, 813–821.

Geay, Y., Bauchart, D., Hocquette, J. F., & Culioli, J. (2001). Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. *Reproduction, Nutrition and Development, 41*, 1–26.

Gerber, N., Scheeder, M. R. L., & Wenk, C. (2009). The influence of cooking and fat trimming on the actual nutrient intake from meat. *Meat Science, 81*, 148–154.

Grau, A., Guardiola, F., Boatella, J., Barroeta, A., & Codony, R. (2000). Measurement of 2-thiobarbituric acid values in dark chicken meat through derivative spectrophotometry: influence of various parameters. *Journal of Agricultural Food Chemistry, 48*, 1155–1159.

Griinari, J. M., & Bauman, D. E. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In M. P. Yurawecz, M. Mossoba, J. K. Kramer, G. Nelson, & M. W. Pariza (Eds.), *Advances in conjugated linoleic acid research* (Vol. 1, pp. 180–200). Champaign, IL, USA: AOCS Press.

Guillevic, M., Kouba, M., & Mourot, J. (2009). Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of French fresh and cooked pork meats. *Meat Science, 81*, 612–618.

Harris, K. B., Harberson, T. J., Savell, J. W., Cross, H. R., & Smith, S. B. (1992). Influences of quality grade, external fat level, and degree of doneness on beef steak fatty acids. *Journal of Food Composition and Analysis, 5*, 84–89.

Jahan, K., Paterson, A., & Spickett, C. M. (2004). Fatty acid composition, antioxidants and lipid oxidation in chicken breasts from different production regimes. *International Journal of Food Science and Technology, 39*, 443–453.

Kramer, J. K. G., Sehat, N., Dugan, M. R., Mossoba, M. M., Yurawecz, M. P., Roach, J. A., et al. (1998). Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion higher-performance liquid chromatography. *Lipids, 33*(6), 549–558.

Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., Taylor, J. F., et al. (1999). Beef customer satisfaction: cooking method and degree of doneness effects on top loin steaks. *Journal of Animal Science, 77*, 637–644.

Maranesi, M., Bochicchio, D., Montellato, L., Zaghini, A., Pagliuca, G., & Badiani, A. (2005). Effect of microwave cooking or broiling on selected nutrient contents, fatty acid patterns and true retention values in separable lean from lamb rib-loins, with emphasis on conjugated linoleic acid. *Food Chemistry, 90*, 207–218.

Murphy, E. W., Criner, P. E., & Gray, B. C. (1975). Comparisons of methods for calculating retentions of nutrients in cooked foods. *Journal of Agricultural Food Chemistry, 23*, 1153–1157.

Nuernberg, K., Kuechenmeister, U., Nuernberg, G., Hartung, M., Dannenberger, D., & Ender, K. (2006). Effect of storage and grilling on fatty acids in muscle of pigs fed plant oils. *European Journal of Lipid Science and Technology, 108*, 554–560.

Park, Y., & Pariza, M. W. (2007). Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International, 40*(3), 311–323.

Prates, J. A. M., & Bessa, R. J. B. (2009). *Trans* and $n-3$ fatty acids. In L. M. L. Nollet & F. Toldrá (Eds.), *Handbook of Muscle Foods Analysis* (pp. 399–417). Boca Raton (Fl, EUA): CRC Press – Member of Francis & Taylor Group.

Raes, K., de Smet, S., & Demeyer, D. (2001). Effect of double-muscling in Belgiam Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. *Animal Science, 73*, 253–260.

Rhee, K. S. (1988). Enzymic and non-enzymic catalysis of lipid oxidation in muscle foods. *Food Technology, 42*, 127–132.

Rodriguez-Estrada, M. T., Penazzi, G., Caboni, M. F., Bertacco, G., & Lercker, G. (1997). Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Science, 45*(3), 365–375.

Roy, A., Chardigny, J. M., Bauchart, D., Ferlay, A., Lorenz, S., Durand, D., et al. (2007). Butters rich either in *trans*-10-18:1 or in *trans*-11-C18:1 plus *cis*-9, *trans*-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. *Animal, 1*(3), 467–476.

Sarriés, M. V., Murray, B. E., Moloney, A. P., Troy, D., & Beriain, M. J. (2009). The effect of cooking on the fatty acid composition of longissimus muscle from beef heifers fed rations designed to increase the concentration of conjugated linoleic acid in tissue. *Meat Science, 81*(2), 307–312.

Savell, J. W., Lorenzen, C. L., Neely, T. R., Miller, R. K., Tatum, J. D., Wise, J. W., et al. (1999). Beef customer satisfaction: Cooking method and degree of doneness effects on the top sirloin steak. *Journal of Animal Science, 77*, 645–652.

Scheeder, M. R. L., Casutt, M. M., Roulin, M., Escher, F., Dufey, P.-A., & Kreuzer, M. (2001). Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. *Meat Science, 58*, 321–328.

Sehat, N., Kramer, J. K., Mossoba, M. M., Roach, J. A., Yurawecz, M. P., Eulitz, K., et al. (1998). Identification of conjugated linoleic acid (CLA) isomers in cheese by gas chromatography, silver ion high performance liquid chromatography, and mass spectral reconstructed ion profiles: Comparison of chromatographic elution sequences. *Lipids, 33*, 963–971.

Serrano, A., Librelotto, J., Cofrades, S., Sánchez-Muniz, F. J., & Jiménez-Colmenero, F. (2007). Composition and physicochemical characteristics of restructured beef steaks containing walnuts as affected by cooking method. *Meat Science, 77*(3), 304–313.

Schmid, A., Collomb, M., Sieber, R., & Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. *Meat Science, 73*, 29–41.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition, 85*, 115–124.

Shanta, N. C., Crum, A. D., & Decker, E. A. (1994). Evaluation of conjugated linoleic acid concentrations in cooked beef. *Journal of Agricultural and Food Chemistry, 42*, 1757–1760.

Sheard, P. R., Nute, G. R., & Chappell, A. G. (1998). The effect of cooking on the chemical composition of meat products with special reference to fat loss. *Meat Science, 49*(2), 175–191.

Smith, D. R., Savell, J. W., Smith, S. B., & Cross, H. R. (1989). Fatty acid and proximate composition of raw and cooked retail cuts of beef trimmed to different external fat levels. *Meat Science, 26*(4), 295–311.

Statistical Analysis System. (2004). *SAS/STAT 9.1 user's guide*. Cary, NC, USA: SAS Institute Inc.

Wahle, K. W. J., Heys, S. D., & Rotondo, D. (2004). Conjugated linoleic acids: Are they beneficial or detrimental to health? *Progress in Lipid Research, 43*, 553–587.

Wahrmund-Wyle, J. L., Harris, K. B., & Savell, J. W. (2000). Beef retail cut composition: 2. Proximate analysis. *Journal of Food Composition and Analysis, 13*, 243–251.

Weihrauch, J. L., Posati, L. P., Anderson, B. A., & Exler, J. (1977). Lipid conversion factors for calculating fatty acid contents of foods. *Journal of the American Oil Chemists' Society, 54*, 36–40.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., et al. (2004). Effects of fatty acids on meat quality: A review. *Meat Science, 66*(1), 21–32.

Woolsey, A. P., & Paul, P. C. (1969). External fat cover influence on raw and cooked beef. 1. Fat and moisture content. *Journal of Food Science, 34*, 554–557.

WHO, World Health Organization (2003). *Diet, nutrition and the prevention of chronic diseases*. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series n 916. Geneva: World Health Organization.

Yang, L., Leung, L. K., Huang, Y., & Chen, Z. Y. (2000). Oxidative stability of conjugated linoleic acid isomers. *Journal of Agricultural and Food Chemistry, 48*(8), 3072–3076.

Yu, L. K., Adams, D., & Gabel, M. (2002). Conjugated linoleic acid isomers differ in their free radical scavenging properties. *Journal of Agricultural and Food Chemistry, 50*(14), 4135–4140.