Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 207–218
Food Chemistry
www.elsevier.com/locate/foodchem

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

M. Maranesi [a], D. Bochicchio [b], L. Montellato [b], A. Zaghini [c], G. Pagliuca [c], A. Badiani [b,*]

[a] *Dipartimento di Biochimica "G. Moruzzi", Alma Mater Studiorum – Università di Bologna, Via Irnerio 48, I-40126 Bologna, Italy*
[b] *Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Alma Mater Studiorum – Università di Bologna, Via Tolara di Sopra 50, I-40064 Ozzano Emilia (BO), Italy*
[c] *Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Alma Mater Studiorum – Università di Bologna, Via Tolara di Sopra 50, I-40064 Ozzano Emilia (BO), Italy*

Received 17 November 2003; received in revised form 26 March 2004; accepted 26 March 2004

Abstract

Proximate composition and fatty acid profiles, conjugated linoleic acid (CLA) isomers included, were determined in separable lean of raw and cooked lamb rib-loins with their subcutaneous and intermuscular fat, prepared as roasts or steaks. Two combinations "cooking method × type of cut" were selected: one is a traditional method for this meat (broiling of steaks), while the other (microwaving followed by final grilling of roasts) is far less widely used. The two methods, similar as regards the short preparation time involved, were also evaluated for cooking yields and true nutrient retention values. The cooking yield in microwaving was markedly higher than in broiling. Significant differences between the two methods were also found in the true retention values of moisture, protein and several fatty acids, again to the advantage of microwaving. On the basis of the retention values obtained, with microwaving there was a minimum migration of lipids into the separable lean, consisting almost exclusively of saturated and monounsaturated fatty acids, while there were small losses of lipids in broiling, almost equally divided between saturated, monounsaturated and ω6 polyunsaturated fatty acids. On the whole, the response to cooking of the class of CLA isomers (including the nutritionally most important isomer *cis*-9,*trans*-11) was more similar to that of the monounsaturated than the polyunsaturated fatty acids.

Keywords: Lamb; Fatty acid composition; Cooking methods; True retention values; Conjugated linoleic acid

1. Introduction

Lamb and goat meat, as well as small ruminant edible products (e.g., cheese and yogurt), are traditional food items in many countries in the Mediterranean basin (Capatti & Montanari, 1999; Noah & Truswell, 2001; Simopoulos & Sidossis, 2000). In Italy, lamb consumption is predominantly regional, meaning a low market penetration (Turrini, Saba, Perrone, Cialfa, & D'Amicis, 2001). Even where the consumption of lamb is customary, it is increasingly regarded as being fatty and therefore unhealthy, wasteful, as well as expensive and time-consuming to prepare (Corcoran et al., 2001). This means that no increase in consumption can be envisaged and yet it has emerged recently that dairy products and muscle foods of ruminant, especially ovine, origin have the highest concentrations of conjugated linoleic acid (CLA) (Chin, Liu, Storkson, Ha, & Pariza, 1992;

* Corresponding author. Tel.: +39-051-2097380; fax: +39-051-2097373.
E-mail address: badiani@vet.unibo.it (A. Badiani).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.043

Fogerty, Ford, & Svoronos, 1988; Fritsche & Steinhart, 1998; Prandini et al., 2001; Zlatanos, Laskaridis, Feist, & Sagredos, 2002), a lipid microcomponent of great interest to nutritionists, so much so that it has justified the recent inclusion of foods of ruminant origin in the list of functional foods (Hasler, 2002).

The acronym CLA refers to a mixture of positional and geometric conjugated dienoic isomers of linoleic acid formed both as intermediates during the reticulo-rumen biohydrogenation of linoleic acid and through the endogenous desaturation of *trans*-11 octadecenoic acid (Griinari & Bauman, 1999). In animal models, CLA has been shown to exhibit anti-carcinogenic, anti-thrombotic, anti-atherogenic and immune modulator properties, as well as to alter body and bone metabolism – in short, pleiotropic effects that, not surprisingly, have been studied for quite some time with special attention to human health (Parodi, 2002). The *cis*-9,*trans*-11 (*c*9,*t*11) isomer (aptly named rumenic acid) is the main CLA occurring naturally in foodstuffs. This isomer, together with *trans*-10,*cis*-12 (*t*10,*c*12), is considered to be biologically active, although research has recently been extended to include minor isomers *trans*-9,*trans*-11 (*t*9,*t*11) and *cis*-9,*cis*-11 (*c*9,*c*11) (Martin & Valeille, 2002). While humans also seem to be able to partially convert dietary *trans*-11 octadecenoic acid to CLA, its main origin is thought to be dietary (Adlof, Duval, & Emken, 2000).

Once it is made known through public nutritional awareness campaigns concerning cooked meat, the enhanced value that CLA confers on lamb may help consumption to increase. At present, however, information is lacking on the response to cooking by CLA in this meat, both as a whole, and as selected isomers. Furthermore, very few works have considered whether and how the fatty acid composition of this meat's intramuscular fat is affected by cooking (Badiani et al., 1998; Hoke, Buege, Ellefson, & Maly, 1999). Lastly, no direct scientific evidence is available concerning the true retention values of fatty acids in general, and selected CLA isomers specifically, contained in lamb. Indeed, knowledge of these coefficients combined with an awareness of the cooking yields may prove extremely useful in the frequent, cost-effective updating of food composition tables and databases, especially in regard to nutrients of more complex determination (Schakel, Buzzard, & Gebhardt, 1997). Consequently, this study was conducted to determine the effect on the content and retention values of fatty acids and other nutrients produced in lamb by two markedly different cooking techniques (microwaving followed by grilling, and broiling), when applied to paired multiple-muscled cuts, traditionally with the fat cover on, and prepared either as roasts (for microwaving) or steaks (for broiling), both methods having been chosen because of their quickness and high practicality.

2. Materials and methods

2.1. Animals and cuts

The study was conducted only on lamb carcasses issuing from the integrated production system "Qualità Controllata" (QC), a quality assurance scheme founded on a voluntary participation, which was established in 1992 by the Emilia-Romagna Region (North-eastern Italy) for several animal and plant foods, and extended since 1997 to include the meat from heavy lambs (slaughter weight 25–50 kg) and wethers (slaughter weight 40–100 kg) (CRPA, 1997). Eight QC heavy lamb carcasses (sex ratio 1:1) were obtained from an exclusive retailer during the summer months. The lambs came from four farms and were intentionally selected to equally represent both the major meat breeds and/or crosses (Appenninica, Biellese, Biellese × Suffolk and Ile de France × Suffolk), and the most common diets adopted under the QC scheme, based on fodder (mainly lucerne hay) and concentrates (either commercial concentrates, or a mixture of maize, barley and faba bean seeds in varying proportions), to attain a growth rate between 200 and 250 g/day. The animals ranged from 4 to 5 months of age; their average slaughter weight, dressing percentage and hot carcass weight (±standard error, SE) were 41.5 ± 1.78 kg, $53.2 \pm 1.91\%$ and 21.9 ± 0.56 kg, respectively. The carcasses, mostly classified as U3 for conformation and fat cover according to the European SEUROP classification scale (EEC, 1992, 1993), were conditioned at ambient temperature for about 4 h prior to chilling at 4 °C. At 48 h post mortem the chilled carcasses were split into halves along the midline and fabricated into retail cuts. The rib-loin, i.e., *costolette* + *lombata* according to the Italian pattern of lamb cutting as illustrated by Swatland (2000), was retained from both sides. Ultimate pH measurements of *M. longissimus thoracis et lumborum* were taken in duplicate at both ends of each rib-loin using a benchtop pH meter (Mod. 420A, Orion Europe, Cambridge, UK) fitted with a spear-type gel electrode (Mod. 52-32, Crison Instruments S.A., Barcelona, Spain) and an ATC temperature probe. The range of pH values obtained (5.48–5.81) ruled out any effects of dark, firm and dry (DFD) meat on muscle composition. All rib-loins were deboned, but left untrimmed of surface adipose tissue. Two thick cross-sectional slices were taken from both the cranial and caudal ends of each cut and were retained as its composite raw reference. The cuts to be microwaved (MW) or broiled came alternately from the left and right side. Each roast intended for MW (average weight ± SE = 1420 ± 129 g) was rolled and wrapped in an elastic netting. The anatomically matched cut (i.e., from the opposite side of the same carcass), intended for broiling, was divided into 11 steaks, approximately 2.5 cm in thickness (overall average weight ± SE = 126 ± 12

g), each of which was fastened with a toothpick. Bilateral symmetry was assumed.

2.2. Cooking and preparation of samples

MW cooking was performed on a ceramic rack in a Pyrex® pan placed in the centre of the carousel (rotary hearth) of a 2450 MHz, 1000 W variable power oven (Mod. MT 243/486, Whirlpool Europe, S.r.l., Comerio, Italy), so as to prevent the roasts from simmering in their own juices. On average, the power control was set at 750 W for the first 9 min/kg weight, then at 350 W for the following 6 min/kg and finally at 350 W + grill for the last 6 min/kg. Such a cooking procedure was developed during preliminary testing to attain a final core temperature of 75 °C (medium, according to Matthews & Garrison, 1975), as checked with a digital thermometer upon removal from the oven. All MW roasts were allowed an uncovered 20-min standing time after cooking. Broiling was performed using a large preheated (200 °C) electric grill (Mod. Barby Q, De'Longhi, Treviso. Italy). The 11 steaks obtained from each rib-loin were arranged on the turnable rack of the grill, with the upper surface of the meat about 6 cm above the heating elements, turned every 2 min and broiled to an internal temperature of 75 °C as checked with a digital thermometer; the overall cooking time was 14 min/side on average. The total cooking time (min) and post cooking rise (°C) were recorded and the heating rate (°C/min) was calculated for each cooking method. Evaporative loss, drip and total cooking losses (% initial raw mass) were determined according to AMSA (1995), as modified by Badiani et al. (2002). Weights of cuts were recorded to the nearest 1 g on a Bel Engineering electronic balance (Mod. Mark 4520, Monza, Italy). Each cooked roast or set of steaks and its raw reference were trimmed of fat cover and surface browning, if any, intermuscular (seam) fat and heavy epimysial connective tissue, to obtain the lean plus intramuscular fat (marbling), i.e., the separable lean, intended to approximate the edible portion of the cut freed of as much fat as possible. The separable lean, made up of *M. longissimus thoracis et lumborum*, the adjacent small muscles and part of the abdominal muscles, was diced, finely ground in three 10 s bursts with an Ovatio 2 food processor (Moulinex, S.p.A., Milan, Italy) and thoroughly mixed between grindings.

2.3. Proximate composition and energy value

The moisture, Kjeldahl nitrogen and ash contents of the homogenised meat samples were determined using the AOAC Methods 950.46 B, 981.10 and 920.153, respectively (AOAC, 2000). Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor. Total lipids were extracted from 10 g of each sample by means of the chloroform:methanol (2:1, v/v) method of Folch, Lees, and Sloane-Stanley (1957), as modified by Michaelsen, Johnson, West, and Leak (1991) and measured gravimetrically. Energy value (kcal) was derived by multiplying the amount of protein and fat by the conversion factors 4 and 9, respectively (Chan, Brown, Lee, & Buss, 1995).

2.4. Fatty acid analysis

2.4.1. Preparation of methyl esters

An aliquot of the fat extract was transferred to a screw-cap test tube and stored at −80 °C until all samples could be analysed for fatty acid and CLA profiles. To determine the fatty acid composition, 100 mg of the extracted lipid was dissolved in 2 ml of hexane and added with 50 μg of heneicosanoic acid (C21:0, Catalogue no. H5149, Sigma-Aldrich, Corp., St. Louis, MO) as an internal standard. An alkaline *trans*esterification was performed using sodium methoxide in anhydrous methanol (15 min at 55 °C), as suggested by Shantha, Decker, and Hennig (1993) to avoid isomerisation of CLA isomers. Both gas chromatography (GC) and silver-ion impregnated high-performance liquid chromatography (Ag^+-HPLC) were carried out on the same methyl ester solution.

2.4.2. GC analysis

A HRGC 8560 Series Mega 2 gas chromatograph (Fisons Instruments, Milano, Italy) equipped with a flame ionisation detector and an automatic injection system (S 800, Fisons Instruments) was used. The column was a SP-2380 fused-silica capillary column (60 m × 0.32 mm i.d., phase thickness 0.20 μm; Catalogue no. 24117, Supelco, Inc., Bellefonte, PA), injected in the split mode with a split ratio of 1:50. Helium was selected as carrier gas at a flow rate of 1.5 ml/min. Both injector and detector temperatures were set at 250 °C. The initial oven temperature was 140 °C, immediately raised by 4 °C/min to 170 °C, followed by a gradient of 1 °C/min to 185 °C, further raised by 4 °C/min to 230 °C and held for 10 min, for a total run time of 44 min. Chrom-Card software for Windows (Version 1.21, 1998, CE Instruments, Milan, Italy) was used for data analysis. Identification was accomplished by comparing the retention time of unknown fatty acid methyl esters (FAME) with those of known FAME standard mixtures (Alltech Associated, Inc., Deerfield, IL; Sigma–Aldrich, Corp.) to which a mixture of CLA methyl esters (*t*9,*t*11; *c*9,*t*11; *c*9,*c*11 and *t*10,*c*12, Catalogue no. 1257, 1258, 1256 and 1254, resp.; Matreya, Inc., Pleasant Gap, PA) had been added. Quantification of methyl esters was based on heneicosanoic acid as an internal standard and on relative peak areas of the fatty acids. The CLA region of the GC chromatogram, which was free of other fatty acids, contained a single peak, occurring between

linolenic acid (C18:3 ω3) and heneicosanoic acid. Since it was most probably the sum of several unresolved CLA isomers (Christie, 2002), it was designated as "total CLA".

2.4.3. Ag^+-HPLC analysis

The CLA isomeric distribution was determined by Ag^+-HPLC with UV detection. The HPLC equipment consisted of a Beckman solvent delivery module 126, coupled to an autosampler Model 507 with a Rheodyne injector fitted with a 20 μl loop and a UV detector Model 166 set at 233 nm (Beckman, San Ramon, CA). The separation was obtained using a ChromSpher 5 Lipids analytical column (250 × 4.6 mm i.d., 5 μm particle size; Chrompack, Bridgewater, NJ) protected by a ChromGuard guard column (50 × 3 mm i.d., 5 μm particle size; Chrompack) and the mobile phase was 0.1% acetonitrile in hexane maintained at a flow rate of 1 ml/min, following the approach outlined by Sehat et al. (1998). The identification of CLA isomers was based on the retention time of the same mixture of CLA methyl esters used in GC analysis and on its coinjection with the sample, as suggested by Yurawecz and Morehouse (2001). HPLC chromatograms contained nine peaks. Three of them were identified as *t*9,*t*11, *c*9,*t*11 (although the coeluting presence of the minor *t*9,*c*11 could not be ruled out), and *c*9,*c*11. The isomer *t*10,*c*12 was not detected. The concentration of each Δ9,Δ11 isomer in lipid extracts was calculated based on its area relative to the area of all peaks, as follows:

$$\text{Concentration of the } \Delta 9, \Delta 11 \text{ isomer} = (\text{total CLA concentration determined by GC} \times \text{area\%})/100.$$

2.5. Nutrient retention values

True retention values (RVs) for all nutrients were calculated using the following formula (USDA, 2002a):

$$\text{RV (\%)} = [(\text{nutrient content per g of cooked food} \times \text{g of food after cooking}) / (\text{nutrient content per g of raw food} \times \text{g of food before cooking})] \times 100.$$

2.6. Analytical quality assurance

Analyses were carried out in duplicate and the reagents were of analytical or HPLC grade. The quality of the analytical results was controlled by analysing the standard reference material "Meat Homogenate" (SRM 1546; NIST, Gaithersburg, MD) for relevant nutrients. For each nutrient, double determinations were carried out five times during the project, following the analytical procedures used in this work. The means determined were always within the certified (or reference) intervals (Welch et al., 2001).

2.7. Statistical analysis

The arcsin transformation was used for proportion data before statistical analysis. Summary statistics (mean and SE of the mean) were computed for each dependent variable. Nutrient contents and retentions were evaluated by analysis of variance (repeated measures design) to test the significance of the effect of the cooking method on the nutrient composition of the separable lean. Means were separated at, or below, the 5% probability level using the Scheffé *post hoc* test. All statistical computation was performed using the Statistica® software package (Release 5, 1997; StatSoft, Inc., Tulsa, OK).

3. Results and discussion

3.1. Heat processing parameters and cooking losses

The cooking methods adopted were equally rapid (cooking times practically identical) but the remaining heat processing parameters (post cooking rise and heating rate) and cooking losses (total losses, as well as evaporative and drip losses) differed (Table 1). This was expected due to the differences between the two cuts as regards shape, surface/volume ratio, size and weight, spatial orientation during cooking of the fat cover, seam fat and intramuscular fat as well as the fibre bundles. In any event, the total losses observed for MW cooking and broiling were within the rather wide ranges provided by Chan et al. (1995) for microwaved and grilled lamb cuts (24–41% and 15–52%, respectively, medium degree of doneness).

The ratio of evaporative to drip losses was about 45:55 for MW cooking, whereas evaporative losses were far greater for broiling (around 97:3). In the case of MW cooking, presumably the evaporative/drip ratio was actually more in favour of drip losses because in all

Table 1
Heat processing parameters and cooking losses for microwaved or broiled lamb rib-loins

Trait[A]	Microwaving	Broiling	*P* value
Cooking time (min)	29 ± 3	28 ± 1	0.9999
Post cooking rise (°C)	9.3 ± 2.3	0	0.0047
Heating rate (°C/min)	2.62 ± 0.21	2.12 ± 0.04	0.0346
Total losses[B] (%)	27.1 ± 1.44	36.5 ± 0.64	0.000047
Evaporative loss (%)	12.3 ± 0.59	35.3 ± 0.64	0.000024
Drip loss (%)	14.8 ± 1.31	1.15 ± 0.07	0.000015

[A] Values represent means ± SE ($n = 8$).
[B] All cooking losses were expressed as a percentage of the initial raw mass.

probability final grilling caused evaporation of some of the cooking juices that collected in the pan. This was probably even more so for broiling, because the electric grill (chosen to resemble the home cooking technique as far as possible) prevented the direct measurement of the abundant juices (most of which were probably rendered subcutaneous and seam fat) produced while cooking, so they were considered evaporative losses.

3.2. Proximate composition and energy value

The separable lean of the cuts analysed in the raw state (Table 2) showed protein and ash contents well within the range of values (15.0–22.6% and 0.90–1.20%, respectively) taken for raw lamb rib-loin (or whenever unavailable, "generic" lamb lean) from food composition tables, data bases and compositional surveys published in several European countries (Chan et al., 1995; Favier, Ireland-Ripert, Toque, & Feinberg, 1995; Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, 2000; Souci, Fachmann, & Kraut, 2000), in the USA (National Live Stock & Meat Board, 1988; USDA, 2002b) and in Australia (Greenfield, Kuo, Hutchison, & Wills, 1987; Hoke et al., 1999). However, the current mean values obtained for the lipid content and energy value were almost at the lower end of the combined ranges from the above sources (2.70–16.5% and 106–209 kcal, respectively), whereas moisture was near the upper end (67.5–76.5%). Therefore, even though the samples used in this study also included the accessory muscles of *M. longissimus thoracis et lumborum*, the basic definition of "extra lean meat" as used in both the UK (Chan et al., 1995) and the USA (US-FDA, 1999) applied.

Both the MW cooking of roasts and the broiling of steaks induced a comparable and statistically significant decrease in lean moisture (Table 2). This was matched by both a significant increase in the protein and lipid content and a consequent rise in the energy value, without significant differences between the cooking techniques used. The composition of the MW cooked and broiled lean revealed a difference only in the ash content; the mean value obtained for MW cooking was identical to that found in the raw cut and significantly lower than that for broiling. Compared to those available for raw meat, there are fewer data sources for comparisons with cooked meat and they are mostly limited to grilled/broiled lamb cuts (Chan et al., 1995; Ensminger, Ensminger, Konlande, & Robson, 1994; Favier et al., 1995; Greenfield et al., 1987; Hoke et al., 1999; National Live Stock & Meat Board, 1988; USDA, 2002b). Only Chan et al. (1995) took MW cooking into consideration and only for chops. The current values obtained for the separable lean of broiled steaks and MW cooked roasts came within the combined ranges based on the above sources for moisture, protein and ash of grilled/broiled cuts (54.6–64.4%, 22.6–31.4% and 1.07–1.55%, respectively). Both cooking techniques resulted in a separable lean with a lipid content and energy value way below the ranges obtained from the literature (7.10–16.0% and 184–249 kcal, respectively) and where only 27–28% of the total energy came from lipids against 73–72% from proteins, confirming the considerable leanness of these cuts once ready for consumption.

Despite the considerably different cooking losses in the MW cooked and broiled cuts, the proximate composition and energy value of their lean were quite similar. This supports the earlier argument that a noticeable proportion of the total losses of the broiled steaks was due to the cover and seam fat rendering from the steaks during cooking. The very spatial orientation of the steaks during cooking makes it possible to rule out significant migrations of fat into the lean, whose increased lipid content in relation to the raw cut may be ascribed to a mere concentration of intramuscular fat. In addition, since there were no significant differences between the lipid content of the two cooked cuts, it may be assumed that such fat migration did not occur in the MW cooked roasts either, despite apparent evidence to the contrary in terms of localisation of the cover and seam fat in the cut being cooked (Jones, Savell, & Cross, 1992; Slover, Lanza, Thompson, Davis, & Merola, 1987). These assumptions were investigated further in light of the fatty acid composition of the lean and of the retentions obtained for the different nutrients under observation.

Table 2
Proximate composition and energy value for raw and cooked lamb rib-loins (g/100 g of lean, except where noted)

Nutrient[A,B]	Raw state	Microwaving	Broiling	*P* value
Moisture	76.0 ± 0.35a	63.8 ± 1.04b	62.3 ± 0.74b	0.000019
Protein	19.4 ± 0.24b	30.4 ± 0.72a	31.2 ± 0.41a	0.000021
Lipids	3.52 ± 0.30b	5.12 ± 0.51a	5.40 ± 0.59a	0.000017
Ash	1.04 ± 0.01b	1.04 ± 0.02b	1.20 ± 0.02a	0.000004
Energy (kcal)	109 ± 2b	168 ± 6a	173 ± 5a	0.000013

[A] Values represent means ± SE ($n = 8$).
[B] Mean values in the same row followed by different letters differ significantly ($P \leqslant 0.05$).

3.3. Fatty acid profile and nutritional implications

In normalised terms (i.e., each fatty acid as a percentage of total FAME), the most represented fatty acids in the lipids extracted from the raw lean, in descending order of concentration, were oleic, palmitic and stearic acids (C18:1 ω9, C16:0 and C18:0, respectively), followed at a considerable distance by linoleic and myristic acids (C18:2 ω6, or LA, and C14:0, respectively), totalling around 84.3% FAME (Table 3). The order of importance and levels of the main fatty acids, as well as the total CLA concentration in intramuscular fat of the raw lean observed in this study were

Table 3
Fatty acid composition (% fatty acid methyl esters) and health-related ratios for raw and cooked lamb rib-loins

Fatty acid[A,B]	Raw state	Microwaving	Broiling	*P* value
C14:0	3.49 ± 0.30b	3.78 ± 0.32a	3.69 ± 0.33ab	0.0039
C15:0	0.46 ± 0.02b	0.54 ± 0.02a	0.52 ± 0.02a	0.000001
C16:0	25.2 ± 0.39b	26.1 ± 0.56a	26.0 ± 0.38a	0.0230
C16:1 ω7	2.68 ± 0.33	2.82 ± 0.25	2.77 ± 0.29	0.7664
C17:0	0.74 ± 0.08	0.79 ± 0.09	0.78 ± 0.09	0.2272
C18:0	14.6 ± 0.81	14.4 ± 0.65	14.7 ± 0.73	0.6056
∑ C18:1 *trans*	2.94 ± 0.29b	3.42 ± 0.33a	3.55 ± 0.36a	0.000033
C18:1 ω9	35.0 ± 0.96	34.2 ± 0.86	34.2 ± 0.92	0.0555
C18:1 ω11	1.00 ± 0.09	1.07 ± 0.12	1.05 ± 0.12	0.1397
C18:2 ω6	5.88 ± 0.46a	5.58 ± 0.43ab	5.45 ± 0.42b	0.0232
C18:3 ω3	0.48 ± 0.06	0.46 ± 0.06	0.46 ± 0.07	0.0539
Total CLA[C]	0.46 ± 0.02	0.49 ± 0.03	0.47 ± 0.04	0.4254
C20:4 ω6	1.60 ± 0.15a	1.45 ± 0.15b	1.44 ± 0.13b	0.0366
C22:4 ω6	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.4756
C22:6 ω3	0.30 ± 0.03	0.26 ± 0.03	0.26 ± 0.04	0.0627
Unidentified Sum	4.90 ± 0.40	4.53 ± 0.32	4.46 ± 0.32	0.4349
∑ SFA[D]	44.6 ± 0.65	45.5 ± 0.68	45.7 ± 0.70	0.0840
∑ MUFA[D]	41.7 ± 1.04	41.6 ± 0.89	41.6 ± 1.12	0.9747
∑ PUFA[D]	8.86 ± 0.60a	8.36 ± 0.58ab	8.21 ± 0.55b	0.0192
∑ ω6 PUFA[E]	7.62 ± 0.62a	7.16 ± 0.59ab	7.01 ± 0.57b	0.0249
∑ ω3 PUFA[F]	0.78 ± 0.09a	0.72 ± 0.08b	0.72 ± 0.11b	0.0282
ω6/ω3	10.8 ± 1.39	10.9 ± 1.39	11.0 ± 1.43	0.4859
PUFA/SFA	0.20 ± 0.01a	0.18 ± 0.01b	0.18 ± 0.01b	0.0087

[A] Values represent means ± SE ($n = 8$).
[B] Mean values in the same row followed by different letters differ significantly ($P \leqslant 0.05$).
[C] Total CLA, sum of the conjugated linoleic acid isomers determined by GC.
[D] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids (CLA excluded).
[E] Sum of C18:2, C20:4, C22:4.
[F] Sum of C18:3, C22:6.

similar to those reported by others in lambs of different breeds and slaughter weights, fed with either forage and concentrates or concentrates alone (Badiani et al., 1998; Bolte, Hess, Means, Moss, & Rule, 2002; Sañudo et al., 2000; Wachira et al., 2002).

Both MW cooking and broiling modified the concentrations of some fatty acids in the cooked lean slightly but significantly compared to the uncooked samples (Table 3). Some saturated fatty acids (SFA) increased significantly, namely myristic (only for MW cooked meat), pentadecanoic (C15:0) and palmitic acids, as well as the sum of *trans* octadecenoic acids (∑ C18:1 *trans*). On the contrary, arachidonic acid (C20:4 ω6, or AA), ∑ ω3 PUFA and the PUFA/SFA ratio decreased significantly in both cooking techniques while LA, ∑ ω6 PUFA and ∑ PUFA decreased significantly only for broiled meat.

What happened to the fatty acids in the intramuscular fat may be accounted for considering that the drip losses were quite substantial in MW cooked roasts. These cuts were rolled up with cover fat towards the outer surface – so they were exposed to the action of fat rendering due to final grilling – and with intermuscular fat inwards, making it particularly sensitive to the action of the microwaves, since heat is generated within the food with this technique (Fox & Cameron, 1990). In apparent contrast to the assumption based on proximate composition, what was observed should lead to the conclusion that there was a slight fat migration into the cooked lean along with the drip losses, consequently imparting to the intramuscular fat a fatty acid composition which would suggest contributions from subcutaneous and intermuscular fat, well known to be richer in SFA and *trans* fatty acids, and poorer in PUFA (Bolte et al., 2002; Demeyer & Doreau, 1999; Enser, Hallett, Hewitt, Fursey, & Wood, 1996). As regards the steaks, it has already been argued that a substantial amount of their high cooking losses was due to the rendering of subcutaneous and intermuscular fat in the form of drip losses; on the basis of the figures in Table 3 it is assumed that part of what derived from the transected intramuscular depots could be added to this. It is likely that, besides producing the desired result of even broiling, the frequent turning of the steaks also caused them to self-baste to a certain extent; here as well, this modified the relative proportion of some fatty acids.

The quantitative fatty acid composition (i.e., fatty acid content, expressed in mg/100 g edible portion) was determined in both raw and cooked meat for the dual purpose of permitting observations of nutritional interest and calculating the true retention values of the fatty acids according to the cooking method applied (see

paragraph below). As expected, cooking produced significant increases in the contents of all fatty acids, but without any statistically significant differences between MW cooked meat and broiled meat (data not shown). After pooling the results of the two methods, the proportion of total energy derived from SFA, monounsaturated fatty acids (MUFA) and PUFA amounted to mean values of 11.4%, 10.5% and 2.04%, respectively; moreover, within MUFA, the energy fraction contributed by oleic acid was clearly predominant (around 82%). These aspects, combined with the modest lipid content of the meats (and therefore the modest contribution supplied by the lipids to total energy), confirmed the possibility of easy introduction into a diet formulated to combine nutrition benefits with practical preparation despite the drawback of the limited contribution made by PUFA in general, and by ω3 PUFA in particular (Eurodiet, 2000). Regardless of the cooking method employed, one serving (100 g) of this cooked meat was found to be capable of contributing an average value of 331 mg of ω6 PUFA, 259 mg of which derived from LA and 66 mg from AA. The mean levels of contribution of ω3 PUFA amounted to only 34 mg/100 g of cooked meat, of which 22 mg were supplied by α-linolenic acid (C18:3 ω3, or ALA) and 12 mg by docosahexaenoic acid (C22:6 ω3, or DHA). These latter values were a negligible contribution compared to the daily human requirements of ω3 PUFA, recently set at 2000 mg ALA + 200 mg very long chain ω3 fatty acids (Eurodiet, 2000). In any case, while it is rather unlikely that lamb would be the first choice among muscle foods for a consumer aiming at increasing his or her ω3 PUFA intake, it is still an important source of CLA.

The mean total CLA content provided by the raw lean examined in this study amounted to 14.8 mg/100 g edible portion. After cooking, the CLA content increased significantly but without a difference between the two cooking methods, rising to a mean total value of 23.2 mg/100 g. Comparison with the available literature is possible by expressing CLA as mg/g fat, so that the lipid content of the meat in examination has no bearing on the data. The mean values obtained in this study were 4.27 mg/g fat for raw meat (range 3.08–4.86) and 4.40 mg/g fat for cooked meat (range 2.29–5.42). The former mean value was within the range provided by both Mir, Rushfeldt, Mir, Paterson, and Weselake (2000) and Ivan et al. (2001) for the rib muscle of control group lambs in feeding trials, and by Chin et al. (1992) for retail lamb meat, with a combined range of 3.13–5.60 mg/g fat.

3.4. True nutrient retention values

As far as concerns the proximate composition and energy value, MW cooking provided higher RVs than broiling (Table 4), which was expected given the former's superior cooking yield. Only our results on broiling can be compared with the RVs reported by Ono et al. (1984) for lambs of two age groups and with the figures calculated from the mean composition values provided by Hoke et al. (1999) for exported Australian lambs; such comparison still refers to multiple-muscled cuts broiled fat on. Moisture and ash RVs were somewhat lower than those reported by these authors, confirming the suggested importance of the drip losses and leaching of the mineral component. Furthermore, with a value around 100%, the protein RV for broiling was confirmed as normal for this nutrient, not susceptible to migration but only to a concentration effect. On the other hand, the protein RV for MW cooking was rather high, resulting in a higher energy retention compared to broiling, a phenomenon for which there is no ready explanation.

Table 4
True nutrient retention values (%) for cooked lamb rib-loins

Nutrient[A,B]	Microwaving	Broiling	*P* value
Moisture	61.0 ± 2.02a	51.6 ± 0.88b	0.0004
Protein	113 ± 1.6a	101 ± 0.9b	0.0002
Lipids	102 ± 5.3	96.2 ± 3.9	0.1066
Ash	72.6 ± 1.78	72.4 ± 1.28	0.9207
Energy	119 ± 2.8a	99.6 ± 1.14b	0.0006
C14:0	115 ± 6.4a	102 ± 4.7b	0.0314
C15:0	125 ± 6.9a	110 ± 4.5b	0.0260
C16:0	109 ± 5.8a	99.1 ± 3.81b	0.0274
C16:1 ω7	117 ± 12	102 ± 6.9	0.2734
C17:0	111 ± 5.9a	100 ± 4.9b	0.0399
C18:0	105 ± 7.6	97.2 ± 5.07	0.0761
∑ C18:1 *trans*	124 ± 7.4	117 ± 6.1	0.2979
C18:1 ω9	104 ± 5.8	94.2 ± 4.30	0.0681
C18:1 ω11	111 ± 4.6a	99.2 ± 2.58b	0.0212
C18:2 ω6	100 ± 5.0a	89.0 ± 4.02b	0.0259
C18:3 ω3	102 ± 5.9	91.2 ± 4.90	0.0650
Total CLA[C]	111 ± 8.2	98.0 ± 8.09	0.0592
C20:4 ω6	94.8 ± 5.70	85.9 ± 4.07	0.1527
C22:4 ω6	97.4 ± 7.62	91.3 ± 8.66	0.4916
C22:6 ω3	92.5 ± 6.27	82.3 ± 6.93	0.1838
∑ SFA[D]	108 ± 6.2a	98.6 ± 4.12b	0.0357
∑ MUFA[D]	106 ± 5.9	96.1 ± 4.16	0.0764
∑ PUFA[D]	100 ± 5.1a	89.0 ± 4.05b	0.0454
∑ ω6 PUFA[E]	98.9 ± 5.01a	88.4 ± 3.95b	0.0410
∑ ω3 PUFA[F]	98.0 ± 5.77	87.3 ± 5.14	0.0645

[A–F] See footnotes in Table 3.

The lipid RVs obtained in this study with the two cooking methods did not vary much from 100%, which can be accounted for by a mere concentration effect or by a substantial equilibrium between cover and seam fat migration into the lean and rendering of intramuscular fat from the lean. The lipid RVs for broiled cuts calculated from the data by Hoke et al. (1999) were higher (111% as an average), while the RVs provided by Ono et al. (1984) were even higher, ranging between 115% and 126%.

RVs of individual fatty acids were higher in MW cooked meat than in broiled meat (Table 4), the differences being significant or at least marginally so

($P < 0.10$) for the majority of SFA and some MUFA and PUFA, relevant sums included. For SFA and MUFA, the mean RVs tended to exceed 100% in MW cooked meat while in broiled meat this phenomenon occurred only for pentadecanoic acid and the sum of *trans* octadecenoic acids. As for PUFA, their RVs in MW cooked meat were around 100% for C18 fatty acids, dropping for C20 and C22 fatty acids, while for broiled meat the values were found to be generally lower. In this regard, total CLA deserved special mention, with an RV more similar to the values obtained for MUFA than for PUFA within cooking method. On the whole, there was an appreciable coincidence with the RVs calculated using the data given by Hoke et al. (1999) for broiled fat-on lamb cuts, although the latter tended to be slightly higher.

In view of the RVs obtained, it is at last possible to complete a hypothesis about the response of the lipid fraction in the cuts under examination. As stated above, what was observed in MW cooked meat could be ascribed to a modest migration of rendered subcutaneous and intermuscular fat into the lean, with which a significant rendering of intramuscular fat from the lean was not associated. This assumption was supported by the high RVs found for *trans* octadecenoic acids and for total CLA, whose greater presence in cover and seam fat compared to intramuscular fat has been ascertained many times (Bolte et al., 2002; Enser et al., 1996; Ivan et al., 2001; Paterson, Weselake, Mir, & Mir, 2002). On the other hand, it has already been argued that, due to the frequent turning of the steaks, the lean of broiled meat was partially affected by rendered subcutaneous and intermuscular fat. To contrast it, there was probably a certain rendering, partly followed by leaching, from the transected depots of intramuscular fat, most likely affecting the triglyceride component rather than the phospholipid component and, therefore, among the PUFA, affecting C18 more than C20 and C22 fatty acids (Armstrong & Bergan, 1992). Due to the modest RVs obtained for C20 and C22, it cannot be ruled out that these fatty acids were partially affected by oxidative degradation (Gandemer, 1992).

A calculation of the amounts of lipids and individual fatty acids that the lean would actually have gained or lost through the effect of cooking, as opposed to the hypothesis of a mere phenomenon of concentration caused by moisture loss (i.e., $RV = 100\%$), reinforced the previous considerations. Comparing the "nutrient contents per g of cooked foods" effectively observed with those calculated by adopting a value of $RV = 100$ in the formula expressed above for the calculation of the retention values, the mean quantity of lipids migrating into the MW cooked meat was estimated at 240 mg/100 g lean against the 180 mg/100 g lean of lipids lost after broiling. For MW cooked meat, the mean contribution estimated for SFA and MUFA to 100 g cooked lean was, respectively, equivalent to 127 mg (mainly palmitic acid) and 87 mg (mainly oleic acid and *trans* octadecenoic acids), while there was a slight loss for PUFA, equivalent to 2 mg/100 g lean, to which CLA did not contribute. As regards broiled meat, the losses estimated for 100 g cooked lean amounted to an average of 42 mg for SFA (mainly stearic acid), 70 mg for MUFA (as a balance between oleic acid, rendering from the lean, against a certain migration into it of *trans* octadecenoic acids) and 50 mg for PUFA (practically only of the ω6 series).

3.5. Conjugated linoleic acid isomers

The qualitative and quantitative picture of the CLA isomers determined in the lamb (Table 5) showed the clear predominance of *c*9,*t*11 (perhaps coeluting with the minor *t*9,*c*11). The relative proportion of *c*9,*t*11 to the total CLA in raw meat was much lower than the only value available for lamb, namely the 92% provided by Chin et al. (1992), while it was more in line with the values more recently found in beef (Fritsche et al., 2000; Fritsche, Rumsey, Yurawecz, Ku, & Fritsche, 2001; Nürnberg et al., 2002; Yurawecz et al., 1998). In the light of such a high incidence for this isomer, the values of the remaining Δ9,Δ11 isomers identified (*t*9,*t*11 and *c*9,*c*11) were decidedly low but, in any case, comparable to the only figure available, namely *t*9,*t*11 for beef (Nürnberg et al., 2002).

In qualitative terms (i.e., with values expressed in mg isomer/g fat), the average content of *c*9,*t*11 found in raw meat was much lower than the data range (7.00–13.87 mg/g fat) available in the literature for lamb (Dufey, 1999; Nürnberg et al., 2001, both on grazing animals). Far greater similarity appeared in the comparison of the data range (1.20–7.40 mg/g fat) established on the basis of studies conducted on raw beef, either retail samples or meat derived from control animals of various trials (Dufey, 1999; Fritsche et al., 2000, 2001; Ma, Wierzbicki, Field, & Clandinin, 1999; Madron et al., 2002; Raes, de Smet, & Demeyer, 2001; Shantha, Crum, & Decker, 1994; Shantha, Moody, & Tabeidi, 1997). However, the content of *c*9,*t*11 expressed in mg/100 g lean is the observation of greatest importance from the nutritional point of view. The average value for the raw meat analysed here fell within the range of 8.4–20.8 mg/100 g lean provided by Nürnberg et al. (2001), thanks to the higher average lipid content in the meat from the lambs used in this study. In any event, and as for total CLA, the *c*9,*t*11 content in raw meat observed in this study varied considerably, from 7.82 to 19.7 mg/100 g edible portion. Other researchers have also reported wide animal-to-animal variations largely, but not exclusively, related to diet, in the *c*9,*t*11 content of foods from ruminants (Bauman, Baumgard, Corl, & Griinari, 1999; Fogerty et al., 1988; Ma et al., 1999; Shantha

Table 5
Concentration, content and true retention values (RV) of Δ9,Δ11 CLA isomers for raw and cooked lamb rib-loins

Isomer[A,B,C]	Raw state	Microwaving	Broiling	*P* value
trans-9,trans-11				
% Total CLA	1.45 ± 0.14	1.58 ± 0.12	1.78 ± 0.07	0.0623
mg/g Fat	0.061 ± 0.006	0.070 ± 0.007	0.078 ± 0.007	0.1233
mg/100 g Lean	0.20 ± 0.01b	0.35 ± 0.04ab	0.42 ± 0.07a	0.0113
RV (%)	–	135 ± 26	143 ± 39	0.6530
cis/trans-9,11				
% Total CLA	74.9 ± 2.32	74.6 ± 2.42	74.7 ± 2.21	0.9962
mg/g Fat	3.20 ± 0.19	3.35 ± 0.28	3.28 ± 0.32	0.7283
mg/100 g Lean	11.3 ± 1.30b	17.2 ± 2.52a	18.1 ± 3.63a	0.0093
RV (%)	–	113 ± 12	100 ± 11	0.1893
cis-9,cis-11				
% Total CLA	0.90 ± 0.13	0.77 ± 0.11	0.78 ± 0.11	0.3683
mg/g Fat	0.037 ± 0.004	0.033 ± 0.003	0.032 ± 0.005	0.5117
mg/100 g Lean	0.13 ± 0.02	0.17 ± 0.02	0.18 ± 0.04	0.1615
RV (%)	–	97.2 ± 11	87.7 ± 14	0.4145

[A] Values represent means ± SE ($n = 8$).
[B] *cis/trans* refers to the Δ9,Δ11 isomers with either a *cis/trans* or a *trans/cis* configuration.
[C] Mean values in the same row followed by different letters differ significantly ($P \leqslant 0.05$).

et al., 1994); the fact it was observed here was not surprising since the animals used were of different breeds or crosses and taken from farms with different feeding conditions.

After cooking, the relative proportion of the various isomers to total CLA and their incidence in each gram of fat did not vary significantly, although there was a marginally significant difference ($P < 0.10$) for *t*9,*t*11 in % total CLA (Table 5). Moreover, regardless of the cooking method employed, the contents of *t*9,*t*11 and *c*9,*t*11 provided by 100 g of lean increased significantly with cooking while a non-significant increase was produced for *c*9,*c*11. In order to determine whether and to what extent these results could be ascribed exclusively to a concentration effect, or whether more complex dynamics needed to be hypothesised, the RVs were calculated for these nutrients as well. The mean value obtained for *t*9,*t*11 with MW cooking was lower than that observed for broiling, while the opposite was true for *c*9,*t*11 and *c*9,*c*11 (Table 5), although the dispersion of data probably prevented the emergence of any significant difference. The *t*9,*t*11 RV was higher than those obtained for *c*9,*c*11 and *c*9,*t*11, the latter value being similar to that found for total CLA, within the same cooking method. Partly in consideration of the RV obtained for total CLA, the existence of a lipid contribution (subsequently found to be very marginal) was previously assumed for MW cooked lean, deriving from the cover and seam fat to the lean. At least in beef, the CLA isomeric profile does not appear to vary in relation to the fat depot examined (Fritsche et al., 2001); this observation, applied to lamb, would exclude a migration of the three isomers into the MW cooked lean of a different amount from their level in the intramuscular fat. Assuming the above, a configuration similar to the one observed for RVs might be explained by assuming that the RVs for these three isomers were the result of a balance; this would suggest an association – in terms of increase – with the contribution made by the rendering of subcutaneous and intermuscular fat, while, in terms of decrease, the occurrence of a certain oxidative degradation cannot be entirely excluded according to a sensitivity that would be of the highest degree for isomers *c*, *c*, and the lowest for *t*, *t* (Yang, Leung, Huang, & Chen, 2000). As for broiled lean, it was affected mostly by a small loss of intramuscular fat rather than by a contribution of melted cover and seam fat caused by self-basting. The *t*9,*t*11 RV attested a contribution of this isomer to the lean while it could be assumed that oxidative events contributed to the *c*9,*c*11 RV, as such events were more likely for the steaks, which were subjected to a higher thermal stress during cooking than the MW cooked roasts. Similarly, it could be assumed that the *c*9,*t*11 RV was the result of an equilibrium where formation by thermal oxidation of LA may have contributed (Shantha et al., 1994). This hypothesis was enhanced by the modest RV of LA itself for broiled lean (see Table 4).

The average *c*9,*t*11 contribution obtainable from 100 g of cooked meat, regardless of the cooking method adopted, was 17.7 mg (9.26–42.5 mg/100 g range); no comparative data for lamb exists in the literature. The only figures that may be compared to those obtained here were provided by Ma et al. (1999) for beef sirloin roast tip and for rib roast, with mean values equal to 28.7 and 77.6 mg/100 g, respectively, both characterised by considerable variability and linked to high or very high lipid contents (9.2 and 27.8%, respectively). At this

point it would be sensible to ask how much one serving (100 g) of this MW cooked or broiled lamb could contribute to the human daily requirement of *c*9,*t*11 – a value which is still not official. Taking as reference the values recently suggested by Ritzenthaler et al. (2001), equal to 620 mg/day of *c*9,*t*11 for adult males and 441 mg/day for adult females, the levels of coverage of such requirements provided by this meat are rather low, more noticeably for men (mean = 2.85%, range 1.49–6.85%) than for women (mean = 4.00%, range 2.10–9.64%). These considerations might suggest the implementation of feeding practices aimed at standardising upwards the average content of *c*9,*t*11 in meat from intensively reared lambs. Nevertheless, the authors share the position of McGuire and McGuire (1999), namely that muscle foods, such as ruminant meats, naturally having comparatively high amounts of *c*9,*t*11 and modest energy values, can usefully complement the consumption of this nutrient without necessarily requiring enrichment, perhaps as a partial substitute for dairy products (especially cheese and ewe cheese in particular), which are certainly the preferred source, but which also have the disadvantage of a higher calorie content.

In conclusion, microwave cooking applied to fat-on lamb rib-loins produced compositional changes that were slighter than those produced by broiling fat-on lamb steaks. The nutritional importance of such differences was, however, very modest. Subsequent to cooking, the quantitatively and nutritionally most important isomer of conjugated linoleic acid, i.e., *cis*-9,*trans*-11, showed true retention values closer to those of the monounsaturated than those of the polyunsaturated fatty acids.

Acknowledgements

Financial support was provided by the Italian Ministry of Education, University and Research (MIUR, "ex-60%" funds). The valuable cooperation of Dr. Stefano Ronchi (A.E.R.PR.O.C., Associazione Emiliano-Romagnola Produttori Ovini Caprini scarl) and the skilful technical assistance of Dr. Matteo Busato are gratefully acknowledged.

References

Adlof, R. O., Duval, S., & Emken, E. A. (2000). Biosynthesis of conjugated linoleic acid in humans. *Lipids, 35*, 131–135.

AMSA. (1995). *Research guidelines for cookery, sensory evaluation, and instrumental tenderness of fresh meat* (p. 47). Chicago, IL: American Meat Science Association and National Live Stock & Meat Board.

AOAC. (2000). *Official methods of analysis* (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

Armstrong, S. G., & Bergan, J. G. (1992). Factors affecting stability and nutritive value of fatty acids: Culinary practices. In C. K. Chow (Ed.), *Fatty acids in foods and their health implications* (pp. 353–363). New York: Marcel Dekker.

Badiani, A., Nanni, N., Gatta, P. P., Bitossi, F., Tolomelli, B., & Manfredini, M. (1998). Nutrient content and retention in selected roasted cuts from 3-month-old ram lambs. *Food Chemistry, 61*, 89–100.

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.

Bauman, D. E., Baumgard, L. H., Corl, B. A., & Griinari, J. M. (1999). Biosynthesis of conjugated linoleic acid in ruminants. In *Proceedings of the American society of animal science*. Available: <http://www.asas.org>. Accessed April 2, 2003.

Bolte, M. R., Hess, B. W., Means, W. J., Moss, G. E., & Rule, D. C. (2002). Feeding lambs high-oleate or high-linoleate safflower seeds differentially influences carcass fatty acid composition. *Journal of Animal Science, 80*, 609–616.

Capatti, A., & Montanari, M. (1999). L'Italia, uno spazio fisico e mentale. In *La Cucina Italiana – Storia di una Cultura* (pp. 3–40). Bari, I: Editori Laterza.

Chan, W., Brown, J., Lee, S. M., & Buss, D. H. (1995). *Meat, poultry and game – fifth supplement to the fifth edition of McCance and Widdowson's the composition of foods*. Cambridge and London, UK: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L., & Pariza, M. W. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *Journal of Food Composition and Analysis, 5*, 185–197.

Christie, W. W. (2002). *Analysis of conjugated linoleic acid (CLA)*. The Scottish Crop Research Institute. Available: <http://www.lipid.co.uk/infores/topics/cla>. Accessed April 4, 2003.

Corcoran, K., Bernués, A., Manrique, E., Pacchioli, T., Baines, R., & Boutonnet, J. P. (2001). Current consumer attitudes towards lamb and beef in Europe. *Options Mediterraneennes. Serie A: Seminaires Mediterraneens, No. 46*, 75–79.

CRPA. (1997). *Disciplinare di produzione integrata: carne ovina di agnellone e castrato* (p. 32). Reggio Emilia, Italy.

Demeyer, D., & Doreau, M. (1999). Targets and procedures for altering ruminant meat and milk lipids. *Proceedings of the Nutrition Society, 58*, 593–607.

Dufey, P.-A. (1999). La viande, source alimentaire d'acides linoléiques conjugués (CLA). *Revue Suisse d'Agriculture, 31*, 173–176.

EEC. (1992). Council regulation no. 2137/92 of July 23, 1992, concerning the community scale for the classification of carcasses of ovine animals and the determination of the community standard quality for fresh or chilled ovine carcasses, which extends the EEC regulation no. 338/91. *Official Journal of the European Communities*, No. L 214, 1–5.

EEC. (1993). Commission regulation no. 461/93 of February 26, 1993, laying down the conditions of enforcement of the community scale for the classification of ovine carcasses. *Official Journal of the European Communities*, No. L 049, 70–74.

Enser, M., Hallett, K. G., Hewitt, B., Fursey, G. A. J., & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science, 42*, 443–456.

Ensminger, A. H., Ensminger, M. E., Konlande, J. E., & Robson, J. R. K. (1994). *Foods and nutrition encyclopedia* (2nd ed.). Boca Raton, FL: CRC Press.

EURODIET. (2000). *Nutrition and diet for healthy lifestyles in Europe: Science and policy implications*. Core report of the EURODIET project steering committee. Available: <http://eurodiet.med.uoc.gr>. Accessed September 26, 2002.

Favier, J.-C., Ireland-Ripert, J., Toque, C., & Feinberg, M. (1995). *Répertoire Général des Aliments: Table de Composition* (2nd ed.). Paris, F: Lavoisier.

Fogerty, A. C., Ford, G. L., & Svoronos, D. (1988). Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk. *Nutrition Reports International, 38*, 937–944.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Fox, B. A., & Cameron, A. G. (1990). Methods of cooking. In *Food science, nutrition and health* (pp. 285–304). Sevenoaks, UK: Edward Arnold Ltd..

Fritsche, J., & Steinhart, H. (1998). Analysis, occurrence, and physiological properties of *trans* fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) – a review. *Fett/Lipid, 100*(6), 190–210.

Fritsche, S., Rumsey, T. S., Yurawecz, M. P., Ku, Y., & Fritsche, J. (2001). Influence of growth promoting implants on fatty acid composition including conjugated linoleic acid isomers in beef fat. *European Food Research and Technology, 212*, 621–629.

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

Gandemer, G. (1992). Les lipides de la viande: vers une estimation precise de leurs apports nutritionnels dans l'alimentation de l'homme. In A. Bernard & H. Carlier (Eds.), *Aspects nutritionnels des constituants des aliments. Influences des technologies* (pp. 25–48). Paris, F: Lavoisier.

Greenfield, H., Kuo, Y. L., Hutchison, G. I., & Wills, R. B. H. (1987). Composition of Australian foods. 33. Lamb. *Food Technology in Australia, 39*, 202–207.

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. M. Mossoba, J. K. G. Kramer, M. W. Pariza, & G. J. Nelson (Eds.), *Advances in conjugated linoleic acid research* (Vol. I, pp. 180–200). Champaign, IL: AOCS Press.

Hasler, C. M. (2002). Functional foods: Benefits, concerns and challenges – a position paper from the American Council on Science and Health. *Journal of Nutrition, 132*, 3772–3781.

Hoke, I. M., Buege, D. R., Ellefson, W., & Maly, E. (1999). Nutrient and related food composition of exported Australian lamb cuts. *Journal of Food Composition and Analysis, 12*, 97–109.

Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN). (2000). *Tabelle di Composizione degli Alimenti, Aggiornamento 2000*. Milano, I: EDRA srl.

Ivan, M., Mir, P. S., Koenig, K. M., Rode, L. M., Neill, L., Entz, T., & Mir, Z. (2001). Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. *Small Ruminant Research, 41*, 215–227.

Jones, D. K., Savell, J. W., & Cross, H. R. (1992). Effects of fat trim on the composition of beef retail cuts – 3. Cooking yields and fat retention of the separable lean. *Journal of Muscle Foods, 3*, 73–81.

Ma, D. W. L., Wierzbicki, A. A., Field, C. J., & Clandinin, M. T. (1999). Conjugated linoleic acid in Canadian dairy and beef products. *Journal of Agricultural and Food Chemistry, 47*, 1956–1960.

Madron, M. S., Peterson, D. G., Dwyer, D. A., Corl, B. A., Baumgard, L. H., Beermann, D. H., & Bauman, D. E. (2002). Effect of extruded full-fat soybeans on conjugated linoleic acid content of intramuscular, intermuscular, and subcutaneous fat in beef steers. *Journal of Animal Science, 80*, 1135–1143.

Martin, J.-C., & Valeille, K. (2002). Conjugated linoleic acids: All the same or to everyone its own function? *Reproduction Nutrition Development, 42*, 525–536.

Matthews, R. H., & Garrison, Y. J. (1975). *Food yields summarized by different stages of preparation*. Agriculture handbook No. 102. Available: <http://www.nal.usda.gov/fnic/foodcomp/Data/Classics>. Accessed March 7, 2003.

McGuire, M. A., & McGuire, M. K. (1999). Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. In *Proceedings of the American society of animal science*. Available: <http://www.asas.org>. Accessed April 4, 2003.

Michaelsen, S. A., Johnson, D. D., West, R. L., & Leak, F. W. (1991). *Determination of cooking characteristics for lean retail beef cuts* (p. 96). Gainesville, FL: Meat Science Section, Department of Animal Science, University of Florida.

Mir, Z., Rushfeldt, M. L., Mir, P. S., Paterson, L. J., & Weselake, R. J. (2000). Effect of dietary supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA content of lamb tissues. *Small Ruminant Research, 36*, 25–31.

National Live Stock & Meat Board. (1988). *Nutrient values of muscle foods* (1st ed., p. 63). Chicago, IL: National Live Stock and Meat Board.

Noah, A., & Truswell, A. S. (2001). There are many Mediterranean diets. *Asia Pacific Journal of Clinical Nutrition, 10*(1), 2–9.

Nürnberg, K., Grumbach, S., Zupp, W., Hartung, M., Nürnberg, G., & Ender, K. (2001). Erhöhung der *n-3* Fettsäuren und der konjugierten Linolsäure im Lammfleisch durch Weidehaltung. *Fleischwirtschaft, 81*, 120–122.

Nürnberg, K., Nürnberg, G., Ender, K., Lorenz, S., Winkler, K., Rickert, R., & Steinhart, H. (2002). *N*-3 fatty acids and conjugated linoleic acids of *longissimus* muscle in beef cattle. *European Journal of Lipid Science and Technology, 104*, 463–471.

Ono, K., Berry, B. W., Johnson, H. K., Russek, E., Parker, C. F., Cahill, V. R., & Althouse, P. G. (1984). Nutrient composition of lamb of two age groups. *Journal of Food Science, 49*, 1233–1239, 1257.

Parodi, P. W. (2002). Health benefits of conjugated linoleic acid. *Food Industry Journal, 5*(3), 222–259.

Paterson, L. J., Weselake, R. J., Mir, P. S., & Mir, Z. (2002). Positional distribution of CLA in TAG of lamb tissues. *Lipids, 37*, 605–611.

Prandini, A., Geromin, D., Conti, F., Masoero, F., Piva, A., & Piva, G. (2001). Survey on the level of conjugated linoleic acid in dairy products. *Italian Journal of Food Science, 13*, 243–253.

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

Ritzenthaler, K. L., McGuire, M. K., Falen, R., Shultz, T. D., Dasgupta, N., & McGuire, M. A. (2001). Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. *Journal of Nutrition, 131*, 1548–1554.

Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I., & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science, 54*, 339–346.

Schakel, S. F., Buzzard, I. M., & Gebhardt, S. E. (1997). Procedures for estimating nutrient values for food composition databases. *Journal of Food Composition and Analysis, 10*, 102–114.

Sehat, N., Yurawecz, M. P., Roach, J. A. G., Mossoba, M. M., Kramer, J. K. G., & Ku, Y. (1998). Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. *Lipids, 33*, 217–221.

Shantha, 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.

Shantha, N. C., Decker, E. A., & Hennig, B. (1993). Comparison of methylation methods for the quantitation of conjugated linoleic acid isomers. *Journal of the AOAC International, 76*, 644–649.

Shantha, N. C., Moody, W. G., & Tabeidi, Z. (1997). Conjugated linoleic acid concentration in *semimembranosus* muscle of grass-fed and grain-fed and zeranol-implanted beef cattle. *Journal of Muscle Foods, 8*, 105–110.

Simopoulos, A. P., & Sidossis, L. S. (2000). What is so special about the traditional diet of Greece – the scientific evidence. *World Review of Nutrition and Dietetics, 87*, 24–42.

Slover, H. T., Lanza, E., Thompson, R. H., Jr., Davis, C. S., & Merola, G. V. (1987). Lipids in raw and cooked beef. *Journal of Food Composition and Analysis, 1*, 26–37.

Souci, S. W., Fachmann, W., & Kraut, H. (2000). *Food composition and nutrition tables* (6th ed.). Stuttgart, D: Medpharm GmbH Scientific Publishers.

Swatland H. J. (2000). *Meat cuts and muscle foods* (pp. 100–101). Nottingham UK: Nottingham University Press.

Turrini, A., Saba, A., Perrone, D., Cialfa, E., & D'Amicis, A. (2001). Food consumption patterns in Italy: The INN-CA study 1994–1996. *European Journal of Clinical Nutrition, 55*, 571–588.

USDA. (2002a). *Composition of foods – raw, processed, prepared, release 15*. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. Available: <http://www.nal.usda.gov/fnic/foodcomp/Data/SR15/sr15_doc.htm>. Accessed March 29, 2003.

USDA. (2002b). *US Department of Agriculture Nutrient Database for Standard Reference, release 15*. Nutrient Data Laboratory. Available: <http://www.nal.usda.gov/fnic/foodcomp/Data/SR15/sr15.html>. Accessed August 20, 2002.

US-FDA. (1999). *The food label. FDA food and drug administration backgrounder*. Available: <http://www.cfsan.fda.gov/~dms/fdnewlab.html>. Accessed May 3, 2003.

Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Enser, M., Wood, J. D., & Fisher, A. V. (2002). Effects of dietary fat source and breed on the carcass composition, $n-3$ polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *British Journal of Nutrition, 88*, 697–709.

Welch, M. J., Colbert, J. C., Gill, L. M., Phinney, C. S., Sharpless, K. E., Sniegoski L. T., & Wood, L. J. (2001). The certification of SRM 1546 – meat homogenate, a new reference material for nutrients in a high protein, high fat matrix. *Fresenius Journal of Analytical Chemistry*, 370, 42–47, plus supplementary material available in electronic form at http://link.springer.de/journals/fjac. Accessed July 29, 2002.

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*, 3072–3076.

Yurawecz, M. P., & Morehouse, K. M. (2001). Silver-ion HPLC of conjugated linoleic acid isomers. *European Journal of Lipid Science and Technology, 103*, 609–613.

Yurawecz, M. P., Roach, J. A. G., Sehat, N., Mossoba, M. M., Kramer, J. K. G., Fritsche, J., Steinhart, H., & Ku, Y. (1998). A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis*-octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. *Lipids, 33*, 803–809.

Zlatanos, S., Laskaridis, K., Feist, C., & Sagredos, A. (2002). CLA content and fatty acid composition of Greek Feta and hard cheeses. *Food Chemistry, 78*, 471–477.