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Full Length Research Paper

Marinating beef with South African red wine may protect against lipid peroxidation during cooking

D. M. Blackhurst[1]*, R-D. Pietersen[2], F. H. O'Neill[3] and A. D. Marais[1]

[1]Division of Lipidology, Department of Internal Medicine, Faculty of Health Sciences, University of Cape Town, Anzio Road, Observatory 7925, South Africa.
[2]Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Francie van Zyl Avenue, Stellenbosch 7505, South Africa.
[3]Biochemistry Division, North-West University, Potchefstroom 2520, South Africa.

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Dietary peroxidised lipids may be harmful to the cardiovascular system. Meat, a complex food, undergoes significant biochemical changes, including lipid peroxidation, during cooking. Wine is often used as an ingredient in the marinating of meat. This study determined whether marinating red meat overnight in red wine has a protective influence against cooking-induced lipid peroxidation in beef. Standardized portions of beef were marinated in red wine. Samples of marinated and unmarinated meat, raw and cooked (microwaved), were analysed for lipid peroxidation products: conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS). The antioxidant capacity of the wine was determined. The oxygen radical absorbance capacity (ORAC) value of the wines was 16.8±10.2 mmol/L trolox equivalents. There was great variability in the baseline peroxidation status of the meat samples. Marinating did not significantly alter the CD, LOOH or TBARS in raw meat. Expressed as changes of the mean values, cooking increased the TBARS in unmarinated meat by 17% (*P* = 0.039). Compared with unmarinated meat, marinating displayed a trend to lower the CD in cooked meat by 20%, (*P* = 0.084). More studies are required to examine the oxidative status of lipids in meat before and after cooking and to establish a beneficial role for marinating meat with wine.

Key words: Conjugated dienes, oxygen radical absorbance capacity (ORAC), polyunsaturated fatty acids, red meat, wine, thiobarbituric acid reactive substances (TBARS).

INTRODUCTION

Man is the only animal that consumes food exposed to higher than physiological temperatures. This is probably a relatively recent evolutionary phenomenon. While cooking may have conferred an advantage by preventing or limiting microbial growth in foods, a longer life expectancy may expose adverse effects of lipid peroxidation. The peroxidation status of edible lipids may influence atherosclerosis independently of the adverse effects that dietary lipids may have on lipoprotein metabolism.

*Corresponding author. E-mail: dee.blackhurst@uct.ac.za. Tel: +27-21-4066108. Fax: +27-21-4066396.

Meat, a complex food, undergoes significant biochemical changes during processing, storage and cooking. Cooking practice introduces varying times and temperatures that will influence lipid peroxidation. Brown et al. (1995) compared the cooking of beef at low (≤ 100°C for 40 min) and high (250°C for 22 min) temperatures and found that the malondialdehyde (MDA) concentration in the meat following the low temperature cooking was 3 to 4 times higher than in the meat exposed to the high temperature. This is attributed to the higher degree of volatilisation of MDA at high temperatures or the increased formation of non–TBA reactive MDA–protein adducts. Microwave cooking has been associated with less peroxidation of meat lipids than oven roasting (Badiani et al., 2002) and also produces fewer fatty acid

compositional changes than broiling (Maranesi et al., 2005). Rodriguez-Estrada et al. (1997) however, found that microwave cooking increased the concentration of a marker of peroxidation, p-anisidine compared with raw meat.

Lipid peroxidation is a complex process involving the degradation of PUFA via products such as conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) including MDA that are considered harmful to human health (Basu et al., 1983). The products may cause colour, nutritional and flavour deterioration (rancidity) of foodstuffs. Their significance in human health has not to date been fully elucidated. Minamoto et al. (1985) reported that ingestion of products of autoxidised linoleic acid stimulated lipid peroxidation in rat livers. Studies on the mechanisms of lipid peroxidation in meat are most often conducted on cooked rather than raw meat (Rhee et al., 1986) because uncooked meat does not undergo peroxidation as readily. Cooking promotes oxidation by raising the temperature and by the release of ferric ions. There is still no consensus on the role of haem–iron versus non–haem iron in lipid peroxidation. Liu (1970) concluded that both types of iron acted as catalysts for lipid peroxidation in beef homogenate.

Several factors influence the rate and degree of lipid peroxidation in meat. The ante–mortem factors include animal species (Kim et al., 2003), gender (Rhee et al., 1986) and age of the animal. Type and quantity of the animal feed are also important. Grass or pasture feeding has been found to increase the concentrations of n–3 unsaturated fatty acids and conjugated linoleic acid (CLA) in beef, compared with concentrate feeding (Varella et al., 2004; Realini et al., 2004). CLA is formed during reticulorumen hydrogenation of linoleic acid and through endogenous desaturation of trans–11 octadecaenoic acid (Griinari and Bauman, 1999). CLA, although a CD, may be anti–atherogenic and anti–carcinogenic (Weiss et al., 2004) with rumenic acid (the cis–9, trans–11 isomer) being the main naturally–occurring CLA in food.

Post–mortem factors include the type of muscle studied, processing and storage. Wilson et al. (1976) found that red muscles are more susceptible to lipid peroxidation than white muscles. Badiani et al. (2002) found that the peroxide value was lower in the infraspinatus beef muscle compared with the semitendinosus muscle. High pressure treatment (often used in the processing of meat) increases TBARS concentrations (Ma et al., 2007). Conditions during storage, such as the temperature, time and oxygen concentration, are also important. A temperature of 4°C has been found to be the most important factor for keeping lipid peroxidation to a minimum, compared with oxygen concentration and storage times (Jakobsen and Bertelsen, 2000). A correlation between the meat haem–protein pigments, myoglobin (major) and haemoglobin (minor) and lipid peroxidation has been shown (Rhee and Ziprin, 1987; Faustman and Cassens, 1990). Exposure to oxygen correlates with the extent of oxidation of meat (Lund et al., 2007). Packaging methods, e.g. aerobic or vacuum packaging, are also important: packaging materials may be permeable to oxygen. Vacuum packaging, with its lower oxygen concentration can greatly extend the shelf–life of raw beef (Smiddy et al., 2002). The concentration of volatiles emanating from peroxidation, for example butane, pentane, hexane, heptane and octane, increases with storage in aerobically packaged meat, with a positive correlation between these volatiles and TBARS (Kim et al., 2003). Irradiation of meat has been found to generate free radicals (Du et al., 2002), verified by increased concentrations of TBARS in irradiated meat compared with non–irradiated meat (Kanatt et al., 2006).

The number of unsaturated bonds in the fatty acids increases the susceptibility to peroxidation of meat (Igene et al., 1979; Kim et al., 2003). These fatty acids reside mostly in phospholipids (PL) in cooked beef, but triacylglycerol (TG) also undergoes lipid peroxidation, albeit to a lesser extent (Igene et al., 1979). Unsaturated fatty acids (UFA) that undergo peroxidation, for example oleic, linoleic and arachidonic acids, make significant contributions to increased rancidity in cooked beef (Gokalp et al., 1983). Cholesterol typically undergoes peroxidation at C7.

The presence of pro– and antioxidants (both endogenous and exogenous) is important. High concentrations of vitamin E (provided by grass grazing for example (Wood et al., 2003)), have been found to inhibit lipid peroxidation (Jensen et al., 1998). Nitrite acts as a potent antioxidant in cooked meat systems (Igene et al., 1979). There is a wide range of different phenolic compounds in wine, as well as a large variation amongst these compounds in the different wines. Resveratrol for example, found mostly in red wines but also in white wines, has been found to have a number of health benefits, due largely to its antioxidant properties. It also has anti-cancer and anti-microbial properties (Signorelli and Ghidoni, 2005; Mahady and Pendland, 2000). Quercetin in red wine has been found to be cardioprotective (Brookes et al., 2002). The oxygen radical absorbance capacity (ORAC) value of a beverage is a measure of its antioxidant capacity and red wine exhibits a high value relative to several other beverages (Seeram et al., 2008). Wine is often an ingredient in the marinating of meat. Marinating is believed to enhance the flavour of the meat and the alcohol and acid in wine may tenderise the meat. Red wine with its higher concentration of polyphenols than white wine, has been shown to have enhanced antioxidant properties and is reported to have greater health benefits (Lugasi and Hóvári, 2003; Das et al., 2007).

The aim of this study was to determine whether

marinating with red wine protects red meat against lipid peroxidation by analyzing CD, LOOH and TBARS in control and marinated meat cooked in a microwave oven.

MATERIALS AND METHODS

Study design

Twenty two different samples of fresh, lean, unfrozen beef were purchased from two South African retail outlets to represent foods relevant to consumers. The samples had been stored in the shop at 4°C in a polystyrene base with a clingfoil wrapping. They included two cuts of beef, porterhouse steak and fillet steak (eleven samples of each) and were trimmed of visible adipose tissue. Two portions of 40 g each (approximate size 4 × 5 × 2 cm) were cut from each sample of meat, taking care that they had a similar appearance in size and content and excluded tissue other than striated muscle. One portion was covered in red wine at 4°C in the dark for 24 h, while the other portion was stored under the same conditions, but in the same volume of potassium phosphate buffer (75 mmol/L, pH 7.4). The South African red wines used in the study all had an alcohol content of 14% by volume. After marinating, each portion was divided into 2 × 20 g samples, resulting in 4 × 20 g samples from each original piece of beef. One sample from each of the marinated and unmarinated portions was cooked in a microwave oven (output power 1000 W, frequency 2450 MHz, Model R–341B, Sharp, Thailand) for 40 s at 50% power, sufficient to cook but not over-cook the meat. Microwave cooking was selected for its moderate and reproducible cooking conditions. The time of cooking was previously established by asking laboratory staff not involved in the project, to declare an agreeable taste. Each sample was placed on ice for 5 min to terminate the cooking reaction. The microwaving procedure resulted in a core temperature of 90 ± 3°C (as determined by a Digitron thermocouple digital thermometer, with a Physitemp micro-probe reading).

Meat samples

A 1 g portion was taken from the centre of each of the meat samples, but was not significantly different from the whole portion in terms of the lipid concentrations and starting lipid peroxidation products (P= 0.416, data not shown). The 1 g portion was minced with scissors and then finely ground in 3 mL potassium phosphate buffer as above, using a mechanical homogenizer (VirTis handishear, the VirTis Company, Gardiner, N.Y.). Lipids were extracted from the homogenised portion by the method of Bligh and Dyer (1959), a modified method of Folch et al. (1957). Briefly, lipids were extracted into a monophasic mixture of chloroform and methanol, which was separated into two phases by the addition of chloroform and water: an upper aqueous phase containing the non-lipid compounds and an organic solvent lower phase containing the extracted lipids. This layer was dried under nitrogen gas and the mass of lipid extract determined. The lipid extract was resuspended in 2000 µl chloroform and divided into 5, 20 and 200 µl aliquots in micro**–**reaction vials which were again dried under nitrogen before being used for lipid and lipid peroxidation analyses.

Lipid peroxidation analyses

Twenty two meat samples were analysed in the unmarinated raw and cooked state as well as in the marinated raw and marinated cooked states. Concentrations of lipid peroxidation products were measured in the 200 µl extracts by spectrophotometric methods using a GBC UV/VIS analyser (Wirsam Scientific and Precision Equipment, South Africa) (CD) or Labsystems Multiskan MS Analyser (AEC Amersham (Pty) Ltd, South Africa) (LOOH and TBARS). Concentrations were calculated using the appropriate molar extinction coefficients. Solvents were purchased from Merck Chemicals and Laboratory Supplies, South Africa.

CD was measured at 234 nm after appropriate dilution in cyclohexane. The molar extinction coefficient of 2.95×10^4 $M^{-1}cm^{-1}$ was used. The inter–assay CV was $< 2\%$. To determine LOOH, lipid extracts were dissolved in chloroform and then assayed in the presence of xylenol orange (3, 3'–bis[N, N–di (carboxymethyl) –aminomethyl] –o–cresolsulfonephthalein) (sodium salt) (Sigma Chemical Co, St Louis, USA) and Fe^{2+} in the FOX assay. Absorbance of the resulting Fe^{3+}–xylenol orange complex was measured at 560 nm (Jiang et al., 1991; Jiang et al., 2002). The molar extinction coefficient is 4.3×10^4 $M^{-1}cm^{-1}$. The inter–assay CV was $< 5\%$.

TBARS were measured according to the method of Asakawa and Matsushita (1980), by reacting with thiobarbituric acid (TBA) (Sigma Chemical Co, St Louis, USA) in the presence of ferric chloride (Fluka Chemie, Switzerland) in a buffer of pH 3.6 containing sodium dodecyl sulphate. Absorbance was read at 532 nm. The molar extinction coefficient is 1.56×10^5 $M^{-1}cm^{-1}$ and the inter-assay CV was $< 12\%$.

Triacylglycerol and PL concentrations were used to normalise the lipid peroxidation concentrations and were measured in the lipid extracts from the meat using standard enzymatic colorimetric kits in a modification of the method described by Zhu et al. (2000). The GPO-PAP (Roche Diagnostics, Germany) and phospholipid B R1 (Wako Chemicals, Germany) kits were used for the TG and PL, respectively. Briefly, 5 and 20 µl lipid extracts were used for the TG and PL determinations, respectively. They were suspended in 50 µl ethanol, followed by mixing with 15 µl 1% (v/v) Triton X–100 and 500 µl 9 g/L NaCl. 125 µl of this mixture was transferred to a Greiner microtitre plate (96–well, transparent, flat-bottomed), and 175 µl enzyme reagent from the TG or the PL kit was added. After 20 min at room temperature, the absorbance at 500 nm was determined in a Labsystems multiskan MS analyser. The concentrations of TG and PL were determined from known standards supplied in the kits. The inter–assay CVs for both TG and PL were $< 3\%$.

ORAC determination of red wine

The method used was modified from the methods described by Cao et al. (1993), Cao and Prior (2000) and Ou et al. (2001). All ORAC analyses on wine were performed on a Varian Cary Eclipse fluorescence spectrophotometer (Varian Australia Pty Ltd) in white flat-bottomed 96–well microtitre plates. Red wine was diluted to 1: 1600 in 75 mmol/L phosphate buffer, pH = 7.4. 50 µl of sample was added to 50 µl buffer, 100 µl fluorescein (3',6'– dihydroxyspiro [isobenzofuran–1[3H],9'[9h]–xanthen]–3 one)(disodium) (96 nmol/L) and 100 µl AAPH (2,2'–Azobis (2**–**methylpropionamidine) dihydrochloride) (107 mmol/L). The reaction was followed from the addition of AAPH until completion, with fluorescence readings being taken at time 0, 2 min, 5 min and every 5 min until 30 min; after which readings were taken every 15 min until 3 h. The reactions were compared with a standard of Trolox (6–hydroxy–2, 5, 7, 8–tetramethylchroman–2–carboxylic acid). The excitation and emission wavelengths were 485 and 520 nm, respectively. The area under the curve (AUC) was determined from a fluorescence/time plot with Graphpad PRISM software (San Diego, USA). Values were measured as concentration of trolox equivalents (TE). The inter–assay CV was $< 8\%$.

Table 1. Lipid peroxidation products and their changes in raw meat, with and without marinating, mean ± SD.

nmol/mg lipid	Raw unmarinated n=22	Raw marinated n=22	P value
CD	46.9±82.4	26.2±10.5 (-44.1%)	0.239
LOOH	14.3±13.5	12.9±15.4 (-9.8%)	0.523
TBARS	46.9±164.4	47.4±163.7 (+1.1%)	0.573

The difference of the mean values is given in parentheses.

Table 2. Lipid peroxidation products and their changes in cooked meat, with and without marinating.

nmol/mg lipid n=22	Cooked unmarinated	Cooked marinated	% change[1] (% change based on the mean)	P value
CD	36.8 ± 25.8	29.3 ± 13.2	7.2 ± 24.6 (-20.4%)	0.084
LOOH	20.0 ± 33.9	14.4 ± 18.9	-44.7 ± 198.0 (-28.0%)	0.132
TBARS	54.8 ± 158.1	37.4 ± 93.0	7.2 ± 54.9 (-31.8%)	0.269

[1] calculated as individual % changes of the 22 samples. The difference of the mean values is given in parentheses.

Statistical analyses

Data are expressed as mean ± SD. All statistical analyses were performed with the use of Graphpad PRISM software (Version 3, San Diego, USA). The Student's t test analysis for paired values was used for normally distributed data. The Student's t test analysis for unpaired values was used to compare the results of the two different cuts of meat. PRISM software was also used to determine the AUC values for the ORAC analysis. Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

The total antioxidant capacity values as determined by the ORAC assay of the South African red wines used in the marinating of the meat samples ranged between 5 and 36 mmol/L TE, with a mean and SD of 16.8 ± 10.2 mmol/L TE. These values are similar to those for red wine published by Sánchez-Moreno et al. (2003).

The lipid peroxidation results from the two cuts of beef were compared, and were found to be statistically similar, thereby allowing the two sets of results to be combined.

The lipid peroxidation parameters displayed large variation. Although, not the primary interest of the investigation, the raw unmarinated meat was compared with the raw marinated meat for lipid peroxidation products. The results given in Table 1 indicate that marinating does not significantly affect lipid peroxidation in raw meat stored at 4°C. Per mg of lipid, raw meat contained the following: CD 46.9±82.4, LOOH 14.3±13.5 and TBARS 46.9±164.4 nmol. Marinating lowered the CD to 26.4± 10.5 nmol/mg lipid, LOOH 12.9±15.4 nmol/mg lipid and TBARS 47.4±163.7 nmol/mg lipid (P = 0.239, 0.523 and 0.573, respectively). Cooking altered the peroxidation status of unmarinated meat in nmol/mg lipid: CD 36.8±25.8, LOOH 20.0±33.9 and TBARS 54.8±158.1, but only the TBARS increase was significant (P = 0.573, 0.298 and 0.039, respectively). A harsher form of cooking, for example oven roasting, might increase the production of lipid peroxidation products and make it easier to assess the impact of the antioxidant effects of the wine but may introduce complexity by different effects on the core and surface. In this study, a central portion of the meat was analysed that reflected heating to approximately 90°C. The decrease in marinated, cooked meat of LOOH and TBARS, compared with those in unmarinated, cooked meat, was not significant (P = 0.132 and P = 0.269, respectively), although there was a trend to decrease the CD by marinating the meat in wine (*P* = 0.084). Cooked, marinated meat displayed the following results: CD 29.3±13.2, LOOH 14.4±18.9 and TBARS 37.4±93.0 nmol/mg lipid, as seen in Table 2 and Figure 1. This suggests a protective effect from the wine, at least for CD.

Red meat is commonly consumed and was therefore the meat of choice for this study. Poultry might resist lipid peroxidation during cooking better owing to the greater MUFA content. Fish is of interest due to a high PUFA content. However, it is not traditional to marinate chicken and fish, although the former may be cooked in wine. Meats are susceptible to fatty acid oxidation, due to relatively high concentrations of unsaturated fatty acids in membrane phospholipids as well as exposure to haem and non–haem iron, especially when barriers are breached during cooking. Although the concentrations of oxidised lipids in foods are usually lower than that required to generate acute toxicity, the cumulative long–term effects of ingestion of small quantities of these compounds are not known. Lipid peroxidation is associated with discolouration (Rhee et al., 1986) and

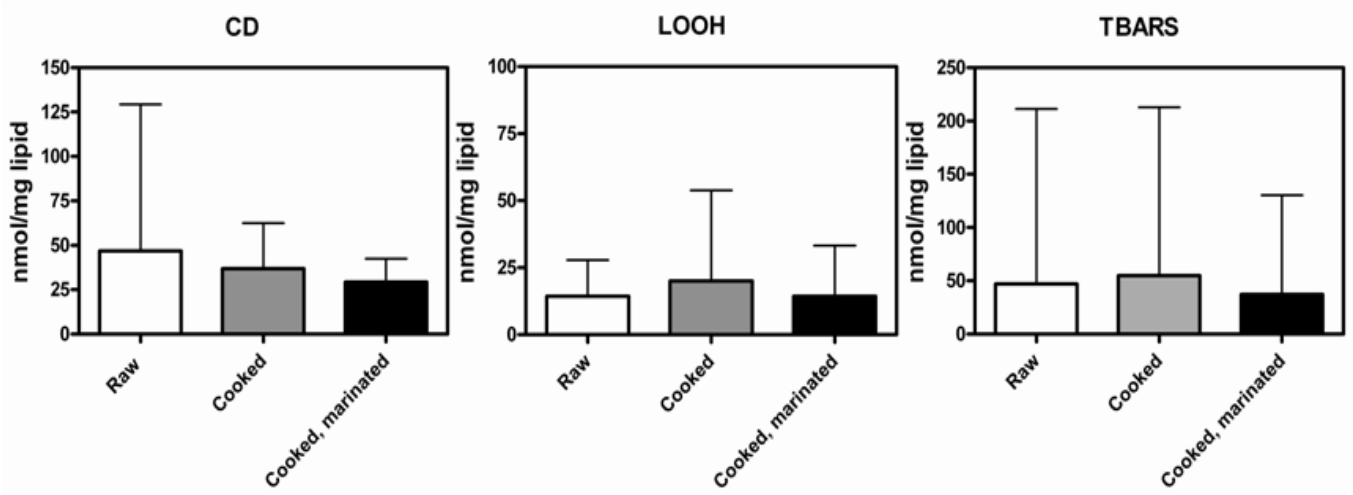

Figure 1. Lipid peroxidation products (CD, LOOH and TBARS) in nmol/mg lipid, in raw meat and after cooking with and without wine marinating. Values are means ± SD (n = 22).

flavour deterioration (Rhee et al., 1998) in uncooked meat, but little is known about peroxidation changes between the abattoir and consumption of meat.

Other studies also found big differences in the quality attributes of beef (Badiani et al., 2002; Jakobsen et al., 2000). The different beef muscles vary considerably in their cooking properties, such as their cooking times. Also, the chill temperature and the time of ageing may influence variation (Maher et al., 2004). The contact with oxygen, pre– and post–cooking, may also be responsible for the oxidative instability of cooked meat (Smiddy et al., 2002; Brewer, 2004) but even when meat is cooked in an oxygen–free atmosphere, lipid peroxidation products can still be detected (Smiddy et al., 2002). Vacuum–packing is a favoured method for packaging meat in order to reduce lipid peroxidation. The high variability in lipid peroxidation recorded in this study warrants studies that track the meat from the abattoir to point of sale and investigate factors that influence the peroxidation.

True marinade recipes contain spices, oils and vinegar that can also influence lipid content, change reactions with different pH and provide additional antioxidants. The effect of wine was specifically evaluated in this study. Its ability to penetrate the meat was not studied. While it is possible that small molecules may diffuse in, it is unlikely that large molecules such as tannins will. Perforating the meat might have enhanced penetration, but was not done because it is not traditional. The volume of marinade covered the meat, limiting oxygen exposure owing to its poor solubility in water.

Oxidation of lipids in the meat may produce lipid hydroperoxides and aldehydes that may react with the meat protein. The aldehydes in particular are a group of very active products and they readily react with the amine groups of lysine, cysteine and glutathione, particularly in the presence of water, causing the formation of insoluble macromolecules such as 2,4,6–trimethyl–1,3,5–dithiazine and 2–pentyl–pyridine (Boskou and Elmadfa, 1999). The amounts of peroxidation intermediates measured in these studies may thus under–represent the peroxidation in that measurements apply only to these products in the lipid extract. No methods were set up to determine reacted peroxidation products with protein or nucleic acids.

The choice of microwave as a cooking method established reproducibility and a relatively gentle and diffuse means of cooking the meat. Grilling is difficult to reproduce and results in layering of temperatures which are often extreme. The choice of cooking container is important, with copper and iron containers possibly enhancing oxidative stress. In this setting, wine may chelate the pro–oxidant ions.

Variability is largely dependent on the meat and the storage conditions, and is possibly modified by wine during the marinating process, but not much during cooking. Conjugated dienes, being the most stable, seem to be the most reliable product for studying the change in lipid peroxidation. For most samples, the variability shown in Figure 2 indicates little difference in the change from raw to cooked meat, with and without marinating. However, a few samples behaved very differently. For CD, the 2 highest cooked samples displayed very different behaviour. These highly variable samples behaved similarly with respect to the LOOH and less so to the TBARS. In these samples, the marinade seemed to make a difference. A third sample responded similarly with regards to the CD, but not to the LOOH and TBARS. A fourth sample displayed much higher concentrations of TBARS, in the raw state as well as after cooking with and

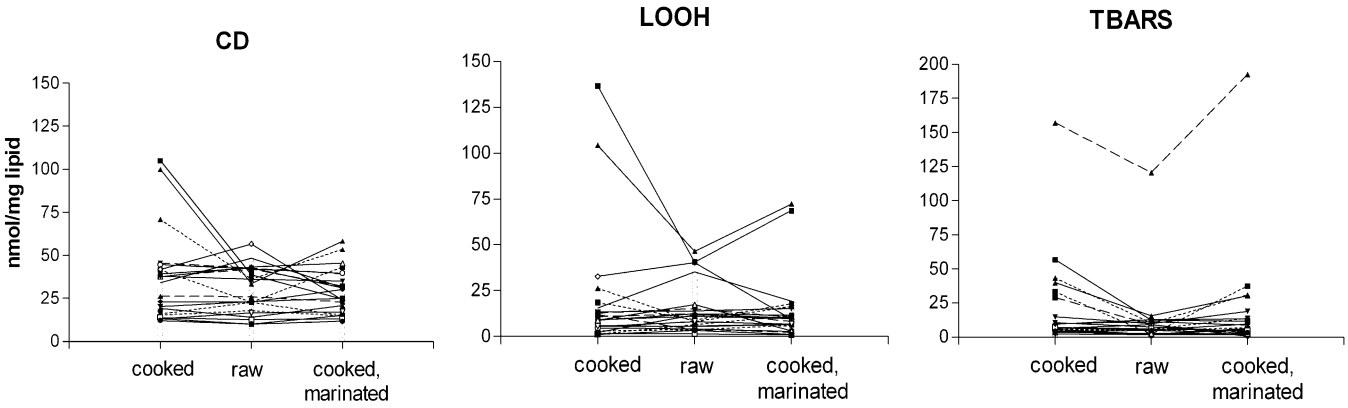

Figure 2. Lipid peroxidation products (CD, LOOH and TBARS) determined in individual beef samples. The 3 panels compare the raw meat with the cooked state as well as the marinated cooked state: n = 22.

without marinade. This particular sample did not seem to respond to marinating, but only with regard to the TBARS, and not the CD and LOOH. Taken together, the trend that emerges is that CD and LOOH, being specific intermediates in the chain of events, may reflect actual peroxidation better than TBARS, which appear to reflect a reserve of PUFA yielding reactive scission products. Future studies in this field might include better standardization and compositional analysis of the meat, the use of abattoir samples with defined storage conditions and more intense cooking conditions.

Conclusions

This study examined the impact of red wine on lipid peroxidation during the storage overnight at 4°C and cooking of relatively fat -free beef. There were 2 main findings from this study. Firstly, there was wide variation inherent in the lipid peroxidation status in the meat for all three products measured. Such baseline variability makes systematic studies difficult. Secondly, marinating with wine showed a trend towards lowering the CD concentration in the meat induced by cooking. It is possible that the red wine marinade may protect red meat from undergoing lipid peroxidation when cooked, but favourable effects may be masked by the high variability of the baseline peroxidation markers.

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