Food Chemistry 184 (2015) 57–64

Contents lists available at ScienceDirect

Food Chemistry

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

The impact of beef steak thermal processing on lipid oxidation and postprandial inflammation related responses

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ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form 25 February 2015
Accepted 18 March 2015
Available online 24 March 2015

Keywords:
Lipid oxidation
Oxidative stress
Beef steak
Pan frying
Sous vide cooking
Conjugated dienes

ABSTRACT

Oxidised lipid species, their bioavailability and impact on inflammatory responses from cooked beef steak are poorly characterised. Oxidised lipid species from pan-fried (PF) and sous-vide (SV) thermally processed beef were determined with UHPLC-ESI/MS. Twenty-three lipid oxidation products increased with thermal processing and differences between the PF and SV steaks were measured. Fifteen oxidised lipids were measured in post-meal plasma after a cross-over randomised clinical study. Postprandial plasma inflammatory markers tended to remain lower following the SV meal than the PF meal. High levels of conjugated dienes were measured in the HDL fraction, suggesting that the protective effect of HDL may extend to the reverse-transport of oxidised lipid species. Oxidised lipids in a single meal may influence postprandial oxidative stress and inflammation. Further studies are required to examine the lipid oxidative responses to increased dietary oxidative lipid load, including the reverse transport activity of HDL.

1. Introduction

Red meat is a rich dietary source of high quality protein, B group vitamins and minerals as well as fatty acids (McAfee et al., 2010). However, many epidemiological studies have linked high consumption of red meat, particularly processed red meat with colorectal cancer (Kim, Coelho, & Blachier, 2013; Magalhaes, Peleteiro, & Lunet, 2012).

Meal ingestion imposes acute oxidative and inflammatory loads which may impact long term disease risk (Le, 2014; van Diepen, Berbée, Havekes, & Rensen, 2013). Oxidative reactions can be initiated by endogenous components of the body, such as metal ions, superoxide radicals or lipoxygenase. Less well understood is the role of oxidised lipid species (Catsburg et al., 2014; Parr, Hjartaker, Lund, & Veierod, 2013). Of the available data, lipid oxidation products in food have been suggested to contribute to oxidative stress (Mannello, Tonti, & Medda, 2009; Yeh et al., 2010). Experimental animal studies have further shown that dietary oxidised lipids are absorbed and incorporated into circulating lipoproteins (Suomela, 2006; Suomela, Ahotupa, & Kallio, 2005). Yet the relationship between dietary oxidised lipids, absorption and lipoprotein transport is not described in humans. There are however data demonstrating the presence of dietary oxidised lipids present within human lipoproteins (Ahotupa, Suomela, Vuorimaa, & Vasankari, 2010; Staprans, Rapp, Pan, Kim, & Feingold, 1994). Lipoproteins, including chylomicrons and LDL, transport the oxidation products into the circulation and may exert pro-oxidative effects in peripheral tissues, whereas lipid oxidation products transported with the HDL are speculated to have a protective and antioxidant effect (Ahotupa et al., 2010).

The present study aimed to investigate the impact of two contrasting thermal processing methods in beef rump steak (pan-frying (PF) and sous-vide (SV)) on the generation of oxidised lipid species. The second aim of this study was to determine whether the oxidised lipid species can be identified in the post-meal circulating lipoproteins following a meal composed of these beef

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http://dx.doi.org/10.1016/j.foodchem.2015.03.059
0308-8146/

rump steaks. Thirdly, the aim was to determine whether these differentially processed beef rump steaks elicited postprandial differences in the oxidative stress and inflammatory responses in a randomised cross-over clinical trial.

2. Materials and methods

2.1. Beef rump

Beef rump steaks were sourced from the Waikato region, New Zealand. Animals were grass fed and slaughtered at around 24 months of age. The parameters of the steak were standardised to 270 ± 20 g and 1.5 cm thickness with 0.25 to 1 cm intramuscular fat layer.

The moisture, ash, protein and lipid content of steaks ($n = 6$) were determined according to Association of Official Analytical Chemists (AOAC, 1995). In brief, the moisture content was measured using a vacuum oven (Heraceus Vacutherm, Waltham, USA) at 100 °C, and ash content with a muffle furnace at 200 °C and 525 °C. Total lipids were extracted using a Soxhlet method using a Soxtherm Extraction Unit (Gerhardt, Königswinter, Germany), and protein measured through sulphuric acid (Mallinckrodt, St Louis, USA) digestion (Labortechnik B-426; Buchi, Flawil, Switzerland) followed by nitrogen distillation and hydrochloric acid (Romil-SA, Cambridge, England) titration. Only the steak lipids were analysed for lipid content, although the meal used for the intervention trial had other lipid sources (i.e. bread). The reason was that this lipid amount was low compared to the steak lipids (1.0 g), was not different between treatments, and was not thermally treated.

2.2. Thermal processing

For PF, a non-stick frying pan was preheated to 240 °C and the steak was grilled on one side for 3 min and 2 min on the alternate side. For SV, a water bath was preheated to 80 °C and vacuum-packaged steak was immersed and maintained within the water bath for six hours. PF and SV steak samples were either consumed immediately for the clinical intervention or were frozen at −20 °C until fatty acid analysis.

2.3. Fatty acid composition of beef steaks

For gas chromatographic (GC) analysis of the fatty acid composition of the beef steaks, samples from three different steaks (5 samples per steak) per thermal treatment were pooled and two replicates per thermal treatment were analysed. The lipids were extracted twice from the meat samples with a modified Folch's chloroform-methanol extraction (Folch, Lees, & Stanley, 1957) after homogenisation with an Ultra Turrax T25 (IKA, Janke and Kunkel, Staufen, Germany). Homogenised meat samples were dissolved in 20 mL of methanol and 40 mL of chloroform and homogenised after each addition with the Ultra Turrax T25. The samples were vacuum filtered and poured into a separatory funnel. The extraction was repeated with the filtration residue. Both the filtrates were poured into the separation funnel. An aliquot of 37.5 mL of 0.88% potassium chloride (KCl) solution was added to the filtrate and the blend was mixed to separate the two phases. The upper phase was removed and the lower phase was washed with 75 mL of KCl–methanol (1:1 v/v) solution and lowered into a boiling flask. The solvent was evaporated in a rotary evaporator. The samples were transferred in chloroform from the boiling flask into an autosampler bottle. Chloroform was evaporated to dryness and the remaining extracts were weighed. The fatty acid methyl esters (FAME) were prepared with a sodium methoxide method (Christie, 1982). In short, the extracted lipids, 1 mg of lipids in 1 mL of hexane, were suspended in 1 mL of dry diethyl ether; then 25 μL of methyl acetate and 25 μL of sodium methoxide were added and the blend was incubated for 5 min while shaken at times. The reaction was stopped with 6 μL of acetic acid. The tubes were centrifuged at 2000*g for 5 min, after which the supernatant was removed and gently evaporated to dryness. The resulting FAMEs were dissolved into 1 mL of hexane. Since fatty acids with uneven carbon number are typical for ruminant meat, no internal standards were used in the procedure.

The FAMEs were analysed with a Shimadzu GC-2010 gas chromatograph equipped with a flame ionisation detector (Shimadzu Corporation, Kyoto, Japan). A DB-23 column was used (60 m, i.d. 0.25 mm, liquid film 0.25 μm, Agilent Technologies, J & W Scientific, Santa Clara, CA). Supelco 37 Component FAME Mix (Supelco, Bellefonte, PA), 68D (Nu-Chek-Prep, Elysian, MN) and GLC-490 (Nu-Chek-Prep, Elysian, MN,) were used as external standards.

2.4. Diene conjugation

For diene conjugation samples from three different steaks per thermal treatment were pooled and three replicates per thermal treatment were analysed. The diene conjugation resembling the amount of lipid peroxidation was measured by dissolving the lipids extracted with chloroform-methanol in 2,2,4-trimethylpentane and measuring the absorbance spectrophotometrically at 234 nm.

2.5. UHPLC-ESI/MS analysis of oxidised lipids

For UHPLC-ESI/MS analysis, the extracted lipids ($n = 2$, descripted in Section 2.3) were filtered with 0.45-μm polytetrafluoroethylene membrane filter and dissolved in 2-propanol for UHPLC-MS analysis. Previously synthesised oxidised triacylglycerol standards were used as references: 22:0-18:1(keto)-22:0, 22:0-18:1(hydroxy)-22:0, 22:0-18:1(hydroperoxy)-22:0, 22:0-9:0(aldehyde)-22:0, 22:0-18:1(epoxy)-22:0, 18:0-18:1(hydroxy)-18:0, 18:0-18:1(epoxy)-18:0, 18:1(hydroperoxy)-18:1-18:0, 18:0-18:2(diepoxy)-18:0, 18:0-18:2(hydroperoxy)-18:0, 18:1(epoxy)-18:1(epoxy)-18:0, 18:0-18:1(hydroperoxy)-18:0, 18:1(hydroperoxy)-18:1(hydroperoxy)-18:0 (underlined double bonds are replaced by the epoxy groups). Internal standard, 1,2,3-tripentadecanoyl-*sn*-glycerol was purchased from Larodan Fine Chemicals AB (Malmö, Sweden).

A Waters Acquity UPLC (Waters co., Milford, MA) was used with Waters Quattro Premier triple quadrupole mass spectrometer equipped with electrospray ionisation (ESI) probe. A Phenomenex (Torrance, CA) Kinetex C18 column (2.1 × 100 mm, 1.7 μm particle size) was used. Water–acetonitrile (1/4, v/v) was used as solvent A and acetone as solvent B at 60 °C column oven temperature. Both solvents contained 0.1% formic acid and 0.1 mM lithium formate.

The capillary voltage was set at 4.5 kV, the cone voltage at 350 V, the extractor voltage at 8 V and the RF lens voltage at 0 V. The source temperature was set at 100 °C and the desolvation temperature at 350 °C. The desolvation gas (N_2) flow was set at 1000 L/h and the cone gas flow at 150 L/h. Full scans of mass range of m/z 190 to 1100 were acquired in positive ionisation mode.

Amounts of oxidised lipids in the samples were evaluated by extracting selected ion chromatograms from the total ion current (TIC) mass chromatograms and comparing the integrated areas of the peaks. Oxidised lipids were identified by retention times and mass spectra.