



Effects in ewe diet of rosemary by-product on lipid oxidation and the eating quality of cooked lamb under retail display conditions

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ABSTRACT

Distilled dietary rosemary leaf (DRL) was tested to prevent lipid oxidation and sensory deterioration of cooked lamb under retail display conditions. Pregnant sheep were fed with a basal diet supplemented by 0%, 10% and 20% DRL. Cooked lamb fillets were stored for 0, 2 or 4 days at a temperature of 4 °C in a display cabinet and re-heated, simulating catering practices. The cooked lamb suffered rapid lipid oxidation and odour and flavour spoilage associated with slight rancidity and warmed-over flavour, and, to a lesser extent, with loss of colour and juiciness. DRL feeding delayed lipid oxidation measured as TBARS and volatile compounds, this being more effective in the first two days of storage. 10% and 20% of DRL provided equal antioxidant capacity. However, DRL feeding hardly prevented sensory deterioration, although incipient rancidity was delayed. Feeding DRL to ewes contributed to extend the shelf life of cooked lamb under retail display conditions.

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1. Introduction

Lipid oxidation reactions are the main determinant of the eating quality and shelf life of chilled-stored cooked meat (Jensen, Skibsted, & Bertelsen, 1998). Prior to consumption, cooked meat can remain for hours or even days in a refrigerated display, exposed to the oxidising action of air and light, which alter the colour, aroma and flavour before microbial spoilage occurs (Ahn, Grün, & Mustapha, 2007). The first sensory signs of cooked meat spoilage include loss of odour and flavour. The terms “warmed-over flavour” (WOF) (Timms & Watts, 1958) and “meat flavour deterioration” (MFD) (Spanier, Vercellotti, & James, 1992) have been coined to describe the overall increase in off-flavour notes and loss of quality in desirable meat flavour. These phenomena become more noticeable when refrigerated cooked meat is re-heated (Lyon, 1993). Lamb is a common ingredient of many pre-cooked dish specialities. Compared to beef or pork, lamb contains high levels of fat, and particularly ω -3 polyunsaturated fatty acids capable of being oxidised (Wood et al., 1999). The shelf life of lamb-based dishes can be extended by the addition of preservatives, but this is a practice that is increasingly being rejected by consumers who demand natural food. An alternative strategy to inhibit meat oxidation involves increasing the level of endogenous antioxidants through

diet. Endogenous antioxidants are metabolized and deposited in muscle, especially tissue membranes in which their antioxidant actions are more effective (Botsoglou et al., 1994; Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008).

The use of agro-industrial by-products in animal nutrition as a source of natural antioxidants is a promising development because by-products hardly increase the cost of animal feeds and EC regulations (EC No. 1831/2003) restrict the use of animal feed additives. Rosemary by-products (*Rosmarinus officinalis* L.) in particular have been successfully used in animal feeds as a source of antioxidants. Experimental diets including rosemary have been tested on broilers (Basmacioğlu, Tokuşoğlu, & Ergül, 2004; Lopez-Bote, Gray, Gomaa, & Flegal, 1998), pigs (Dal Bosco, Castellini, & Cardinali, 2005; Haak et al., 2006), turkeys (Botsoglou, Govaris, Giannenas, Botsoglou, & Papageorgiou, 2007; Govaris et al., 2007), ostriches (Abou-Arab & Abu-Salem, 2010) and sheep (Moñino et al., 2008; Nieto, Díaz, Bañón, & Garrido, 2010). Rosemary diets inhibited lipid oxidation in refrigerated raw meat. However, their antioxidant effectiveness on lipids in cooked meat is doubtful (Botsoglou et al., 2007; Dal Bosco, Castellini, & Cardinali, 2005; Haak et al., 2006).

Distilled rosemary leaf (DRL) is a by-product of essential oil, which is rich in antioxidant polyphenols. Moñino et al. (2008) supplemented the diets of ewes during gestation and lactation with 10% or 20% DRL, so that active concentrations of rosemary polyphenols were reached in lamb muscle, without any detriment to

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animal performance. These polyphenols include acids such as carnosic, rosmarinic, caffeic, ferulic and coumaric, plus carnosol, hesperidin, naringin, luteolin, apigenin and genkwanin. In further studies, diets supplemented with DRL also led to an increase in the shelf life of raw lamb packed in an oxygen-rich atmosphere and stored under retail conditions (Nieto et al., 2010). DRL diets preserved colour, odour and flavour, delayed rancidity, inhibited lipid oxidation, and even moderately reduced total viable counts. However, the studies failed to clarify whether the endogenous antioxidants from rosemary were effective in preventing lipid oxidation in cooked lamb under retail display conditions. Cooked lamb is a more oxidised substrate than raw lamb. Therefore, a greater concentration of antioxidants in muscle may be needed. Furthermore, polyphenols from rosemary can be degraded by heating, thereby decreasing their potential antioxidant activity on cooked meat. The possibility of producing lamb with a high level of endogenous antioxidant from rosemary represents an opportunity to prolong preservation of cooked dishes. For this reason, the aim was to investigate whether dietary DRL supplements given to pregnant ewes delays lipid oxidation and sensory spoilage of the subsequent cooked lamb meat.

2. Materials and methods

2.1. Animals and diet

Twenty-seven pregnant ewes (Segureña breed) were randomly assigned on the basis of their age (3.2 years old) and body condition (2.6 ± 0.1) into three homogeneous groups. Body condition was calculated following Russell and Doney (1969). The ewes were given a basal diet consisting of 1.3 kg feed per animal and day. For further details of the chemical composition of their experimental diets, including polyphenol content, refer to Moñino et al. (2008) and Nieto et al. (2010). For the experimental DRL diets, the basal diet was replaced by 10% and 20% of DRL, in the form of a pellet made from 50% barley and 50% DRL. Steam-distilled rosemary leaves were obtained from a local company (Nutrafur-Furfural Español S.A, Murcia, Spain). Leaves were steam-distilled for 3 h using a distillation system with a stainless steel steam boiler. Dietary total polyphenols were 749 mg kg^{-1} (Control), 2062 mg kg^{-1} (R10) and 3375 mg kg^{-1} (R20), including gallic acid, caffeic acid, ferulic acid, coumaric acid, naringin, hesperidin, luteolin, rosmarinic acid, apigenin, genkwanin, carnosol and carnosic acid (Moñino et al., 2008). The sheep given these diets were fed for 240 days, coinciding with their gestation and lactation periods, in order to study the quality of the subsequent lamb meat. Both the sheep and lambs were intensively reared on a research farm (CIFEALorca, Murcia, Spain). Following the practices recommended by Segureño farmers, all the lambs were weaned when they reached the weight of $13 \pm 1 \text{ kg}$ and were fed with commercial fattening pellets until they reached the slaughter weight of $25 \pm 2 \text{ kg}$. Finally, the lambs were slaughtered in a local slaughterhouse according to EC Regulations. The carcasses were stored at 2°C for 24 h in a cooling room.

2.2. Meat sampling

The legs were removed from the carcasses, boned by a professional butcher, vacuum packed, frozen and stored at -20°C for 1–3 months. The frozen boned legs were brought up to -5°C and cut with a slicing machine (V220/380, Mobba, Badalona, Spain) producing 1.5 cm thick rounds of variable diameter depending on the muscle section. Fillets from the thigh (*gluteus*, *quadriceps*, *biceps femoris*, *semimembranosus*, *semitendinosus*, *adductor* and others minor muscles) were sampled.

Once defrosted, the meat was cooked on a double electric hot plate (Media Liscia, Silanos, Milano, Italy) at 150°C until they reached an internal temperature of 72°C for two minutes, as controlled by a portable T200 thermometer (Digitron Instrumentation Ltd., Merd Lane, Hertford, United Kingdom). After cooking, the lamb fillets were packaged in a transparent polystyrene stray Aer-pack B5–37, covered with an oxygen-permeable polypropylene film ($650 \text{ cm}^{-3} \text{ m}^{-2} \text{ h}^{-1}$ per 23 h) (Raelma Industries Madrid Ltd., Spain) and stored at 4°C for 0, 2 and 4 days in a display cabinet (Helkama, Finland) lit by white fluorescent light (650 lux), simulating retail display conditions for catering use. Samples were analysed in triplicate.

2.3. Analysis of thiobarbituric acid-reactive substances

Lipid oxidation was expressed as thiobarbituric acid-reactive substances “TBARS” (mg MDA kg^{-1}), as determined by Botsoglou et al. (1994), using a UV2 spectrophotometer (Pye Unicam Ltd., Cambridge, United Kingdom).

2.4. GC-MS analysis

2.4.1. Reagents

Pure reference standards for hexanal, 1-penten-3-ol, heptanal, 1-pentanol, octanal, 1-hexanol, nonanal, 1-octen-3-ol, 1-heptanol, (*E*)-2-nonenal and (*E*)-2-octen-1-ol were supplied by Acrös Organics (Geel, Belgium).

2.4.2. Solid-phase microextraction

The headspace volatile constituents of cooked lamb meat were isolated using a solid-phase microextraction “SPME” device (Supelco Inc, Bellefonte, PA, USA) as described by Steffen and Pawliszyn (1996). The fibre used was coated with $100 \mu\text{m}$ of Carboxen-Polydimethylsiloxane. The SPME fibre was preconditioned prior analysis at 280°C for 60 min. The headspace sampling technique was used as follows: Samples of 1 g of minced cooked lamb meat were put into a 4 mL vial, and held in a water bath at $(37 \pm 1^\circ\text{C})$ throughout the assay. In order to achieve equilibrium between each sample and its headspace before SPME analysis, the samples were maintained in this condition for 5 min. According to the method described by Estevez, Morcuende, Ventanas, & Cava, (2003), the fibre was exposed above the sample headspace for 30 min. This sampling method was selected because in such conditions most of the analytes might have reached equilibrium between the three phases that constitute the system. After the extraction time, the SPME device was removed from the meat sample vial and inserted directly into the injection port of the GC-MS.

2.4.3. GC-MS analysis of SPME samples

Once the analytes were adsorbed onto the coatings, they were subjected to analysis by GC-MS. For this portion of the work, a Hewlett–Packard 5890 Series II Plus Gas Chromatograph, equipped with a DB-Wax 52 CB (Polyethylenglicol–Carbowax) column with $30 \text{ m} \times 0.32 \text{ mm i.d.}$ and $1.0 \mu\text{m}$ film thickness was used. The stationary phase was supplied by Agilent Technologies (Palo Alto, CA, USA). Helium was used as the carrier gas (constant pressure, β -ionone eluting at 47.02 min). The analytes were desorbed at 250°C for 3 min in the injection port of the chromatograph. The volatiles were desorbed immediately onto the column with no need for cryofocusing, as the 3 min. desorption time was sufficient to ensure against sample-to-sample interference. To improve the recovery of highly volatile components, a split less injection was applied during the first 0.3 min of injection. After this time the split ratio was set to 50:1. The GC was linked to an Agilent Model 5972 inert mass spectrometry detector (Agilent, Palo Alto, CA). For the DB-Wax.52 CB column, the initial oven temperature was set at

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