Meat Science 110 (2015) 62–69

Contents lists available at ScienceDirect

Meat Science

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

Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere

Jordi Ortuño, Rafael Serrano, Sancho Bañón *

Department of Food Science and Technology and Nutrition, Faculty of Veterinary Science, University of Murcia, Espinardo, 30071 Murcia, Spain

ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form 8 July 2015
Accepted 9 July 2015
Available online 12 July 2015

Keywords:
Polyphenols
Vitamin E
Lipid oxidation
Protein oxidation
Sensory evaluation
Microbial quality

ABSTRACT

The antioxidant and antimicrobial effects on lamb meat of the dietary use of rosemary diterpenes and vitamin E were compared. Thirty fattening lambs were assigned to three diets: (C) control; (R) C plus 600 mg kg^{-1} carnosic acid and carnosol at 1:1 w:w; or (E) C plus 600 mg kg^{-1} α-tocopherol. The deposition of the dietary supplements in the muscle was determined. Microbial quality (total viable counts, Lactic Acid Bacteria, *Enterobacteriaceae*, *Escherichia coli* and *Salmonella spp*), oxidative stability (CIELab color, malondialdehyde and total carbonyls) and sensory attributes (appearance and odor) were determined in loin stored at 2 °C under 70% O_2/30% CO_2 atmosphere. Microbial quality was ensured by packaging and chilling. The E-diet was more effective ($P \leq 0.05$) than the R-diet in preventing meat oxidation, although the latter had antimicrobial effects on meat. The shelf life of lamb (assessed as the loss of freshness) could be increased by 5 (R-diet) or 10 (E-diet) days.

1. Introduction

The extension of raw meat shelf life is a long-held aim of the meat industry as it would lead to great savings (Gill, 1996). Current strategies to preserve meat mainly involve the use of chilling systems, protective packaging and preservatives. High O_2/CO_2 modified atmosphere packaging (MAP) is commonly used to extend the shelf life of red meat, such as lamb, in retailing conditions: CO_2 levels of 20–30% in atmosphere are often effective at inhibiting microbial spoilage (Gill & Tan, 1980; Stiles, 1991), whereas high O_2 levels delay metmyoglobin formation prolonging meat redness (McMillin, 2008). However, MAP also promotes lipid oxidation due to the pro-oxidizing action of oxygen, which generates rancid off-odors. The shelf-life of chilled raw lamb loin kept in high O_2/CO_2 MAP would be around 8–9 days, by which time browning and/or rancidity reduces meat acceptance, even though the microbial quality is ensured (Bañón, Méndez, & Almela, 2012; Camo, Beltrán, & Roncalés, 2008; Ripoll, Joy, & Muñoz, 2011). Therefore, improving the antioxidant status of meat could help to increase its shelf life.

Since 2008, the addition of antioxidants to meat cuts has not been permitted by the European Commission Regulation 1333/2008 on food additives. However, there is still the possibility of depositing antioxidants in the muscle by modifying the diet, thus increasing lipid and pigment stability during the meat retailing time. Vitamin E is the most widely used dietary antioxidant in animal feeding to prevent meat oxidation. The supplementation of sheep diet with vitamin E has been shown to offer protection against meat oxidation (Kasapidou et al., 2012; Kerry, Sullivan, Buckley, Lynch, & Morrissey, 2000; Lauzurica et al., 2005; López-Bote, Daza, Soares, & Berges, 2001; Turner, McClure, Weiss, Borton, & Foster, 2002; Wulf et al., 1995). The most biologically active form of vitamin E, α-tocopherol, is not degraded in the rumen but it is deposited in the muscle and fat tissues. The deposition of α-tocopherol in the muscle prevents lipid and pigment oxidation since it acts directly on the cell membranes (Higgins, Kerry, Buckley, & Morrissey, 1998). In order to achieve optimum protection in lamb meat effect, the minimum level of α-tocopherol for dietary inclusion has been established at around 500 mg kg^{-1} feed (López-Bote et al., 2001). In further studies, supplementation of the lamb diet with 250–1000 mg α-tocopherol kg^{-1} feed extended the shelf life of meat kept under MAP by up to 4 days due to its reduction of lipid and haem pigment oxidation, although the resultant sensory traits were not assessed. Moreover, vitamin E was seen to have no effect on microbial inhibition on meat (Álvarez et al., 2008; Lauzurica et al., 2005; Ripoll et al., 2011)

In recent years, several alternative dietary strategies based on plant phenolic antioxidants have been successfully checked for improving lamb meat preservation (Andrés et al., 2014, 2013; Jerónimo et al.,

* Corresponding author at: Department of Food Science & Technology & Human Nutrition, Veterinary Faculty, University of Murcia, Campus Espinardo, 30071 Murcia, Spain.
E-mail address: sanchoba@um.es (S. Bañón).

http://dx.doi.org/10.1016/j.meatsci.2015.07.011
0309-1740/

2012; Luciano et al., 2009; Rivas-Cañedo et al., 2013; Simitzis, Ilias-Dimopoulos, Charismiadou, Biniari, & Deligeorgis, 2013; Simitzis et al., 2008), and, in particular, by using rosemary and/or its derivatives (Bañón et al., 2012; Morán, Andrés, Bodas, Prieto, & Giráldez, 2012; Morán, Rodríguez-Calleja, et al., 2012; Morán et al., 2013; Nieto, Díaz, Bañón, & Garrido, 2010; Ortuño, Serrano, Jordán, & Bañón, 2014; Serrano, Ortuño, & Bañón, 2014). Carnosic acid and, in particular, carnosol, the main active diterpenes in rosemary, can be deposited in lamb muscle at sufficient levels to have antimicrobial and antioxidant effects on meat (Jordán, Castillo, Bañón, Martínez-Conesa, & Sotomayor, 2014; Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008). Among the tested rosemary derivatives used in animal feeding, oil-free extracts provided effectual and steady results, probably due to their lack of heterogeneity and the possibility of adjusting the desired proportion of active compounds. For example, raw lamb shelf life was extended by up to 4 days when the lamb diet was supplemented with 200–600 mg diterpenes kg^{-1} feed (Bañón et al., 2012; Ortuño et al., 2014; Serrano et al., 2014).

The supplementation of lambs with dietary rosemary extract (DRE) or vitamin E seems to yield similar benefits in terms of lamb preservation, although few comparative studies are available. When Caputi-Jambrenghi et al. (2005) used 500 and 1000 mg kg^{-1} of a non-specified DRE on air-packaged (AP) lamb, the results were poor compared with those obtained with 500 mg kg^{-1} α-tocopherol. Neither the use of 600 and 1200 mg carnosic acid kg^{-1} feed did exert comparable antioxidant effects to 600 mg kg^{-1} vitamin E on lamb under MAP (Morán, Andrés, et al., 2012; Morán, Rodríguez-Calleja, et al., 2012). The lack of carnosol in the DRE used by these authors could have limited the transfer of rosemary diterpenes to lamb muscle (Jordán et al., 2014). Therefore, there are still issues to be clarified concerning the use of dietary treatments based on vitamin E or DRE, particularly as regards their antioxidant and antimicrobial effectiveness on meat.

The aim of the present study was to compare the effects of DRE containing carnosic acid and carnosol and an equivalent dose of α-tocopheryl acetate on the shelf life of lamb loin in retailing conditions (high O_2/CO_2 MAP, refrigeration and fluorescent lighting).

2. Material and methods

2.1. Dietary supplements

Vitamin E (DL-α-tocopheryl acetate) and a DRE (containing carnosic acid plus carnosol) were used for the experimental lamb diets. Vitamin E (Microvit™ E Promix 50) was provided by Lorca Alimentación Animal, S.A., Murcia, Spain. DL-α-tocopheryl acetate was obtained by adsorbing vitamin E oil on a silica support to obtain a powder with a vitamin E purity of 500 I.U. g^{-1}. DRE was provided by Nutrafur-Furfural Español S.A., Murcia, Spain. The extract was obtained by successive extraction, drying and concentration stages using oil-free rosemary leaf and different solvents, including acetone and/or ethanol–water mixtures, as described by Del Baño et al. (2003). The resulting DRE was a dry (7.2 g water per kg extract) greenish-brown powder containing 0.31 kg rosemary diterpenes per kg extract (0.16 and 0.15 kg carnosic acid and carnosol, respectively).

2.2. Feed manufacturing

The same dosage level (600 mg kg^{-1} feed) of rosemary diterpenes or vitamin E was incorporated, together with other additives (vitamins, minerals, etc.), to the respective experimental feeds for fattening lambs. The temperature and pressure during the whole pelleting process (17 min duration) were 70–75 °C and 2 bar, respectively. After pelleting, the remaining contents of active antioxidants in the feed were established in 515 mg kg^{-1} diterpenes (252:263 carnosic acid:carnosol) and 525 mg kg^{-1} vitamin E. The HPLC methods for determining rosemary diterpenes and vitamin E in feed will be described in Subsection 2.5.

2.3. Animals and diets

Thirty weaned *Segureña* lambs (15 males and 15 females) with 13 ± 1 kg of live weight were selected from a collective feedlot. Lambs were individually identified and weighed, following which they were randomly assigned to one of three dietary treatments (10 lambs per treatment): lambs fed on a basal diet supplemented with 0.5 mg DRE kg^{-1} feed (R), 0.5 mg kg^{-1} α-tocopheryl acetate (E) or not supplemented (C) (Table 1). The lambs were fattened in individual pens located in an experimental farm from the period February to May. All handling practices followed the recommendations of the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes, and all of the animals were able to see and hear other lambs. All the animals were fed ad libitum with the corresponding fattening feed until they reached a live weight of 24 ± 1 kg. The fattening period lasted 50 ± 8 days. Finally, it was checked that the experimental diets given to lambs did not affect animal performance (average daily gain, conversion index or carcass weight) (data not shown).

2.4. Meat sampling

The lambs were slaughtered in a local abattoir according to EC Regulations and the carcasses were chilled at 2 °C for 48 h. After chilling, a professional butcher removed the *Longissimus thoracis et lumborum* (LTL) muscle from both sides of the carcasses. Two batches composed of six loins (two loins from the same lamb carcass per each diet) were processed weekly until all thirty lamb carcasses had been analyzed. Meat sampling was carried out in a commercial butchery. The loins were filleted (1.5 cm thick) and packaged in polystyrene trays B5-37 Aerpack (Coopbox Hipania, Lorca, Murcia, Spain), in COMBIVAC 95 bags (Alcom Food Packaging, Girona, Spain) composed of polyamide with a polyethylene sealing layer. Gas permeability of bag was: 50, 150 and 10 cm^3 ^{2} per 24 h bar for oxygen, carbon dioxide and nitrogen, respectively (measured at 23 °C and 75% R.H.). The air in the packs was replaced by a modified atmosphere composed of 70% O_2 and 30% CO_2 (v:v) (EAP20, Carburos Metálicos, Barcelona, Spain) using a discontinuous INELVI VISC 500 packer (Industrial Eléctrica Vilar, Barcelona, Spain).

Table 1
Ingredients and chemical composition of the experimental basal diet used in fattening lambs.

Ingredients (g/kg fresh weight)	
Maize	336.0
Barley	321.0
Soybean meal	180.0
DDGs	80.0
Wheat bran	25.0
Sugarcane molasses	20.0
Calcium carbonate	16.2
Crude soy oil	5.0
Sodium chloride	4.0
Sodium bicarbonate	3.0
Vitamin D3 (E-671) (IU/kg DM)	2000
Vitamin A (E-672) (IU/kg DM)	10000
Vitamin E (mg/kg DM)	30.0
Nutritional information (g 100 g^{-1})	
Crude protein	17.05
Ether extract (crude lipids)	3.72
Crude fiber	4.06
Crude ash	6.23
Calcium	0.86
Phosphorus	0.40
Sodium	0.26
Magnesium	0.20