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Use of dietary rosemary diterpenes to extend the preservation of sulphited-lamb products

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

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ABSTRACT

The use of dietary antioxidants is proposed for enhancing the preservative effects of sulphite in minced lamb products. Lamb diet was supplemented with 400 mg rosemary diterpenes (carnosic acid plus carnosol at 1:1 w:w ratio) per kg feed during the fattening stage. The patties were formulated combining meat from different sources (lambs given feed supplemented with rosemary extract and control lambs) and SO_2 addition levels (0, 150, 300 and 450 mg kg^{-1}). Several physical–chemical (reflectance, pH, WHC, carbonyls and volatiles from lipid oxidation), microbial (viable and lactic acid bacteria and coliforms) and sensory (appearance and odor) traits were determined in patties kept at 2 °C and packed under 70/30 O_2/CO_2 atmospheres. Dietary antioxidants extended the shelf life from 7.9 to 12.3 days in patties made with 450 mg kg^{-1} SO_2, but had little effect at lower SO_2 doses. Greater inhibition of browning, lipid oxidation, odor deterioration and rancidity was achieved by using supplemented lamb. The processing of lamb meat reinforced with endogenous diterpenes from rosemary seems to be a promising strategy for manufacturing sulphited raw products.

1. Introduction

Worldwide food safety strategies aim at linking the entire chain of food production and consumption. The use of natural antioxidants in animal feeding is considered as part of the "farm-to-fork" strategy involving animal production, meat processing, sale and consumption. Dietary antioxidants may be effective at improving the antioxidant status of meat, contributing to color and flavor stabilization and rancidity prevention, among other benefits. Dietary antioxidants are metabolized and deposited in muscle, especially tissue membranes, where their antioxidant actions are more effective (Botsoglou et al., 1994). Another possible application of dietary antioxidants might be in reducing potentially toxic preservatives, in particular, sulphites or nitrites, in meat products. Both preservatives degrade more slowly in substrates containing antioxidants and so lower concentrations may be required to achieve the same degree of preservation in meat products (Roller et al., 2002; Bañón et al., 2007).

Sulphur dioxide (SO_2), generally known as sulphite, is widely used as a preservative in minced raw meat due to its antimicrobial, antioxidant and, in particular, myoglobin stabilizing properties (Shayne, 2005). However, the potentially allergic and respiratory reactions associated with SO_2 ingestion, along with the increasing awareness of consumers as regards food safety, make necessary to reconsider its use as meat preservative. The Food and Agriculture and World Health Organisations established a maximum

* Corresponding author at: Department of Food Science and Technology and Human Nutrition, Veterinary Faculty, University of Murcia, Campus Espinardo, 30071 Murcia, Spain. Tel.: +34 868 888265; fax: +34 868 884147.
E-mail address: sanchoba@um.es (S. Bañón).

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permissible daily ingestion of 0–0.7 mg SO_2 per kg of body weight and considered especially important to minimize SO_2 in food with a high thiamine content, such as red meat (FAO/WHO, 1986). European Community Directive (95/2/EC) authorizes a maximum of 450 mg SO_2 per kg in meat products.

Phytochemicals, particularly, phenolic compounds, are being used as dietary antioxidants in different livestock species. Rosemary (*Rosmarinus officinalis*, L.) is recognized as a source of bioavailable polyphenols, such as carnosic acid and carnosol, for sheep (Moñino et al., 2008). Rosemary essential oils (Vasta et al., 2013; Smeti et al., 2013) oil-free leaf (Nieto et al., 2010) and oil-free extracts (Bañón et al., 2012; Morán et al., 2012; Ortuño et al., 2014) have been tested as dietary supplements for sheep. Depending on the method used, rosemary-based diets could induce antioxidant and, to a lesser extent, antimicrobial effects on lamb meat. Among rosemary derivatives, the use of typified dietary rosemary extracts (DRE) avoids the problems derived from the heterogeneity found in the phenol content of raw rosemary (Sotomayor et al., 2009). Supplementing the lamb diet with 200–400 mg carnosol-enriched extract per kg feed was seen to be effective in improving the antioxidant status of lamb meat (Jordán et al., 2014).

Several ingredients, such as green tea, grape seed, ascorbate or chitosan, have been successfully tested for reducing the quantity of SO_2 required for preserving minced raw meat, although certain sensory limitations were reported (Bañón et al., 2007; Serrano and Bañón, 2012). On the other hand, different rosemary extracts or oleoresins showed antioxidant and/or antimicrobial effects when they were added as ingredients to raw ground beef and pork (Rojas and Brewer, 2008), lamb patties (Baker et al., 2013) or buffalo and chicken patties (Naveena et al., 2013). The possibility of reducing SO_2 was not considered in the above studies. Indeed, no dietary studies on reduced-SO_2 meat products are available, although the dietary use of vitamin E was seen to be effective in extending the shelf-life of reduced-nitrite cooked ham (Dineen et al., 2001). Thus, dietary strategies involving rosemary could also help to limit the use of SO_2 in raw meat products. The aim of the present work was to study the possibility of enhancing the preservative actions of sulphites in lamb patties through supplementation with a DRE containing carnosic acid and carnosol. The possibility of reducing the SO_2 dose required to preserve the patties was also considered.

2. Materials and methods

2.1. Experimental design

The shelf life of lamb patties from two combined treatments (Dietary Rosemary Extract "DRE" × SO_2 added at different concentrations) was compared. Lamb diet was supplemented or not with carnosic acid and carnosol mixes during the fattening stage. Two homogeneous groups of nine lambs per each dietary treatment were selected and slaughtered to obtain the meat. Meat from leg was processed. The patties were formulated combining meat from two different sources (lambs given feed supplemented or not with 400 mg DRE kg^{-1}) and four different SO_2 addition levels (0, 150, 300 and 450 mg kg^{-1}). Nine patty manufacturing trials including the eight treatment levels were made. One boned leg from each diet group (Control "C" and DRE "R") was sampled during nine different trials (18 lamb legs in total). The resulting minced meat from each lamb leg was mixed and divided into four homogeneous batches to prepare the samples with different SO_2 addition levels. Ninety-six patties (15 ± 0.5 g) from each leg were used for sampling (6 patties × 4 SO_2 levels × 4 control days). The patties were packed and kept in retailing conditions. Several physical–chemical (Instrumental color, pH, Water Holding Capacity "WHC"), total carbonyls and volatiles from lipid oxidation), microbial (total viable bacteria, lactic acid bacteria and total coliforms) and sensory (appearance and odor) quality traits were determined.

2.2. Diets and animal production

Lamb diet was supplemented or not with DRE during the fattening stage. A typified DRE containing 0.3 kg diterpenes (carnosic acid and carnosol at 1:1 w:w) per kg extract was used (Nutrafur-Furfural, Murcia, Spain). The DRE was incorporated to the feed for lambs during pelletizing process (1.8 kg DRE per 1000 kg feed). After the pelleting process, the carnosic acid and carnosol contents were quantified by HPLC-MS (Moñino et al., 2008) in 186 and 213 mg kg^{-1} feed, respectively. The lambs (*Segureña* breed) were fattened in different pens located in the same farm during two different time periods (from November to March). Nine lambs per each dietary treatment were randomly selected from a larger group and were slaughtered (25 ± 1 kg slaughter weight) to obtain the meat. For more detailed information on DRE, diets and sheep rearing, see Ortuño et al. (2014).

2.3. Meat processing and sampling

The lambs were slaughtered in a local abattoir according to EC Regulations and the carcasses were chilled at 2 °C for 72 h in a cooling room. The legs were removed from the carcasses, boned by a professional butcher, vacuum packed, frozen and stored at −20 °C for a maximum of 3 months. The frozen boned legs were thawed at 2 °C overnight and minced (2 mm plate) using a P3298 cutter (Braher International, San Sebastian, Spain). Minced meat from *gluteus, quadriceps, biceps femoris, semimembranosus, semitendinosus, adductor* and other minor leg muscles were mixed for 5 min using an RM-60 mixer (Mainca Granollers, Spain). Twenty grams of sodium chloride salt per kg meat were added to the minced meat.

Eight different patty formulations were established combining the two diets (C and R) and four SO_2 addition levels (0, 150, 300 or 450 mg SO_2 kg^{-1} meat). The resulting treatments were denominated as C0, C150, C300, C450, R0, R150, R300 and R450. SO_2 was added in the form of sodium metabisulphite (Juan Martinez Perez, Murcia, Spain) using 5 mL of deionized water to dissolve it. The same amount of water was added to the non-sulphited patties. The mix, containing minced meat, sodium chloride, sulphite solution (or equivalent of water) was mixed for 1 min using an RM-60 mixer and then 15 ± 0.5 g patties were formed manually.

The patties were assigned to different control days and packed in polystyrene trays, B5-37 Aerpack (Coopbox Hipania, Lorca, Murcia, Spain), covered by 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 mL^2 per 24 h bar for oxygen, carbon dioxide and nitrogen, respectively (measured at 23 °C and 75% R.H.). The samples were packed in a modified atmosphere (MAP) composed of 70% O_2 and 30% CO_2 (EAP20, Carburos Metálicos, Barcelona, Spain) in a discontinuous INELVI VISC 500 packer (Industrial Eléctrica Vilar, Barcelona, Spain). The meat/gas ratio was approximately 0.03 kg meat per liter O_2/CO_2. After sealing, the atmosphere inside the bags was checked using a Dan Sensor gas meter (WITT Gasetechnik, Witten, Germany). No significant variation in the gas mixture was detected during storage. The packed patties were kept at 2 ± 1 °C for 0, 4, 8 or 12 days in a Climacell 707 display cabinet (MMM Medcentre Einrichtungen, München, Germany) continuously illuminated with white fluorescent light (800 lx), simulating retail display conditions. Samples were analyzed in triplicate.

2.4. Physical–chemical analysis

Patties spoilage was monitored by reference to different physical–chemical parameters related with meat discoloration (instrumental color and pH), exudation (WHC) and oxidation (total carbonyls and volatile lipid oxidation markers). Objective color was measured using a CR-200/08 Chroma Meter II (Minolta Ltd., Milton Keynes, United Kingdom) with illuminant D65, 2° observer angle, and aperture size of 50 mm and calibrated against a standard white tile. Reflectance measures were taken directly on the meat surface after a