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Effects of oregano extract on oxidative, microbiological and sensory stability of sheep burgers packed in modified atmosphere

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ABSTRACT

The aim of this study was to evaluate the effects of oregano natural extract added to sheep burgers packaged in a modified atmosphere for 20 days at 2 ± 1 °C, seeking antioxidant properties and healthiness appeal. Antioxidant capacity of the oregano extract was determined by Folin-Ciocalteau, DPPH• and FRAP methods. Three treatments of burgers were prepared: without antioxidant (control, CO), with 50 ppm BHT and with 1000 ppm oregano extract (ORE). The proximate composition (moisture, fat, protein and ash) of the meat product was determined and its stability was assessed through physicochemical (pH value, colour, lipid and protein oxidation, free fatty acids and volatile compounds profile), microbiological [total viable counts (TVC), *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria (LAB)] and sensory analysis (5-point rating scale). ORE treatment presented similar counts of TVC and LAB and, also, an equivalent capacity to slow lipid and protein oxidation after 20 days, in comparison to BHT. The total amount of volatile compounds increased during storage ($P < 0.01$) and all treatments showed a decrease ($P < 0.001$) on a* value. However, ΔE_{0-20} was higher ($P < 0.05$) for CO treatment, indicating visual colour changes perceived by consumers. The presence of natural extract prevented the loss of sensory qualities in sheep burgers up to 15 days of storage, being that changes in off-odour were consistent with the microbial results that indicate burgers spoilage. In conclusion, oregano extract presented antioxidant effects quite similar to BHT and thus, can be considered a viable solution for the production of sheep burgers with a healthier appeal.

1. Introduction

The lipid oxidation, microbial growth and colour changes are the main factors that influence the deterioration in meat and meat products. During storage, the oxidative processes promote major impacts on flavour, texture and nutritional value, and consequently, a loss in consumer acceptability (Rodríguez-Carpena, Morcuende, & Estévez, 2011).

The packaging system under modified atmosphere (MAP) is considered a very effective method to guarantee the quality and safety of foods, including fresh meats. The gases most commonly used are O_2 and CO_2 and can be applied individually or in combinations, according to the type of food and the desired effect (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007), being the efficiency dependent of numerous extrinsic factors, among them, the composition of gas and the ratio between them. However, lower temperatures are also necessary for greater efficiency and acceptance of the product by the consumer, enabling the prevention of oxidation of the bioactive components of food. For colour maintenance, storage under MAP with concentrations of oxygen up to 80% has been extensively studied (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007), as well as packages containing 20–30% CO_2, which may reduce the microbiological spoilage efficiently (Fernandes et al., 2014), and possibly also prevent the formation of carbonyl by blocking the amino group of the amino acids lysine or arginine (Jongberg, Skov, Torngren, Skibsted, & Lund, 2011).

As for delaying the occurrence of oxidative processes, synthetic antioxidants are commonly added during processing, aiming to increase the shelf life. But today, due to greater consumers awareness and concern regarding food safety and their high

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demand for a healthier diet, phenolic compounds have been explored as potential natural antioxidants for possible replacement of synthetic ones (Jennings & Akoh, 2009). The addition of plants and extracts that present antioxidant properties in meat products is a good opportunity to promote the benefic ingestion of a healthier food containing functional ingredients, without causing drastic changes in the eating habits of the population, taking advantage of the growing concern of consumers regarding their health (Hayes, Stepanyana, Allena, O'Grady, & Kerry, 2011; Karabagias, Badeka, & Kontominas, 2011).

Several authors have reported the effectiveness and the antioxidant potential of oregano, which is among the most active natural extracts (Aranha & Jorge, 2012), mainly due to the action of phenolic-type compounds present in *Origanum vulgare* L., which is generally the species most studied (Spiridon, Bodirlau, & Teaca, 2011). Oregano extract has high antioxidant properties, including when added to meat products (Velasco & Williams, 2011). Fernandes et al. (2015) proved that oregano extract showed a high antioxidant activity when compared to other herbs, and can replace synthetic antioxidant in lamb burgers without compromising the sensory acceptability.

In commercial herds for production of lambs, sheep are generally used until 6 or 7 years of age. These matrices, in advanced age, are discarded of the production systems slaughtered, but the carcass does not have the ideal quality characteristics as the young animals (lambs) (Zeola et al., 2005). As the sheep meat presents inferior properties, which directly influences their acceptance *in natura*, the preparation of meat products is a relevant alternative to encourage a better use of this meat, in order to increase the profitability of the ovine industry.

Therefore, due to the interest in antioxidants from natural sources and the lack of information about oregano extract added to sheep meat products, this study aimed to evaluate the antioxidant effects of this extract in association with MAP during storage of sheep burgers under refrigeration for 20 days.

2. Material and methods

2.1. Oregano extract

To obtain the extract, the methodology used was previously described by Fernandes et al. (2015). For extraction, the acetone/water/glacial acetic acid 70:28:2% (v:v:v) was used at ratio plant:solvent 1:20 (w:v). Finally, lyophilised extract was solubilised in water at a ratio of 1:4 (v:v). All the procedures were carried out at 45 °C. The extraction was carried out in triplicate and the aqueous extracts were kept under frozen storage (−20 °C).

2.2. Determination of phenolic content and antioxidant activity of the oregano extract

2.2.1. Estimation of total polyphenol content

Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method following Fernandes et al. (2015). A volume of 100 μL of plant extract was used, being the analyses performed in triplicate, and the results were expressed as mg gallic acid equivalents (GAE)/g of dry weight (dw).

2.2.2. Free radical-scavenging ability by the use of a stable DPPH• radical

The DPPH• radical-scavenging activity was determined according to the same study to determine polyphenol content, as extract aliquots of up to 170 μl were tested among 3.150 μL of 72 μM methanolic solution of DPPH• radical. Absorbance measurements commenced immediately and the decrease in absorbance at 515 nm was determined after 3 h. Analyses were performed in triplicate and the results were corrected for dilution and expressed as EC_{50} (μg/mL), based on a standard curve with a methanolic solution of trolox 500 μM.

2.2.3. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric reducing antioxidant power (FRAP), following the study cited previously; the assay was based on the reducing power of a compound (antioxidant). The FRAP reagent (3.400 μl) and sample solutions (100 μl) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 30 min. Analyses were performed in triplicate and the results were expressed as μmol trolox equivalent/g dw.

2.3. Preparation of sheep burgers

Three treatments of ground meat burgers [control (CO), butylated hydroxytoluene (BHT) 50 ppm and oregano extract (ORE) 1000 ppm] were manufactured in the pilot plant of *Centro Tecnolóxico da Carne*. Considering the concentration of the 28.57% of oregano extract, the addition of 1000 ppm was equivalent to 3.50 mL/kg of meat product. Sheep burgers of 100 g ($n = 2$ per treatment and storage time) were manufactured using crossbred sheep meat cuts and with low commercial value. The age of animals was from 6 to 12 years. After defrosting for 12 h, these cuts were minced, using a 6 mm plate in a refrigerated mincer machine (TOP-114, Talleres Ramon, S.L., Spain). The meat was mixed and compressed manually with 10 g of NaCl per kg of meat and 50 mg/kg of BHT or 1000 mg/kg of natural extract. Burgers were produced in moulds of 10 cm diameter and 1 cm height in a burger-maker (A-2000, Gaser, Girona, Spain). Sheep burgers were packed in 300 mm thick polystyrene trays, which were sealed with polyethylene film 74-mm thick and permeability <2 mL/(m^2 bar/day) suitable for gas mixtures (Viduca, Alicante, Spain). The packaging was carried out using a heat sealer (LARI3/Pn T-VG-R-SKIN, Ca.Ve.Co., Palazzolo, Italy), after injection of the gas mixture containing 80% O_2 + 20% CO_2. The trays were stored at 2 ± 1 °C under light to simulate supermarket conditions, being placed over metal shelving and receiving lux values in the range of 15–20 depending on the tray position (HT 306, Digital luxometer, Italy). The light source was conventional, so any wavelength or range, in this case UV, was not filtered. Analyses were carried out at 0, 5, 10, 15 and 20 days of storage period. The pH, colour, lipid oxidation, and microbial spoilage values were determined in duplicate for every sampling point.

2.4. Proximate composition of sheep burgers

The determination of moisture, protein (Kjeldahl N × 6.25) and ash were quantified according to the International Standards recommended—1442:1997 (ISO, 1997), 937:1978 (ISO, 1978) and 936:1998 (ISO, 1998), respectively—and the fat according to AM 5-04 (A.O.C.S., 2005). The analyses were determined in duplicate for each repetition in the zero time.

2.5. Microbial analysis of sheep burgers

Samples of 10 g of sheep burgers were aseptically weighed and homogenised with 90 mL of 0.1% sterile peptone water in a masticator blender (IUL Instruments, Barcelona, Spain) for 2 min at room temperature. Serial decimal dilutions were prepared for each sample in 0.1% peptone solutions (Merck, Darmstadt, Germany), and 1 mL or 0.1 mL of the samples in appropriate dilutions, in duplicate, were poured and spread for total count and selective agar