Meat Science 116 (2016) 253–259

Contents lists available at ScienceDirect

Meat Science

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

The application of natural antioxidants via brine injection protects Iberian cooked hams against lipid and protein oxidation

Mónica Armenteros [a], David Morcuende [b], Jesús Ventanas [a], Mario Estévez [b,*]

[a] SiPA, University of Extremadura, Av. de las Ciencias s/n, 10003 Cáceres, Spain
[b] IPROCAR Research Institute, University of Extremadura, Av. de las Ciencias s/n, 10003 Cáceres, Spain

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 15 February 2016
Accepted 16 February 2016
Available online 17 February 2016

Keywords:
Spices
Fruits
Cooked hams
Lipid oxidation
Protein oxidation

ABSTRACT

In response to the increasing consumers' mistrust in synthetic additives, the meat industry is focused on searching sources of natural antioxidants. Two different sources of natural antioxidants i) a mixture of garlic, cinnamon, cloves and rosemary essential oils and ii) a *Rosa canina* L. extract, were compared with a commercial antioxidant additive (Artinox®) for their ability to control protein and lipid oxidation in cooked hams after a settling period of 30 days and at the end of a chilled storage (150 days). The mixture of essential oils was the most effective against lipid oxidation while *R. canina* L. extracts were the most effective in controlling protein carbonylation at day 150. Accordingly, the use of these antioxidants via brine injection is a successful strategy to enhance the oxidative stability of cooked hams without modifying their physicochemical properties.

1. Introduction

Cooked ham is a delicatessen meat product highly appreciated among European consumers (Toldrá, Mora, & Flores, 2010). The type and volume of injected brine, the rate and length of tumbling and the cooking time and temperature are among the most relevant technological factors having an impact on the quality of the final product (Delahunty, McCord, O'Neill, & Morrissey, 1997). This complex processing system could affect the oxidative stability of muscle lipids and proteins, and hence, the nutritional and sensory properties of cooked hams (St. Angelo et al., 1987). The heating process disrupts the muscle cell structure, inactivates antioxidative enzymes and releases catalytic iron from myoglobin leading to an intense prooxidant environment in which both, lipids and proteins, can be affected (Estévez, 2011; Kanner, 1994). Additionally, prior to consumption, cooked ham is commonly chilled or frozen and during this period, the oxidizing action of air and light may alter the color, aroma and flavor of hams and hence, consumers' acceptability (Ahn, Grün, & Mustapha, 2007). A recent study (Utrera, Armenteros, Ventanas, Solano, & Estévez, 2012) reported the potential impact of protein oxidation on the color and texture of cooked hams elaborated from previously frozen raw material.

In order to inhibit oxidative reactions and as a result, protect muscle foods against their unpleasant effects, additives and ingredients with antioxidant potential are commonly used in the meat industry. Concerns over the safety of synthetic compounds and consumer's interest in the so-called natural ingredients, has prompted scientists to search alternative natural antioxidants derived from natural sources such as fruits, vegetables, seeds and spices (Cando, Morcuende, Utrera, & Estévez, 2014; Ganhão, Morcuende & Estévez, 2010; Rodríguez-Carpena, Morcuende, & Estévez, 2012; Yoo, Lee, Lee, Moon, & Lee, 2008). The potential of spices as natural antioxidants has been extensively reported and generally the Labitae family and particularly rosemary (*Rosmarinus officinalis*), are well known for their antioxidant properties (Nissen, Byrne, Bertelsen, & Skibsted, 2004). The effectiveness of rosemary essential oils as antioxidants has been demonstrated in a large variety of meat products including refrigerated beef, frozen pork patties or frankfurters (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2003; Estévez, Morcuende, & Cava, 2005; Estévez, Ramírez, Ventanas, & Cava, 2007; Estévez, Ventanas & Cava, 2007; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001). Other spices like cinnamon (*Cinnamomum verum*) or clove (*Syzygium aromaticum*) have been shown to decrease lipid oxidation as effectively as certain synthetic antioxidants in cooked meat products (Dwivedi, Vasavada, & Cornforth, 2006; Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007; McCarthy et al., 2001). Clove has been used as a condiment and reported to be rich in hydrolysable tannins and eugenol, which is known to show strong antioxidant activity (Ito, Murakami, & Yoshino, 2005). Cinnamon is rich in essential oils (mainly cinnamaldehyde and eugenol) which possess antimicrobial properties and also cinnamic aldehydes which have potential antioxidant properties (Murcia et al., 2004). However, the characteristic aroma of these spices limits their use (Craig, 1999). Garlic (*Allium sativum*) has also been proposed as a source of natural antioxidants. Garlic extracts have been reported to inhibit lipid oxidation

* Corresponding author.
E-mail address: mariovet@unex.es (M. Estévez).

http://dx.doi.org/10.1016/j.meatsci.2016.02.027
0309-1740/

by acting as hydroxyl radical scavengers in several meat products like dry-fermented sausages (Aguirrezábal, Mateo, Domínguez, & Zumalacárregui, 2000; Yang, Yasaei, & Page, 1993). Moreover, a wide variety of wild Mediterranean fruits and berries are found in the Mediterranean forest with powerful antioxidant activity. For instance, rose hips (*Rosa canina* L.) are rich in phenolic compounds and ascorbic acid and have been recently reported to be effective enhancers of the oxidative stability of meat patties (Ganhão, Morcuende, et al., 2010). However, only few studies have attempted to elucidate the effectiveness of mixtures of natural antioxidants against lipid and protein oxidation (Ahn et al., 2007). Additionally, the technological challenge of inoculating such phytochemicals in intact meat products such as cooked hams needs to be approached.

Accordingly, the aim of this work was to investigate the effects of a spice mixture (garlic, cinnamon, cloves and rosemary), and a phenolic-rich extract from *R. canina* on the physico-chemical properties and lipid and protein oxidation in cooked hams after a settling period of 30 days and at the end of a chilled storage (150 days). These effects were compared with those displayed by a commercial natural antioxidant (Artinox®) based on a mixture of citrate and erythorbate.

2. Material and methods

2.1. Materials

The raw material, left hind legs from pure *Iberian breed* × *Duroc* pigs were supplied by Consorcio de Jabugo, S.A. The antioxidant blend of essential oils of garlic, cinnamon, cloves and rosemary was obtained from Aditivos Industriales, S.A. (San José, Costa Rica), whereas the commercial antioxidant namely Artinox® consists of a combination of additives (sodium citrate: sodium erythorbate; 1:1 w/w) from Chemital S.A. (Terrasa, Barcelona, Spain). Fruits from rose hip (*R. canina* L.) were collected at full ripeness in the Cáceres region (Spain) and subsequently frozen at −80 °C until used. Other chemicals and reagents were purchased from Merck (Merck, Darmastadt, Germany), Panreac (Panreac Química, S.A., Barcelona, Spain) and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

2.2. Preparation of rose hip extracts

For the preparation of rose hip extracts, 30 g of sample including peel and pulp were cut into pieces while the seeds were carefully removed. The fruit was ground, dispensed in a falcon tube and homogenized with 10 volumes (*w*/*v*) of absolute ethanol. The homogenates were centrifuged at 2600 ×*g* for 10 min at 6 °C. The supernatants were collected and the residue was re-extracted once more following the procedure previously described. The two supernatants were combined, evaporated using a rota-evaporator and redissolved using 250 g of distilled water. Water solutions from rose hips were prepared and stored under refrigeration until used for the manufacture of cooked hams (less than 24 h). No insoluble fragments or residues were observed in the water solutions.

2.3. Animals

Thirty pure *Iberian breed* × *Duroc* pigs were free-range reared and fed on commercial diet based on grains and soybeans following the traditional livestock farming for Iberian pigs. The animals were slaughtered at 154 kg live weight. Carcasses were produced and deboned right after slaughter (hot deboning). Thirty left hind legs were obtained and subsequently vacuum-packed by reducing pressure to 10 mbar and using HF100 in the upper film with 34.0 cm^3/m^2 permeability to O_2 at 23 °C and 85% HR and HF200 in the lower film with 18.0 cm^3/m^2 permeability to O_2 at 23 °C and 85% HR (Mobepack Company, Salamanca, Spain) Vacuum-packed legs were kept frozen at −20 °C until their manufactured (5 months).

2.4. Manufacture of cooked hams

The above mentioned left hind legs were used as raw material for the manufacture of cooked hams. The frozen legs were thawed in a cold chamber by keeping overnight at +4 °C, similarly to the industrial process. The legs were deboned while subcutaneous and intermuscular fat, connective tissue and rind were removed. Hams were injected with brine to increase their weight by 21% and to obtain 0.3% pentasodium tripolyphosphate, 0.05% sodium ascorbate, 1.8% NaCl and 0.01% sodium nitrite in the injected hams. Cold brine (−2 °C) was injected using a multineedle injector equipped with a spraying system at constant pressure (Movistick 60PC, Metalquimia, Girona, Spain). Hams were then placed in a vacuum tumbler (Thermomat 8X, Metalquimia, Girona, Spain) at +4 °C at a pressure of 200 mbar. The tumbling schedule was set for the hams to rotate a total of 2000 times at 14 rpm. After a 48 h maturation period, the hams were packed in bags (CN330, Sealed Air, Italy), molded in 7 L capacity stainless steel molds (INOX.SERIE 444, Inoxnisge, Barcelona, Spain), placed in an automatic steam oven (FDC Cookline, Metalquimia, Girona, Spain) and cooked to an internal temperature of +66 °C using an external temperature of +68 °C. Upon completion of the cooking procedure, cooked hams were vacuum-packed following the aforementioned procedure for fresh hind legs and kept for five months at +4 °C. Microbiological analyses (anaerobic, enterobacterias, and *Listeria monocytogenes*) guaranteed the safety of the products during the whole storage (data not shown).

2.5. Experimental setting

Depending on the addition of different ingredients through the injected brine, hams were randomly divided into three groups (n = 10) and each group was assigned to one of the following three treatments: Treatment 1 (T1), treated with essential oils of garlic, cinnamon, cloves and rosemary (1 g per kg of meat); Treatment 2 (T2), treated with Artinox® powder (3 g per kg of meat), and Treatment 3 (T3), treated with the experimental rose hip extract (300 mL per kg of meat) as antioxidant. Prior to the injection, the different sources of antioxidants were dissolved in distilled water and mixed with the brine. Treatments 1 and 3 were equivalent in total phenolic content (≈500 mg GAE/100 g sample) as measured by the Folin–Ciocalteu method described by Singleton and Rossi (1965) and were selected in accordance to preliminary studies to guarantee protection against oxidation phenomena. The addition of Artinox® followed the producer recommendation. For comparative purposes, some data from a previous study (Utrera et al., 2012) was used as a Control (no added antioxidants) as the raw material and processing were identical to those applied to the present samples. The cooked hams (n = 10 per treatment) were randomly divided into two groups for sampling 1) after a settling period of 30 days (n = 5) and 2) at the end of the chilled storage (150 days; n = 5). At sampling times, semimembranosus muscles from cooked hams were manually removed and subjected to the physico-chemical and lipid and protein oxidation analyses.

2.6. Physico-chemical analyses

2.6.1. Proximate composition, water activity and pH of cooked hams

Moisture, total protein, heme iron, nitrite and chloride content were determined using AOAC methods (AOAC, 2000). The method of Bligh and Dyer (1959) was used for isolation of fat from each sample. The water activity (a_w) was determined by using a Lab Master a_w system (Scientific Solutions, Hamilton Drive Mentor, OH, USA) and the pH of the cooked hams was analyzed using a pH meter Crisom (Crison Instruments, S.A., Barcelona, Spain).

2.6.2. Fatty acid profile

Fatty acid methyl esters (FAMEs) were prepared by acidic esterification in the presence of sulfuric acid, following the method of López-Bote,