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Dog rose (*Rosa canina* L.) as a functional ingredient in porcine frankfurters without added sodium ascorbate and sodium nitrite

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ABSTRACT

The effect of dog rose (*Rosa canina* L.; RC), rich in polyphenols and ascorbic acid, on lipid and protein oxidation, colour stability and texture of frankfurters was investigated. Four treatments were prepared: with 5 or 30 g/kg RC extract and without sodium ascorbate and sodium nitrite (5RC and 30RC, respectively), a positive control (with sodium ascorbate and sodium nitrite; PC) and a negative control (without sodium ascorbate, sodium nitrite or RC extract; NC). Hexanal values were much higher throughout storage in NC compared to RC and PC frankfurters ($P<0.001$). The RC extracts protected against protein oxidation, but not as efficiently as PC ($P<0.05$). In the RC treated frankfurters, lower a* values were measured compared to PC due to the lack of sodium nitrite. In conclusion, dog rose can act as a natural antioxidant in frankfurters, but not as full replacer for sodium nitrite.

1. Introduction

Lipid and myoglobin oxidation is a well-known phenomenon in meat products, which results in discolouration, off-odours and off-flavours during storage (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Recently, protein oxidation has been linked with impaired meat quality, such as loss in juiciness and increased toughness of meat (Lund, Heinonen, Baron, & Estévez, 2011). Oxidation leading to degradation of lipids, proteins and pigments is one of the primary mechanisms of meat deterioration and can be prevented by including antioxidants in meat products. However, increased public concern over the safety and toxicity of synthetic additives, challenges the meat industry to find natural alternatives.

Natural alternatives can be antioxidant-containing extracts from herbs and spices (Yanishlieva, Marinova, & Pokorny, 2006). The main components contributing to the antioxidant effect of these extracts are phenolic compounds, due to their hydrogen-donating capacity and metal-chelating potential (Rice-evans, Miller, Bolwell, Bramley, & Pridham, 1995). The rose hips of dog rose (*Rosa canina* L., RC), are rich in phenolic compounds and ascorbic acid (Demir & Ízcan, 2001) and are therefore believed to be potential natural antioxidants. According to Ganhão, Estévez, Kylli, Heinonen, and Morcuende (2010), the main phenolic compounds present in the RC extracts are procyanidins and catechins. In fact, RC extracts have shown high antioxidant activities *in vitro* (Ganhão, Estévez, et al., 2010) and the addition to porcine burger patties has resulted in positive effects on colour stability, texture and on delaying lipid and protein oxidation (Ganhão, Morcuende, & Estévez, 2010a). However, the relative efficiency of phenolic-rich extracts when applied in different food matrices cannot be predicted even for very well-characterised extracts (Nissen, Byrne, Bertelsen, & Skibsted, 2004). Therefore, although RC has shown some promising beneficial effects on burger patties, the use of RC in different meat products needs to be further investigated.

Among other synthetic additives, the use of sodium nitrite should be revised according to consumers' opinion. However, this ingredient is very important for the meat industry as it plays a key role in colour development, fat oxidation, flavour and microbiological safety. It is particularly important for cooked meat products, such as frankfurters, as their characteristic pink colour originates from nitrosylhemochromogen, a reaction product of nitrite and denatured myoglobin (Pegg & Shahidi, 1997). However, the potential health risks related to residual nitrite levels and the formation of harmful N-nitrosamines in meat and meat products has led to demand for a significant decrease in the use of sodium nitrite (Honikel, 2008). As RC contains considerable amounts of nitrates (Cakilcioglu & Khatun, 2011), replacing sodium nitrite by nitrate could result in lower residual nitrite concentrations, reducing the risk of N-nitrosamine formation during ingestion. In addition, as RC also contains high amounts of ascorbic acid, its use could result in less sodium ascorbate being added during manufacturing.

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The objective of this research is to investigate the potential of RC as a functional ingredient in porcine frankfurters without added sodium ascorbate and sodium nitrite in terms of texture and colour, lipid and protein oxidative stability.

2. Materials and Methods

2.1. Extraction of dog rose

Fruits of dog rose (*R. canina* L., RC) were collected at full ripeness in June in the Cáceres region (Spain) and immediately frozen at −80 °C. For the extraction, whole fruits were ground and 5 or 30 g were weighed for low and high concentrated frankfurters, respectively. The ground fruits were homogenized 1:4 (w/v) in distilled water using an Ultra-Turrax. Subsequently, the homogenates were centrifuged (1048 ×*g*, 7 min, 4 °C) and the supernatant was filtered and collected. The residue was re-extracted once more with distilled water (1:2 w/v) following the procedure previously described and the filtered supernatant was combined with the first supernatant. Finally, distilled water was added to the residue, shaken by hand, filtered and an amount of that filtrate was added to the combined supernatant until 180 ml of extract was obtained. The total phenolic content and antioxidant capacity using the 1,1-diphenyl-2-picryl hydrazyl radical assay (see below) were determined and the extracts were stored in refrigeration until the manufacturing of the frankfurters (less than 24 h).

2.2. Manufacture of frankfurters

The experimental frankfurters were manufactured in a pilot plant and the same formulation was used for all frankfurters. For each treatment 1 kg of frankfurters was prepared. The basic recipe was as follows (g/kg raw batter): 700 g porcine meat, 100 g back fat, 180 g distilled water or extract, 20 g sodium chloride and 5 g sodium di- and triphosphates (all from ANVISA, Madrid, Spain). Four different types of frankfurters were considered: a negative control (NC) consisting of a basic recipe, without sodium nitrite or sodium ascorbate; a positive control (PC) consisting of a basic recipe with 0.1 g/kg sodium nitrite and 0.5 g/kg sodium ascorbate and 2 experimental frankfurters (5RC and 30RC) manufactured with the basic recipe and to which the 180 g of distilled water was replaced by 180 g of *R. canina* L. extract (from 5 g or 30 g whole fruits, respectively, see extraction procedure). The meat was chopped into small cubes (1 cm^3) and mixed with the sodium chloride (and for PC also sodium nitrite and sodium ascorbate). Then, the meat was minced in a cutter (Stephan UMC 5 Electronic) for 2 min at 3000 rpm, together with water or extract. After that, the fat was added and minced for 4 min until a homogeneous raw batter was obtained. Finally, the mixture was stuffed into 18 mm diameter cellulose casings, hand-linked at 15 cm intervals and given a thermal treatment for 30 min in a hot water bath (70 °C). After cooling in an ice bath, the frankfurters were wrapped with an oxygen permeable polyvinylchloride film, dispensed in polypropylene trays and subsequently stored for 60 days at 2 °C in the dark. At each sampling (days 1, 20, 40 and 60), four frankfurters per treatment were taken out of the refrigerator. A portion of the frankfurters was used for colour and texture measurements and the remainders were frozen at −80 °C until analysis.

2.3. Total phenolics content and antioxidant activity of the extracts

The Folin Ciocalteau reagent was used for the quantification of total phenolics present in the RC extracts, as described by Turkmen, Sari, and Velioglu (2006) with minor modifications as follows: 0.2 ml extract was mixed with 1 ml of Folin Ciocalteau reagent (10% in distilled water). After 5 min, 0.8 ml of sodium carbonate (7.5% in distilled water) was added and the samples were allowed to stand for 2 h at room temperature in darkness. The absorbance was measured at 740 nm. A standard curve with gallic acid was used for quantification. Results were expressed as mg of gallic acid equivalents (GAE) per ml extract and analysed in duplicate.

The antioxidant activity of the RC extracts was evaluated by the 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay (Kähkönen & Heinonen, 2003). Briefly, aliquots of 0.033 ml were mixed with 2.0 ml DPPH solution (6×10^{-5} M in methanol). The reaction mixture was stirred and kept in the dark for 6 min at room temperature. The absorbance was measured spectrophotometrically at 517 nm using methanol as a blank. The antioxidant activity against the DPPH radical is expressed as percentage of radicals scavenged after 6 min reaction time (%) and was analysed in duplicate.

2.4. Proximate composition of the frankfurters

Moisture and crude protein content of the frankfurters were determined (AOAC, 2000) and the method of Folch, Lees, and Sloane-Stanley (1957) was used for isolating the fat. Analyses were carried out in duplicate and results are expressed as g/100 g frankfurter.

2.5. Volatile compounds

Lipid oxidation was assessed by determining the lipid-derived volatiles hexanal, heptanal, octanal and nonanal according to Estévez, Morcuende, Ventanas, and Cava (2003). One gram of homogenized frankfurter was placed in a 2.5 ml vial and the SPME fibre (divinyl-benzene–carboxen–polydimethylxilosane, 50/30 μm) was exposed to the headspace while the sample equilibrated during 30 min immersed in water at 37 °C. Analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass-selective detector (Agilent model 5973). Volatiles were separated using a 5% phenyl–95% dimethyl polysiloxane column (30 m, 0.25 mm id., 1.0 μm film thickness; Restek). Compounds were positively identified by comparing their mass spectra with those from standard compounds run on the same conditions. The area of each peak was integrated using ChemStation software and the total peak area was used as an indicator of lipid-derived volatile generated from the samples. Samples were analysed in quadruplicate and results were provided in arbitrary area units (AAU×10^6).

2.6. Analysis of AAS and GGS

The protein oxidation products AAS and GGS were analysed according to Utrera, Morcuende, Rodriguez-Carpena, and Estévez (2011). Briefly, the frankfurters were minced and subsequently homogenized 1:10 (w/v) in 10 mM phosphate buffer containing 0.6 M NaCl. Aliquots of 0.2 ml were taken and proteins were precipitated twice using trichloroacetic acid (10%). Then, protein carbonyl groups were derivatized using: 250 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0) containing 1% sodium dodecyl sulfate and 1 mM diethylenetriaminepentaacetic acid, 250 mM MES buffer containing 50 mM p-amino benzoic acid (ABA) and 250 mM MES buffer containing 100 mM $NaCNBH_3$. The mixture was incubated at 37 °C for 90 min and the proteins were subsequently precipitated and simultaneously washed. Afterwards, the precipitates were hydrolysed with 6 N HCl at 110 °C for 18 h and the hydrolysates were dried in vacuo at 40 °C using a Savant speed-vac concentrator. Hydrolysates were finally reconstituted with 0.2 ml Milli-Q water and analysed using HPLC (15 cm×4.6 mm×5 μm COSMOSIL 5 C18-AR-II RP-HPLC column) with fluorescence detection (excitation and emission wavelength of 283 and 350 nm respectively). The mobile phase was a mixture of 50 mM sodium acetate buffer (pH 5.4) and acetonitrile, varying gradually the acetonitrile concentration from 0% to 8% at a flow rate of 1.0 ml/min. Identification of both derivatized semialdehydes in the FLD chromatograms was carried out by comparing their retention times with those from standard AAS and GGS (synthesised *in vitro* according to Akagawa et al. (2006)). Samples were analysed in quadruplicate and results are