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Effect of sodium ascorbate dose on the shelf life stability of reduced nitrite liver pâtés

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ABSTRACT

The effect of sodium ascorbate (SA; 500, 750, 1000 mg/kg) and sodium nitrite (SN; 40, 80, 120 mg/kg) doses on the shelf-life stability of liver pâtés was investigated in a full factorial design. Clear dose-dependent responses of the added SN or SA were found for the concentrations of nitrite, ascorbic acid and dehydroascorbic acid in the raw batters and in the cooked pâtés before and after 48 h of chilled display. Decreasing the SN dose to 80 mg/kg had no negative impact on the colour stability (a* value) and lipid oxidation (TBARS), and no additional antioxidant effect of SA was noticed. Lowering SN to 40 mg/kg resulted in proper colour formation, but the colour stability was inferior and lipid oxidation increased. Yet, increasing the amount of SA, at this low SN dose, resulted in lower TBARS values. Decreasing the SN dose to 80 or 40 mg/kg had no distinct effect on protein oxidation, which was however only measured by carbonyl content.

1. Introduction

Liver pâté, a traditionally cooked and widely consumed meat product in many countries, consists of a comminuted mixture of liver and fat to which additives are added. Due to its chemical composition and manufacturing process, liver pâté is considered as being a product highly susceptible to oxidation (Estévez, Ramírez, Ventanas, & Cava, 2007). Liver pâté is rich in fat and non-haem iron, with the latter being considered as the most important pro-oxidant in meat systems (Kanner, 1994). In addition, mincing and cooking makes meat products more susceptible to oxidation compared to fresh meat, due to the facilitated interaction between free fatty acids and oxygen in the presence of catalysts such as heat and metalloproteins (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Oxidation leads to several changes in fat components and meat pigments, thereby reducing the quality of the product in terms of taste, colour and shelf-life. In the past, the main focus on oxidation in meat and meat products was on colour and lipid oxidation, but nowadays also protein oxidation seems to influence specific meat quality traits (Lund, Heinonen, Baron, & Estévez, 2011).

Two important ingredients influencing the oxidative stability of liver pâté are nitrite and ascorbic acid (AA). Nitrite plays an important role during meat processing, colour development, lipid oxidation, flavour formation and microbiological safety (Honikel, 2008). After adding nitrite to a batter of meat, the nitrite has different fates: it is partially oxidized to nitrate by sequestering oxygen, bound to myoglobin and bound to proteins or other substances (Honikel, 2008). The rate of nitrite depletion is dependent on different factors such as pH, initial nitrite concentration, processing technique and storage temperatures, meat-to-water ratio and the presence of antioxidants (Kilic, Cassens, & Borchert, 2002). According to Honikel (2008) residual nitrite levels in meat products vary between 5 and 20% of the ingoing amount. The antioxidant AA works as a potent radical-scavenging component, improving the oxidative stability and colour formation of meat and meat products (Perlo et al., 1995, Sahoo & Anjaneyulu, 1997). Also, AA can act synergistically with tocopherols by regenerating and restoring their antioxidant properties (Niki, Noguchi, Tsuchihashi, & Gotoh, 1995) and interacts with nitrite (Izumi, Cassens, & Greaser, 1989). When nitrite is added to a batter, an equilibrium reaction occurs between nitrite and nitrous acid. From the nitrous acid, nitric oxide can be formed by the action of endogenous meat enzymes or reducing agents (Ranken, 1981). For the colour formation, nitric oxide reacts almost instantly with metmyoglobin forming nitrosylmetmyoglobin. The nitrosylmetmyoglobin is subsequently reduced to the red cured

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colour nitrosylmyoglobin. As AA is a strongly reducing agent, it plays an important role in the colour formation of cured meat products since it accelerates the reducing steps (Ranken, 1981).

The World Cancer Research Fund/American Institute for Cancer Research (2007) suggests that dietary nitrites are to be considered as human carcinogens, because they may be converted to carcinogenic N-nitroso compounds (Demeyer, Honikel, & De Smet, 2008). Consumers are concerned about the possible harmful effects of nitrite and the meat industry is challenged to reduce the concentrations of nitrite in their meat products. Regarding the formation of N-nitroso compounds, knowledge about the residual nitrite concentration could be just as important as focusing on the initially added nitrite concentration.

Because of the significant role of AA in the colour development and oxidative stability of meat products and the concerns about residual nitrite, a partial replacement of nitrite with AA in meat processing could be valuable. Therefore, the objective of this study is to investigate the effect of increased sodium ascorbate supplementation on the residual nitrite levels, colour stability, lipid and protein oxidation as well as antioxidant concentrations of reduced nitrite liver pâtés.

2. Materials and methods

2.1. Experimental set-up and sampling

The experiment consisted of a 3×3 full factorial design with three levels of sodium nitrite (SN, E250) (Kerry Ingredients and Flavours, Bornem, Belgium) (40, 80 and 120 mg/kg) combined with three levels of sodium ascorbate (SA, E301) (Kerry Ingredients and Flavours, Bornem, Belgium) (500, 750 and 1000 mg/kg) added to the batters. In commercial conditions, 120 mg/kg SN and 500 mg/kg SA are generally used (according to the information given by the supplier). The basic composition of each batch was based on a commercial recipe (g/kg): 290 g pork liver, 380 g pork subcutaneous fat, 290 g broth (the boiling water in which the fat was cooked), 18.0 g sodium chloride, 5.0 g dextrose, 10.0 g sodium caseinate and spices (2.0 g white pepper, 0.5 g nutmeg, 0.5 g ginger, 0.2 g cardamom, 0.5 g onion powder). All spices were purchased from RAPS (Beringen, Belgium) and the other additives were from Kerry Ingredients and Flavours (Bornem, Belgium).

The preparation of the batters and cooking of the pâtés were performed on three subsequent days, with the preparations for all levels of SA and per SN dose done on one day. Beforehand, separate mixtures of raw livers and subcutaneous fat from several commercial slaughter pigs (Impens NV, Melle, Belgium) were made. These mixtures were divided in three batches to be used for the three processing days, frozen until −21 °C and stored for maximum three days. For the preparation of the batter, first the cold liver part was minced for 8 min at 3000 rpm (Stephan vertical cutter–mixer, model UM12-F/3, consisting of two blades aligned at 180° to each other), curing salts (sodium nitrite and sodium chloride) were added and the cutting process was continued under vacuum for 2 min at 1500 rpm. The cured liver was kept refrigerated (1–7 °C) during the preparation of the fat. The fat was scalded for 20 min in boiling water until it reached a temperature of 40 °C. The fat was then minced and homogenised with sodium caseinate and broth for 5 min at 51 °C in the Stephan vertical cutter–mixer. Subsequently the cured liver and the other additives were added to this warm emulsion in the cutter. The mixture was further homogenised for 3 min until a homogeneous raw batter of 40 °C was obtained. Finally, the ready batters were manually distributed into metal cans until completely full (250 g, can height: 6 cm) and these were then hermetically closed using a can sealing machine (Indosa, type M160).

Three cans per treatment were immediately frozen (−21 °C) for further analysis, and are referred to as the batter samples. The other cans (six per treatment) were cooked in saturated steam conditions at 75 °C for 90 min. The cans were rapidly cooled in an ice bath and were stored in the dark at 4 °C. After 7 days, the cans were opened and two slices of 2 cm thickness were sampled after removing 1 cm of the top and bottom layer. Six slices per treatment were immediately vacuum packed and stored at −21 °C for further analysis. Six other slices per treatment were wrapped in an oxygen permeable polyethylene film (purchased from a local supermarket (Delhaize), thickness 0.010 mm), and placed in an illuminated chilled cabinet (1000 lx, 4 °C). Permeability characteristics of the film were not available from the supplier, but according to Massey (2002), this kind of low density polyethylene film has an oxygen gas permeability in the range of 255–470 $cm^3 \times mm \times m^{-2} \times 24\ h^{-1} \times atm^{-1}$ and a water vapour transmission rate in the range of 1.25–1.85 $g \times mm \times m^{-2} \times 24\ h^{-1}$. After 48 h of chilled display, the polyethylene film was removed and the samples were vacuum packed and stored at −21 °C until further analysis. All analyses were performed in duplicate.

2.2. Composition analyses

Dry matter, crude protein and crude fat content were analysed on three pooled pâté samples according to the ISO 1442–1973, ISO 937–1978 and ISO 1444–1973 methods, respectively. The pH was measured on minced samples using a glass pH-electrode.

The AA and dehydroascorbic acid (DHAA) content was determined according to the method of Zapata and Dufour (1992) and Dodson, Young, and Soliman (1992). This assay is based on the reaction of DHAA with orthophenylenediamine (OPD). Briefly, AA and DHAA were extracted using methanol/water (5/95; v/v) containing 0.1 M citric acid and 0.2 mM EDTA. DHAA is able to react with OPD, but AA first needs to be converted into DHAA, using active carbon. By measuring total DHAA (i.e. present DHAA and DHAA formed from converted AA), and DHAA present in the samples, the AA concentration was calculated. Samples were analysed by reversed phase HPLC (Agilent, Waldbronn, Germany), using a Pursuit XRS C18 column (15 cm × 4.6 mm × 5 μm; Varian, Sint-Katelijne-Waver, Belgium) with fluorimetric detection at an excitation and emission wavelength of 350 and 430 nm respectively. The mobile phase was a mixture of methanol/water (5/95; v/v), containing 5 mM cetrimide and 50 mM KH_2PO_4 (pH 4.6). The elution was performed at a flow rate of 1.0 ml/min. Quantification was done by comparison of peak areas with those obtained from a standard solution of converted (L)-ascorbic acid. Results were expressed as mg AA or DHAA/kg batter or pâté.

The residual nitrite was determined by the ISO 2918–1975 reference method. After a reaction with sulfanilamide and naftylethylenediamine, nitrite was measured spectrophotometrically at 538 nm. The nitrite concentration was calculated based on a standard curve obtained with SN and expressed as mg/kg batter or pâté.

The α-tocopherol content was determined according to the method of Desai (1984) with slight modifications. After saponification and n-hexane extraction, all samples were analysed by reversed phase HPLC (GE Healthcare, Diegem, Belgium), using a Supelcosil LC18 column (25 cm × 4.6 mm × 5 μm; Sigma-Aldrich, Bornem, Belgium). The mobile phase was a mixture of methanol/water (97/3; v/v) and the elution was performed at a flow rate of 2.0 ml/min. UV-detection was accomplished at a wavelength of 292 nm. The α-tocopherol content of the samples was determined by comparison of peak areas with those obtained from a standard curve of α-tocopherol. The results were expressed as mg α-tocopherol/kg batter or pâté.

2.3. Oxidative stability measurements

Lipid oxidation was assessed by measuring the 2-thiobarbituric acid-reactive substances (TBARS), with the extraction method using perchloric acid (0.64 M) as described by Ventanas, Estévez, Tejeda, and Ruiz (2006). In this method, malondialdehyde (MDA), a secondary oxidation product, forms a coloured complex with 2-thiobarbituric acid (TBA). This complex was determined spectrophotometrically at 532 nm. Results were expressed as mg MDA/kg batter or pâté.