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Underlying chemical mechanisms of the contradictory effects of NaCl reduction on the redox-state of meat proteins in fermented sausages

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ABSTRACT

NaCl reduction remains a significant challenge for the food industry. The influence of NaCl reduction on the oxidative damage to meat proteins during processing of experimental fermented sausages was studied. Sausages with low (0.55%, LS), medium (1.1%, MS) and high (2.2%, HS) NaCl concentrations were produced and analyzed at fix times (days 1, 18, 42 and 54 of processing) for proteolysis, protein oxidation, volatiles and texture. NaCl reduction led to a 30% lower oxidation of TRP but promoted proteolysis (from 19.5 to 26.7 mg free amino acids/g sample in HS and LS, respectively at day 54), protein carbonylation (from 0.62 to 1.18 nmol carbonyl/mg protein in HS and LS, respectively at day 42) and formation of Schiff bases (from 119 to 179 fluorescence units in HS and LS, respectively at day 54). NaCl reduction led to a significantly lower detection of Strecker aldehydes (from 8.8 to 28.6 area units in LS and HS, respectively at day 42) and a significant increase of hardness (from 117 to 143 N in HS and LS, respectively at day 54) in fermented sausages. The redox state of proteins is affected by ionic-driven effects and proteolysis with this having consequences on relevant quality traits.

1. Introduction

Sodium chloride (NaCl) plays an essential role in processed meat products from three different points of view: processing technology, preservation, and sensory attributes (Ruusunen & Poulanne, 2005). It is well known that NaCl enables myofibrillar protein solubilization, enhances water holding capacity (WHC) of meat and allows the gel formation in fermented sausages leading to the desirable texture (Ruusunen & Poulanne, 2005). In addition, NaCl decreases water activity and hence, contributes to controlling the growth of microorganisms responsible for spoilage and safety concerns (Ruusunen & Poulanne, 2005). NaCl also modulates the biochemical and enzymatic reactions during ripening, having an influence on the flavour of the final product (Toldrá, 1998). Flavor is also influenced by NaCl as provides salty taste, enhances savory and meaty flavours and improves the release of volatile aroma compound from the food matrix (Ruusunen & Poulanne, 2005).

Currently, consumers and health authorities are concerned about the harmful effects of a high level of sodium intake owing to the alleged adverse cardiovascular effects (EFSA, 2005). Actually, in most Western countries, the intake of sodium is higher than recommended by the European Food Safety Agency (EFSA, 2005) and the World Health Organization (WHO, 2003). Taking into consideration that the mean daily sodium intakes are in excess of dietary needs (about 1.5 g sodium/day in adults) (EFSA, 2005) and that processed meat products are believed to contribute to that excess, meat scientists have proposed strategies to reduce and/or replace sodium chloride by other salts. However, NaCl reduction compromises the quality of the final product and consumer acceptability (Ruusunen & Poulanne, 2005). In particular, NaCl reduction leads to products with an excess of proteolytic activity due to the intense action of endopeptidase enzymes resulting in defective textures like softness (García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2004; Gou, Morales, Serra, Guàrdia, & Arnau, 2009). Additionally an intense exopeptidase activity would generate excessive content of low molecular weight nitrogen compounds (peptides and free amino acids), affecting the flavour of the final product by enhancing bitter and metallic tastes (Toldrá, 1998). As far as lipid oxidation is concerned, salt has been described as a pro-oxidant agent in several studies (Rhee, Ziprin, & Ordóñez, 1987). Lipid oxidation is a main source of volatile compounds which are implicated in the development of the typical flavor of dry-cured meats (Toldrá, 1998).

On the contrary, the effect of NaCl on protein oxidation during

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meat fermentation has been scarcely covered (Soladoye, Juarez, Shand, Phyllis & Estévez, 2015). In fact, only recent studies have covered the impact of sodium chloride on the occurrence and extent of protein oxidation in meat systems and its influence on WHC and gelling capacity of pork (Liu, Xiong, & Chen, 2011; Li, Xiong, & Chen, 2013). Protein oxidation is manifested in muscle foods in a variety of chemical modifications including loss of thiols and tryptophan, carbonylation, carboxylation, formation of Schiff bases and disulphide bonds (Estévez, 2011; Soladoye et al., 2015). Recently, the oxidation of proteins has been found to occur during meat fermentation and has been linked to color, texture and flavor changes in dry-cured hams and fermented sausages (Fuentes, Estévez, Ventanas, & Ventanas, 2014; Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Villaverde, Morcuende, & Estévez, 2014a). To which extent salt reduction has an influence on the extent and nature of protein oxidation and the potential influence of that on the quality of fermented sausages is currently unknown. To fulfil this objective, it is of great importance to clarify the link between protein oxidation and other biochemical reactions with proven impact on relevant quality traits, namely proteolysis.

The present study was designed to elucidate the impact of NaCl reduction on protein oxidation in fermented sausages and to assess the impact of the chemical changes on the quality of the final product.

2. Material and methods

All chemicals and reagents used for the present work were purchased from Panreac (Panreac Quimica, S.A., Barcelona, Spain), Merck (Darmstadt, Germany), and Sigma Chemicals (Sigma–Aldrich, Steinheim, Germany). Food grade additives were purchased from Anvisa (Anvisa S.A., Madrid, Spain). Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA, USA). Porcine meat (longissimus dorsi muscle) and porcine back-fat was obtained from a local slaughterhouse.

2.1. Manufacture of dry-cured fermented sausages

Porcine meat and porcine back-fat were cut into cubes and chopped separately. To the general recipe (90% meat and 10% back-fat), different NaCl concentrations were considered resulting in three treatments, namely LS (low concentration of salt; 0.55%), MS (medium concentration; 1.1%) and HS (high concentration; 2.2%). The three treatments were produced in triplicate as independent batches in a random order. The ingredients were finely minced using a 'Mainca' cutter machine (Barcelona, Spain) until a homogeneous batter was obtained (5 min/2000 rpm/^{Ta}<8 °C). The batter was subsequently stuffed in 3.5 cm diameter collagen casings to yield 2 kg of raw sausage per treatment and production batch. This emulsion-type of sausage was made to guarantee the homogeneity of the drying process and the uniformity of the final product.

Experimental sausages were subjected to a drying process in ripening chambers under controlled temperature (T) and relative humidity (RH). Four samplings were made during the processing coinciding with key points of the ripening stages (modifications of the thermo-hygrometric conditions). The first sampling (T1) was performed the day following the manufacture (T: 5 °C; RH: 88%). The second sampling (T2) was performed 18 days after processing (T: 7 °C; RH: 84%). The third sampling (T3) was performed 42 days after processing (T 22° C; RH: 80%). The last sampling (final product, T4) was made 54 days after processing (T: 15° C; RH: 82%). Final products suffered in average a moisture loss of 40%. Upon sampling, sausages were vacuum-packaged and frozen (−80 °C) until completion of all analyses (less than two weeks).

2.2. Proteolytic activity determination

2.2.1. Water soluble nitrogen (WSN) content

Ten grams of meat were homogenized twice with 50 mL deionized water and centrifuged at 5000 g, 4 °C for 10 min. Combined supernatants were filtered through Whatman No. 1 filter paper and subsequently subjected to the Kjeldhal method (AOAC, 2000) for nitrogen quantification.

2.2.2. Non-protein nitrogen (NPN) content

Twenty-five mL of the above mentioned filtrate were mixed with 25 mL of 20% trichloroacetic acid (TCA), settled at room temperature for 30 min, centrifuged at 5000 g at 4 °C for 10 min and filtered through Whatman No. 4 filter paper. NPN was also quantified using the Kjeldhal method (AOAC, 2000).

2.2.3. Free amino acid (FAA) content

An aliquot of the TCA-precipitated filtrate was added to 3 mL of o-phthaldehyde reagent. Forty mg of o-phthaldehyde were dissolved in 5 mL ethanol, 25 mL of 0.1 M sodium tetraborate, 0.1 mL of β-mercaptoethanol and brought to volume (50 mL) with deionized water. The absorbance was read at 340 nm against the o-phthaldehyde reagent. The FAA content was calculated using tyrosine as standard and expressed as mg tyrosine/100 g dry matter.

2.3. Analysis of tryptophan (TRP) fluorescence

The decrease of TRP fluorescence was measured on a Perkin Elmer LS 55 luminescence spectrometer (PerkinElmer, Beaconsfield, UK). The emission spectra were recorded from 300 to 400 nm with the excitation wavelength established at 283 nm. Prior to the analysis, 1.5 g of sample from each treatment were diluted (1:10) with 8 M urea in 100 mM sodium phosphate buffer, pH 3.5. N-acetyl-L-tryptophanamide (NATA), a tryptophan analogue, was used as model compound to describe changes in the tryptophan fluorescence due to its equivalent fluorescent behavior and higher stability in water solutions (Utrera & Estévez, 2012a). A standard 0.5 μM NATA solution was prepared in 0.05 M Tris–HCl buffer pH 7.5, made from purified water. TRP content was calculated from a standard curve of NATA. The linearity ($R^2 = 0.9955$; $p < 0.05$) between NATA concentration (ranged from 0.1 to 0.5 μM) and fluorescence intensity was statistically significant. The percent of TRP loss was calculated as $[(FL_1 - FL_4)/FL_1] \times 100$, where FL_1 is the fluorescence emitted by samples at T1 and FL_4 is the fluorescence emitted by samples at T4.

2.4. Analysis of AAS

The analysis of AAS by high performance liquid chromatography (HPLC) was performed following the procedure described elsewhere (Utrera & Estévez, 2012b). Briefly, 400 μL of a sample homogenate (1:10 in 8 M urea in 100 mM sodium phosphate buffer, pH 3.5) were dispensed in 2-mL plastic tubes, treated with a cold 10% TCA solution and centrifuged at 5000 g for 5 min at 4 °C. Precipitated proteins were treated with p-amino benzoic acid (ABA) in the presence of $NaBH_3CN$ as detailed in the aforementioned paper. Upon derivatization, samples were washed twice with 10% TCA and diethyl ether-ethanol (1:1). Finally, the pellet was treated with 6 N HCl and kept in an oven at 110 °C for 18 h until complexion of hydrolysis. The hydrosylates were dried *in vacuo* in a centrifugal evaporator. The generated residue was reconstituted with 200 μL of milliQ water and then filtered through nylon syringe filters (0.45 μm pore size, Pall Corporation, USA) for HPLC analysis. An aliquot (1 μL) from the reconstituted protein hydrosylates was injected and analyzed in a Shimadzu 'Prominence' HPLC apparatus