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Prevention of rancidity and discolouration of Iberian dry cured sausage using proteases

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ABSTRACT

Fifty sausages were manufactured using different proteases (batch 1: *without protease*; batches 2 and 3: *with validase FP II* at 0.5 and 1 g kg^{-1}, respectively; batches 4 and 5: *with validase FP Concentrate* at 0.5 and 1 g kg^{-1}, respectively). Effect of the proteases on sausage quality was evaluated. Moisture content of the samples was lower ($p < 0.001$) in batch 1 than in batches with protease. Samples with protease presented a greater proteolysis. The protease FP Concentrate caused a higher proteolysis and amino acid content. Batches with protease presented lower values of hardness ($p < 0.001$), on the contrary, samples without protease presented higher hexanal content ($p < 0.001$). Moreover, batch 4 and 5, showed the highest oxidative stability ($p < 0.001$). Regarding to parameters related to red colour (a^* and Hue*), batches 4 and 5 presented significant higher values of them ($p < 0.001$). Finally, sausages with proteases presented higher acceptance than control samples ($p < 0.001$), probably caused by an optimum odour ($p < 0.001$). Thus, a greater proteolysis in batches 4 and 5 prevents rancidity and discolouration of these samples.

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1. Introduction

Sensory quality of meat products is determined by parameters such as colour, texture and flavour (Keeton, 1994). Although fat is mainly responsible of these parameters, lipid oxidation is the major quality deteriorative process of these products (Sammet et al., 2006; Summo, Caponio, & Pasqualone, 2006; Zanardi, Dorigoni, Badiani, & Chizzolini, 2002). In these sense, lipid oxidation results in a variety of breakdown products which produces off-odours and flavours (Faustman & Cassens, 1990; Kanner, 1994). On the other hand, numerous studies have observed the relationship between lipid oxidation and pigment oxidation (Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Guerrero Legarreta, 2008; Kanner & Harel, 1985; Young & West, 2001). In this regard, previous papers have shown that the inclusion of antioxidants in meat products is necessary. Although synthetic antioxidants have been traditionally used (Ansorena & Astiasarán, 2004), approaches using natural antioxidants are very well appreciated (Badr & Mahmoud, 2011; Ismail, Lee, Ko, & Ahn, 2008; Sebranek, Sewalt, Robbins, & Houser, 2005).

Proteases are proteolytic enzymes that produce the hydrolysis of proteins generating polypeptides, small peptides and free amino acids. This enzymatic activity has been used by the meat industry with various technological purposes such as improving meat sensory (Bruna, Fernández, Hierro, Ordóñez, & de la Hoz, 2000; Kilara, 1985; Toldrá, 2004) or increasing meat tenderness (Gerelt, Ikeuchi, & Suzuki, 2000; Melendo, Beltrán, & Roncalés, 1997). Moreover, in a previous study carried out in our laboratory, the use of commercial proteases in a meat product such as dry cured sausage, reduced lipid oxidation probably as a result of the increase in active peptides and free amino acids with antioxidant effect during processing (Broncano, Timón, Parra, Andrés, & Petrón, 2011). In this sense, other authors corroborated these results such as Sun et al. (2009) and Vaštag, Popović, Popović, Petrović, and Peričin (2010) who described that the final products of proteolysis (low and medium weight peptides, oligopeptides and free amino acids) presented antioxidant effect in protein extracts from fermented sausages.

Iberian dry cured sausage is a Spanish traditional pork product with great palatability, with enormous economic importance for the industry in this country. However, a relatively high unsaturated fat content in this product can increase lipid oxidation susceptibility, consequently causing quality deterioration, as it has been described in other Iberian meat products (Muriel, Ruiz, Ventanas, Petrón, & Antequera, 2004).

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The aim of this work is to produce Iberian dry cured sausage of high quality by adding proteases. The addition of proteases in this product could be a new non artificial method to improve the quality of this product preventing rancidity and discolouration. However, study of other parameters on final products, such as texture or acceptability by consumers, is necessary in case proteases might cause an intense proteolytic degradation.

2. Material and methods

2.1. Samples

This study was carried out using fifty samples of Iberian dry cured sausages which were manufactured in the pilot plant of the School of Agricultural Engineering of Badajoz. Five batches of sausages were made: a control batch (batch 1, $n = 10$), where no protease was added, batch 2 ($n = 10$), with added validase FP II (fungal protease from *Aspergillus oryzae*, 0.5 g kg^{-1}), batch 3 ($n = 10$), with added validase FP II (fungal protease from *A. oryzae*, 1 g kg^{-1}), batch 4 ($n = 10$), with added validase FP Concentrate (fungal protease concentrate from *A. oryzae*, 0.5 g kg^{-1}) and batch 5 ($n = 10$), with added validase FP Concentrate (fungal protease concentrate from *A. oryzae*, 1 g kg^{-1}). Commercial enzymes were purchased from Valley Research (Valley Research Iberica, Madrid, Spain) and used following commercial specifications.

Sausages were made from a mixture of pork meat from Iberian pigs supplied by Señorío de Montanera industry (Badajoz, Spain), as it was explained before by Broncano et al. (2011). After processing, sausages were minced, vacuum packed, and stored at −80 °C until analysis.

2.2. Materials

The solvents used were of the following origin and quality: Synthesis grade, extra pure, PRS and reagent grade, and they were purchased from Panreac Química S.A. (Barcelona, Spain) and Scharlau Chemie S.L. (Barcelona, Spain).

Standards required for the assays included: malondialdehyde (MDA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ammonium sulphate and L-leucine were purchased from Scharlau Chemie S.A. (Barcelona, Spain).

A spectrophotometer (Thermo Fisher Scientific Model BioMate 3) was used.

2.3. Methods

2.3.1. Physico-chemical analysis

Moisture content was determined according to the official method for analysis of meat products ISO (1973) by dehydration at 102 °C until constant weight.

Total protein (Kjeldahl N × 6.25) was determined following the official method: ISO (1978).

Total lipids were extracted from 5 g of minced sausage according to the method of Folch, Lees, and Sloane Stanley (1957), using chloroform:methanol (2:1) as solvent. The extracts were dried in a rotating vacuum evaporator and weighted to determine the total quantity of lipids.

2.3.2. Non-protein nitrogen, proteolysis index, peptidic nitrogen amino acid nitrogen analysis

Non-protein nitrogen (NPN) was determined by the Nessler method using 4 g of sample after protein precipitation with 0.6 mol l^{-1} perchloric acid. The proteolysis index was calculated as the quotient between NPN and total protein multiplied by 100 as described by Astiasaran, Villanueva, and Bello (1990). Amino acid nitrogen (AN) was determined from the 0.6 mol l^{-1} perchloric acid protein precipitation fraction after peptide precipitation with 10 g 100 ml^{-1} sulfosalicilic acid (Benito, Rodríguez, Córdoba, Andrade, & Córdoba, 2005). The determination of peptidic nitrogen (PN) was made as AN prior to acid hydrolysis of the NPN fraction with 6 mol equ l^{-1} HCl (24 h at 120 °C) and correction for AN.

2.3.3. Texture analysis

Texture profile analysis (TPA) of the samples was performed at room temperature, using a $TA.XT_{plus}$ texture analyser (Stable Micro Systems, Godalming, UK) equipped with a cylindrical probe of 50 mm in diameter. This procedure involved cutting slices approximately 1.5 cm thick that was compressed twice to 25% of their original height. Force-time curves were recorded at a cross-head speed of 2 mm s^{-1}. Hardness (N), springiness (cm), cohesiveness, gumminess (N) and chewiness (N × cm) were evaluated at the end of the ripening process.

2.3.4. Hexanal content

Hexanal was quantified by headspace-solid phase micro-extraction (SPME) and GC/MS (gas chromatograph AGILENT 6890, coupled to a mass selective detector AGILENT 5973 Network) extensively described by Muriel, Andrés, Petrón, Antequera, and Ruiz (2007).

2.3.5. Colour measurement

The following colour coordinates were determined in samples: lightness (L^*), redness (a^*, red ± green) and yellowness (b^*, yellow ± blue). The colour parameters were determined using a Minolta CR-300 colorimeter reflectance spectrophotometer (Minolta Camera Co., Osaka, Japan) (Illuminant D65/0° standard observer and 0.8 cm port/viewing area). a^* and b^* values were used to calculate spectral colour (hue = arctan $[b^*/a^*]$) and colour saturation (chroma = $[a^{*2} + b^{*2}]^{0.5}$). Before use, the colorimeter was standardized using a white tile (mod CR-A43). The measurements were repeated on three randomly selected locations on each sausage slice and averaged for statistical analysis.

2.3.6. Sensory analysis

At the end of ripening, an acceptance test supplemented with a diagnosis of attributes was made. A balanced hedonic scale of nine points (from 0 (extremely dislike) to 9 (extremely like)) and an intensity scale of seven points for colour, hardness and rancid odour (from 0 (too low) to 7 (too high), being 4 right colour, hardness or odour) were used for the analysis. Afterwards, prior to data analyses, the data were converted into the data format of emotional response test (from −4.5 (extremely dislike) to +4.5 (extremely like) and from −3.5 (too low) to +3.5 (too high) being 0 right attribute).

120 untrained tasters, 68 men and 52 women ranging in age from 19 to 50 years, recruited from students and teachers of the Agricultural Engineering School (Extremadura University), who were regular consumers of sausage, participated in the test. The test was realized in a room of the School where light and temperature were constant and always at 11:00 h approximately. Samples were randomly provided to the panelists.

2.4. Statistical analysis

Means and standard error of the mean ($n = 10$ within each batch) were obtained from the analytical experiments. Results were analysed using an ANOVA test using the GLM procedure of SPSS 15.0 (SPSS Institute Inc., Cary, NC). When statistically significant differences were found, Tukey's test were performed. A Pearson's correlation test was performed to evaluate any relationship between analysed parameters. The level of significance was set to $p < 0.05$.

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