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Influence of partial replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ on lipolysis and lipid oxidation in dry-cured ham

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ABSTRACT

Sodium intake above nutritional recommendations may involve harmful consequences to health such as the increased risk of cardiovascular diseases. Dry-cured ham constitutes a product with a relatively large amount of sodium. Thus, to obtain a healthier product for consumers with reduced sodium content, two formulations containing KCl alone (formulation II) or mixed with $CaCl_2$ and $MgCl_2$ (formulation III) have been proposed to partially replace NaCl. Lipolysis and lipid oxidation occurring in hams processed with these formulations have been studied since they have direct influence on the final flavor. No significant differences in acid lipase activity or lipid oxidation were found at the end of the process between the alternative formulations and formulation I (control with 100% NaCl). Differences in some free fatty acids, generated along the processing, were detected among treatments and at the end of dry-curing. Data suggests a slight trend towards a major lipolysis during treatment III.

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1. Introduction

Salt is the major source of sodium in the diet and it is well known that an excessive sodium intake increases blood pressure in genetically salt susceptible individuals, and thus the risk of cardiovascular diseases and early death (Stamler, 1997). Salt intake, mainly in developed countries, exceeds nutritional recommendations. The Food Standards Agency in the United Kingdom and The National Academy of Science in the USA recommend the consumption not higher than 6 g of salt per day, however, the current intake of salt for the population is 9–12 g per day (He & MacGregor, 2010). This high intake is mainly due to the recent large increase in the consumption of highly salted processed foods. Because of this, reducing salt involves a very important target for the food industry, but more studies are necessary to obtain healthier products with microbial, biochemical and sensory characteristics more similar to traditional products.

Sodium chloride (NaCl) is the most important ingredient in the processing of dry-cured ham period. This ingredient contributes to microbial stability due to reduction of water activity, allows the solubilization of muscle proteins and provides a pleasant salty taste to the final product (Martin, 2001). A strategy to reduce salt content consists of its partial replacement by other chloride salts (potassium chloride, KCl; calcium chloride, $CaCl_2$; and magnesium chloride, $MgCl_2$) which has been studied in ham and several meat products like dry-cured pork loin (Aliño et al., 2010; Armenteros, Aristoy, Barat, & Toldrá, 2009a) and dry-fermented sausages (Gimeno, Astiasarán, & Bello, 1998). Some studies have been done on the kinetics of penetration and diffusion of these salts. Thus, hams prepared with calcium and magnesium need more time post-salting to reach the same water activity conditions as 100% NaCl salted hams (Blesa et al., 2008; Aliño, Grau, Fuentes, & Barat, 2010; Aliño, Grau, Toldrá, et al., 2010). The influence on the microbiota of dry-cured ham during the post-salting stage has also been studied, and no differences in microbial counts have been reported in hams prepared with these experimental salt formulations (Blesa et al., 2008). Some biochemical processes taking place during dry-curing, like the creatine to creatinine conversion (Mora, Hernández-Cázares, Sentandreu, & Toldrá, 2010) or muscle proteases activity (Armenteros, Aristoy, & Toldrá, 2009) were not affected by partial NaCl substitution with Ca, Mg or K. However, the effect of these salt formulations on lipolysis, especially acid lipase activity, and lipid oxidation throughout the dry-curing process of ham has not been studied.

Lipid breakdown generates free fatty acids (FFA) which oxidation drives to volatile compounds that have been reported to contribute to dry-cured ham flavor (Toldrá, 2006). Consequently, control of lipolysis and lipid oxidation is essential to obtain a final product with an optimal flavor (Toldrá, Flores, & Sanz, 1997). Several combinations of salts as partial replacers of sodium chloride could affect the activity of lipolytic enzymes changing the FFA profile and

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consequently affecting the generated flavor compound's profile. However it is well known that potassium, calcium and magnesium addition to meat products is mainly limited by their bitter taste, off-flavors, or metallic and astringent sensations. In fact, some authors (Askar, El-Samahy, & Tawfic, 1994; Gou, Guerrero, Gelabert, & Arnau, 1996; Gelabert, Gou, Guerrero, & Arnau, 2003) have reported 40% KCl as the maximum at which bitterness would be acceptable. Nevertheless, in a recent study, differences in sensory analysis between dry-cured loins with 50% substitution of NaCl by KCl and controls with 100% NaCl were found to be non-significant (Armenteros, Aristoy, Barat, & Toldrá, 2009b). Respect to the addition of $CaCl_2$ and $MgCl_2$, the sensory quality of dry-cured loins with formulations containing 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$ were reported to have no significant differences in relation to the control loins with 100% NaCl (Armenteros, Aristoy, Barat, et al., 2009a).

With respect to oxidation of lipids, partial replacement of NaCl by these alternative salts could affect oxidation. Several studies in meat products recognize NaCl as a prooxidant agent and have proposed some oxidative action mechanisms. According to Kanner, Harel, and Jaffe (1991) NaCl acts displacing iron ions from binding macromolecules and Hernández, Park, and Rhee (2002) concluded that its addition seems to decrease glutathione peroxidase activity.

The use of alternative chloride salts for the partial substitution of NaCl could affect acid lipase activity, free fatty acids generation and lipid oxidation. Therefore, the aim of this study was to determine how KCl, $MgCl_2$ and $CaCl_2$ may influence these reactions along the processing of dry-cured ham.

2. Materials and methods

2.1. Dry-cured ham preparation

Fifty-seven fresh hams with an average weight of 10 ± 1 kg from 6-month old pigs (Landrace × Large White) were selected in a local slaughterhouse. All hams were frozen in an industrial freezer at −40 °C and stored for at least 30 days at −20 °C under vacuum package. Motilva, Toldrá, Nadal, and Flores (1994) saw that the use of thawed hams did not affect lipolysis at the end of the dry-curing process. Hams were thawed in a cold chamber at 3 °C for 5 days. Three of these hams were used to characterize the raw material. The remaining hams were divided into three groups. Each group was salted by a vacuum salting treatment using the following formulations: formulation I: 100% of NaCl salt; formulation II: NaCl and KCl at 50% each; and formulation III: 55% NaCl, 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$. The hams were placed in plastic bags (95 μm thick) with the respective salt formulations and each bag was vacuum-packaged at 760 mm Hg in a machine from Tecnotrip (Terrassa, Barcelona, Spain) and thermosealed. The amount of the salt mixture was 4% of the total weight of ham; 100 ppm of $NaNO_2$ and 200 ppm of KNO_3 were also added as curing agents. Each ham was placed in a tray and remained for 13–15 days at 3 °C. After salting, hams were removed from the bags, washed to remove the excess of salt from their surfaces and maintained in a chamber at 4 °C and 75%–85% of relative humidity, for different times (depending on formulation) to obtain a complete salt homogenization. Thus, hams salted with 100% NaCl remained 50 days in this post-salting stage, while hams salted with formulations II and III remained up to 80 days to get a correct salt penetration. These differences in post-salting times were characterized previously by Blesa et al. (2008) and Aliño, Grau, Fuentes, and Barat (2010) when water activity and salt penetration were measured at 20, 50 and 80 days in hams with the same formulations as ours. Salt penetration (sodium, potassium, calcium and magnesium) was determined at different positions in the cross section of hams by ion chromatography using a PC-controlled Compact IC 761 equipment (Metrohm®, Herisau, Switzerland). Afterwards, hams were hung in a chamber and submitted to higher temperatures (14–20 °C) and lower relative humidity (until 70%) until the end of the process (11 months), constituting the ripening period.

2.2. Sampling

Samples (60 g) of *Biceps femoris* muscle were removed from hams at different times from the beginning of the process: 0 (fresh ham), 20 and 50 (in the post-salting period), and 90, 219 and 330 days (in the ripening period). Immediately after sampling all samples were vacuum packed and stored at −20 °C for further chemical and enzymatic analyses. These samples were divided into 4 subsamples, 15 g each, for the analysis described below. Three hams were each tested for every time and formulation, except for 50 days, where four hams per batch were tested.

2.3. Free fatty acids analysis

Total lipids were extracted from 5 g of minced *Biceps femoris* (previously the intermuscular and subcutaneous fat of the sample were trimmed with a knife) according to the method by Folch, Lees, and Stanley (1957), using dichloromethane–methanol (2:1) instead of chloroform:methanol (2:1) as solvent. Free fatty acids were determined in total lipids as described by Needs, Ford, Owen, Tuckley, and Andreson (1983) using heneicosanoic acid (C21:0) as internal standard, and fatty acids were methylated as described by Morrison and Smith (1954).

The analyses were carried out in a Fisons 8000 series gas chromatograph (FISONS Instruments, Rodano, Italy) equipped with a split injector and a flame ionization detector. The capillary column (100 m length, 0.25 mm i.d., 0.2 μm film thickness) was a HP-88 (Agilent, Barcelona, Spain). The oven temperature was maintained at 140 °C for 10 min, increased to 190 °C at 4 °C/min and maintained for 10 min, then increased to 220 °C at a rate of 2 °C/min and maintained for 5 min, and finally increased to 230 °C at a rate of 2 °C/min and maintained 20 min. The head pressure of the helium carrier gas was 35 psi and the split ratio was 2:1. The individual fatty acids were identified by comparing their retention times with those of standard fatty acid methyl esters. For the quantification, the response factors of the standards with respect to the internal standard (C21:0) were used. The results were expressed in mg of fatty acid/100 g of muscle dry basis.

2.4. Assay of lysosomal acid lipase activity

Five grams of minced *Biceps femoris* (previously the intermuscular and subcutaneous fat of the sample were trimmed with a knife) from samples described in Section 2.2, were homogenized in 25 ml of 50 mM disodium phosphate buffer, pH 7.5, containing 5 mM EGTA by using a Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland) (3 × 30 s at 27,000 rpm with cooling in ice). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C and the resulting supernatant filtered through glass wool and collected for the enzyme assay. All operations were carried out at 4 °C.

Enzyme assays were performed as previously described by Motilva, Toldrá, and Flores (1992) with slight modifications. The reaction mixture consisted of 50 μl of enzyme extract and 250 μl of reaction medium containing 0.24 mM of 4-methylumbelliferyloleate (Sigma-Aldrich Co., St. Louis, MO) as specific substrate. The reaction medium consisted of 0.1 M citric acid/0.2 M disodium phosphate containing 0.8 mg ml^{-1} bovine serum albumin (BSA) and 0.05% (by vol.) of Triton X-100 at pH 5. The reaction mixture was incubated at 37 °C for 20 min and the fluorescence was measured at $t = 0$ and at $t = 20$ min, at $\lambda_{ex} = 355$ nm and $\lambda_{em} = 460$ nm by using a multiscan fluorometer (Fluoroskan Ascent FL, Thermo Electron Corporation, Labsystems, Helsinki, Finland). One unit of activity (U) was defined as the amount of enzyme hydrolyzing 1 μmol of substrate/h at 37 °C. Four replicates were measured for each experimental point.

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