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Biochemical and Sensory Properties of Dry-Cured Loins as Affected by Partial Replacement of Sodium by Potassium, Calcium, and Magnesium

Mónica Armenteros,[†] María-Concepción Aristoy,[†] José Manuel Barat,[‡] and Fidel Toldrá*,[†]

[†]Instituto de Agroquímica y Tecnología de Alimentos (CSIC). P.O. Box 73, 46100 Burjassot (Valencia), Spain, and [‡]Departament of Food Technology, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

An attempt to decrease the NaCl content in dry-cured products through the use of three different salting treatments (II: 55% NaCl, 25% KCl, 15% $CaCl_2$, 5% $MgCl_2$; III: 45% NaCl, 25% KCl, 20% $CaCl_2$, 10% $MgCl_2$; and IV: 30% NaCl, 50% KCl, 15% $CaCl_2$, 5% $MgCl_2$) in comparison to a control (I: 100% NaCl) was assayed to evaluate the biochemical and sensory characteristics in the final product. Most proteolytic enzyme activities from the loins submitted to the experimental salting treatments, especially treatments II and IV, remained higher than those salted traditionally (control). The higher aminopeptidase activity was also reflected in a larger release of free amino acids. Finally, a sensory paired comparison test revealed that those loins salted with the treatment II were not significantly different from the loins salted traditionally (100% NaCl), so that this treatment could be successfully used for sodium reduction.

KEYWORDS: dry-cured loin; low sodium; low salt; potassium chloride; magnesium chloride; calcium chloride

INTRODUCTION

Sodium chloride is the main curing agent used in the manufacture of processed meats because it contributes to characteristic flavor and texture as well as improvement of shelf life and protein solubilization (*1*). Moreover, it is known that salt is also controlling the proteolytic and lipolytic phenomena inside the meat product. Indeed, salt has shown a strong inhibitory action on cathepsins and other proteases such as alanyl aminopeptidase (AAP) and also of neutral lipase and acid esterase (*2*−*5*). Hence, the use of low salt concentrations in meat products is difficult because it could lead to a defective texture in the final product, such as softness (*6*−*8*).

On the basis of the scientific information and following health recommendations, the meat industry is trying to develop low-salt products because meat products represent a relatively relevant part of the dietary sodium intake. Furthermore, the demand for low-salt-meat products by consumers is increasing in order to reduce the risk of cardiovascular diseases, such as hypertension, which is related to an excessive intake of sodium (*9*, *10*).

Nowadays, different strategies have been attempted to reduce sodium content in meat products, mainly by replacing the NaCl with other chloride salts such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), or calcium chloride ($CaCl_2$) (*11*−*15*). However, KCl, the most used NaCl substitute, imparts a bitter taste at high concentrations, and consequently, its use is restricted (*16*). Indeed, the maximum level of NaCl that could be substituted in fermented sausages (*17*) and dry-cured loins (*13*) without imparting undesirable flavor and texture attributes was 40%. More recently, Armenteros et al. (*11*) have reported substitutions of up to 50% by KCl in dry-cured loin without affecting the sensory properties nor the proteolytic and lipolytic activity.

The substitution of Na^+ by $CaCl_2$ and $MgCl_2$ salts may generate off-flavors (*18*, *19*). Lately, Gimeno et al. (*20*) replaced sodium content with calcium ascorbate in dry-fermented sausages and obtained an acceptable product without affecting attributes such as texture and color. The majority of the studies carried out with divalent salts have focused on the fortification of food products such as yogurt, fruit juice, or even beef frankfurter with magnesium and particularly with calcium to provide an opportunity for increasing their dietary intake (*21*, *22*). $CaCl_2$ has also been traditionally used in infusion, injection, or marination to improve meat tenderness (*23*). However, experimental studies conducted to examine the taste of these divalent cations in meat products have concluded that magnesium and calcium could lead to bitter tastes, off-flavors, or metallic and astringent sensations. Nevertheless, the presence of sodium chloride together with these cations could suppress these unpleasant tastes, especially bitterness (*24*, *25*).

The main objective of this work is to reduce the sodium content in dry-cured loin by partial substitution with a mixture of calcium and magnesium chlorides in addition to potassium chloride without affecting the biochemical and sensory properties of the product.

*To whom correspondence should be addressed. Phone: +34963900022. Fax: +34963636301. E-mail: ftoldra@iata.csic.es.

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MATERIALS AND METHODS

Samples. Fifteen fresh loins from *Landrace* × *Large White* pigs with an average weight of 2.6 ± 0.8 kg were obtained from a local slaughterhouse in the area of Valencia. All of the loins were vacuum-packed and immediately frozen in an industrial freezer at −40 °C and stored by 5 days at −20 °C. Then, the frozen loins were thawed in a cold chamber at 3−4 °C for 5 days, similarly to usual industrial process (*26*, *27*). Three of the loins were sampled and analyzed in order to characterize the raw material. The remaining 12 loins were submitted to the traditional loin processing. Thus, the loins were randomly divided into four groups with three loins in each group. Loins from the first group were salted by rubbing with the traditional NaCl (100% NaCl, treatment I) and were used as controls of the sensory and biochemical parameters, whereas the other groups were salted in the same way but with partial substitutions of NaCl by other salts. So, the second group was salted with 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$ (treatment II); the third group with 45% NaCl, 25% KCl, 20% $CaCl_2$, and 10% $MgCl_2$ (treatment III); and the fourth group with 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$ (treatment IV). The salting stage was carried out at 3 ± 1 °C and 90% relative humidity (RH) during a total of 6 days. The amount of the salt mixture was 2% of the loin weight also containing 150 ppm of KNO_3 and 150 ppm of $NaNO_2$ as curing agents.

After completion of salting, all of the loins were stuffed into artificial casings and immediately hung in a chamber for postsalting and initial ripening at 3 °C and 90% RH during 5 days. Subsequently, the loins were transferred to a drying chamber at 10 °C and 85% RH for 7 days and then at 12 °C and 75% RH until the loins achieved 35% weight loss, equivalent to 47% moisture usually found in commercial loins. Then, samples were vacuum-packed and kept frozen at −20 °C until their analysis.

Determination of Sodium, Potassium, Calcium, and Magnesium Content. Samples (10 g of dry-cured loins) were homogenized 1:10 (p/v) with Milli-Q water in a stomacher (IUL Instruments, Barcelona, Spain) for 10 min at 4 °C and then centrifuged at 10 000*g* for 20 min at 4 °C. The supernatants were filtered through glass wool and immediately frozen at −20 °C until used. Previous to the analysis, samples were thawed and filtered through nylon membrane filters (0.45 μm) and injected using the Metrohm 838 Advanced Sample Processor (Metrohm Ltd., Switzerland). The sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) contents in sample solutions were determined by using an Advanced Compact IC 861 (Metrohm Lt., Herisau, Switzerland) ion chromatograph (IC) equipped with a conductivity detector to monitor the separation. A software IC Net 2.3 (Metrohm Lt., Herisau, Switzerland) was used for data collection and processing. The concentration of each cation was determined from respective calibration curves, whereas the calibration for the assay was established using a triplicate set of standard solutions of Na^+, K^+, Ca^{2+}, and Mg^{2+} (Fluka, Switzerland, Sigma, St. Louis, MO). The results (means of three determinations) were expressed as mg/100 g of sample.

Sensory Analysis. At the end of the ripening stage, one loin for each salting treatment (treatments I, II, III, and IV) was selected for the sensory evaluation. The loin salted with the treatment I (100% NaCl) was used as control. The selected loins were cut into thin slices, approximately 5 mm thick, using a commercial slice machine and then placed in a dish and equilibrated at room temperature before serving. The samples were assessed by a nontrained panel consisting of 48 members, including university students, professors, and staff recruited at the Institute of Agrochemistry and Food Technology (Valencia, Spain). The evaluation sessions were conducted in a sensory analysis room at constant temperature (24 °C) on two different sessions. A glass of mineral water and unsalted toasted bread were available to the assessors. No information about the samples was provided to the panelists.

A paired-preference test (ISO-5495, 1983) (*28*) was carried out to determine panelist's preferences for each pair of samples (each sample against the standard) with respect to the color, aroma, texture, taste, and overall quality. Two pairs of samples were tested per panelist and session. Sensory evaluations were recorded by computer software using Compusense *five* release 4.6 (Compusense Inc., Guelph, ON, Canada).

Measurement of Enzyme Activities. The enzyme extract for cathepsin assays was prepared from 2.5 g of muscle homogenized in 25 mL of 50 mM sodium citrate buffer, pH 5.0, containing 1 mM EDTA and 2% (v/v) Triton X-100, whereas 4.0 g of muscle was employed for peptidase (aminopeptidases and dipeptidylpeptidases) assays in 20 mL of 50 mM phosphate buffer, containing 5 mM EGTA, pH 7.5. In all cases, the extracts were homogenized using a polytron homogenizer (Kinematica AG, Switzerland) 3 × 10 s at 27 000 rpm in a bath ice and then centrifuged at 10 000*g* for 20 min at 4 °C. The resulting supernatants were filtered through glass wool and used for further enzymatic assays.

Subsequently, muscles enzyme activities were measured by fluorometric assays using aminoacyl-7-amido-4-methyl coumarine (aa-AMC) (Sigma, St. Louis, MO) as fluorescent substrates. Cathepsins were assayed as previously described by Toldrá and Etherington (*29*). Thus, cathepsin B (EC 3.4.22.1.) and B+L (EC 3.4.22.15) were assayed at pH 6.0 using 0.05 mM *N*-CBZ-Arg-Arg-AMC and 0.05 mM *N*-CBZ-Phe-Arg-AMC, respectively, as substrates and cathepsin H (EC 3.4.22.16) at pH 6.8 using L-Arg-AMC as substrate. Aminopeptidase activities, alanyl- (AAP) (EC3.4.11.14), arginyl- (ARG) (EC 3.4.11.6), and leucyl- (LAP) (EC 3.4.11.18) aminopeptidase, were evaluated by following the method described by Flores et al. (*2*), using 0.1 mM Ala-AMC at pH 6.5, 0.1 mM Arg-AMC at pH 6.5, and 0.25 mM Leu-AMC at pH 9.5, respectively, whereas methionyl aminopeptidase (MAP) (EC 3.4.11.18) activity was assayed at pH 7.5 using 0.15 mM Ala-AMC as substrate (*30*). The determination of dipeptidyl peptidase activities (DPP) I (EC3.4.14.1), II (EC 3.4.14.2), III (EC 3.4.14.4), and IV (EC 3.4.14.5) was carried out as previously described by Sentandreu and Toldrá (*3*), using 0.5 mM Gly-Arg-AMC at pH 5.5, 0.5 mM Lys-Ala-AMC at pH 5.5, 0.5 mM H-Arg-Arg-AMC at pH 8.0, and 0.25 mM Gly-Pro-AMC at pH 8.0 as substrates.

In all cases, for each assay, 50 μL of extract was diluted with 250 μL of the respective reaction buffer and incubated in a multiplate incubator at 37 °C for 15 min. The fluorescence was determined using excitation and emission wavelengths of 355 and 460 nm, respectively, in a multiscan fluorometer (Fluoroskan Ascent FL, Thermo Electron Corporation, Labsystems, Helsinki, Finland). Four replicates were performed for each enzyme asssay, except when determining dipeptidyl peptidase activity, where three repetitions were done for each enzymatic assay. One unit of enzyme activity (U) was defined as the amount of enzyme which hydrolyzes 1 μmol of substrate per min at 37 °C. The results showed the residual enzymatic activity inside the loin measured by the incubation of the enzyme under standard conditions.

Protein Analysis. The extraction of sarcoplasmic and myofibrillar proteins was carried out by the procedure described by Molina and Toldrá (*31*). Dry-cured loin (10 g) was homogenized 1:10 (v/w) with 30 mM phosphate buffer, pH 7.4, for 4 min in a stomacher. The mixture was centrifuged at 10 000*g* for 20 min at 4 °C, and the supernatant containing the sarcoplasmic proteins was filtered through glass wool and stored at 4 °C until used. This operation was repeated with the pellets up to three times with the phosphate buffer to remove all sarcoplasmic proteins. The resulting pellet was resuspended in 9 volume (w/v) of 100 mM phosphate buffer at pH 7.4, containing 0.7 M potassium iodide and 0.02% sodium azide, homogenized in a stomacher for 8 min and then centrifuged at 10 000*g* for 20 min at 4 °C. The supernatant containing the myofibrillar proteins was collected. The protein concentrations of both sarcoplasmic and myofibrillar protein extracts were determined by the method of Smith et al. (*32*), using bicinchoninic acid as reagent and the bovine serum albumin (Sigma, St. Louis, MO) as standard.

The protein profile was achieved by sodium dodecyl sulfate−polyacrylamine gel electrophoresis (SDS−PAGE) using the method described by Toldrá et al. (*33*). The sarcoplasmic and myofibrillar extracts (100 μL) were denatured by mixing 1:1 (v/v) with the sample buffer consisting of 50 mM Tris buffer, pH 6.8, containing 8 M urea, 2 M thiourea, 75 mM dithiotreitol (DTT), 3% (w/v) sodium dodecyl sulfate (SDS), and 0.05% bromophenol blue. The mixture was heated at 100 °C for 4 min and stored at −20 °C until use. Denatured protein samples were diluted with the sample buffer up to 1 mg/mL protein concentration, and then, 12 μL of each sample was injected in each line into the gel. SDS−PAGE was carried out using 12% polyacrylamide gels and stained with Coomassie brilliant blue R-250. Standard proteins from BioRad (Hercules, CA) were simultaneously run for molecular mass estimation. Finally, the gels were scanned with Fujifilm LAS 1000 Intelligent Dark Box II (Fujifilm, S.A., Spain).

Free Amino Acids Analysis. Dry-cured loin samples for free amino acid analysis were extracted and deproteinized following the method

Table 1. Sodium, Potassium, Calcium, and Magnesium Content (mg/100 g) in Dry-Cured Loins Submitted to Four Types of Salting Treatments: (I) Control, 100% NaCl; (II) 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$; (III) 45% NaCl, 25% KCl, 20% $CaCl_2$, and 5% $MgCl_2$; and (IV) 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$[a]

parameter	raw meat	I	II	III	IV
Na^+	$55.83^a \pm 0.65$	$2944.23^c \pm 88.65$	$1685.13^b \pm 180.43$	$1634.85^b \pm 134.57$	$1414.78^b \pm 125.76$
K^+	$359.58^a \pm 1.66$	$597.00^b \pm 17.72$	$1235.29^c \pm 61.34$	$1350.66^d \pm 39.38$	$1886.32^e \pm 77.69$
Ca^{2+}	$13.48^a \pm 0.30$	$81.91^b \pm 9.16$	$166.78^{cd} \pm 17.80$	$212.29^d \pm 20.55$	$162.33^c \pm 7.30$
Mg^{2+}	$9.49^a \pm 0.05$	$43.46^b \pm 1.93$	$92.11^c \pm 8.10$	$120.75^e \pm 8.18$	$91.73^c \pm 4.26$

[a] Results are expressed as means of the three replicates ± SE. Means with different superscript letters in the same row are significantly different ($p < 0.05$).

described by Aristoy and Toldrá (*34*). Samples (5 g) were cut, minced, and subsequently homogenized with 0.01 M HCl 1:5 (w/v) in a stomacher for 8 min at 4 °C and then centrifuged at 10 000*g* for 20 min at 4 °C. The supernatant was filtered through glass wool and stored at −20 °C until required. Next, 200 μL of thawed samples plus 25 μL of an internal standard solution (1 mM norleucine in 0.01 M HCl) was deproteinized with 600 μL of acetonitrile. Samples were centrifuged (10 000*g* for 5 min), and free amino acids in the supernatants were derivatized to their phenylthiocarbamyl derivatives according to the method of Bidlingmeyer et al. (*35*). Derivatized samples were analyzed by reverse-phase HPLC in a 1100 Agilent chromatograph (Agilent, Palo Alto, CA) by using a Waters Nova PacK C18 column (3.9 × 300 mm) (Waters Corporation, Milford, USA). The separation was achieved in 65 min at 52 °C using a gradient between two solvents: 70 mM sodium acetate at pH 6.55 containing 2.5% of acetonitrile (solvent A) and water/acetonitrile/methanol, 40:45:15 (solvent B), as described by Flores et al. (*36*). Separated amino acids were detected at 254 nm.

Free Fatty Acids Analysis. Lipids were extracted from 5 g of dry-cured loin samples according to the method of Folch et al. (*37*) but using dichloromethane/methanol 2:1 as solvent. The free fatty acid composition was determined by gas chromatography of the corresponding methyl esters according to the method of Needs et al. (*38*) with slight modifications. Free fatty acids were purified from the total lipids using an anion exchange resin (Amberlyst A26, Rohm & Haas, Delf, The Netherlands) as described by Countron-Gambotti and Gandemer (*39*). The derivatives were obtained in accordance with the method of Berry et al. (*40*), and the analysis of the fatty acid methyl esters (FAME) was carried out as described by Navarro et al. (*41*) using a Fison 816 gas chromatograph (GC) (Fisons Instruments, San Carlos, CA) equipped with a flame ionization detector. The split ratio used was 1:50. The capillary column was a DB-225 (J&W Scientific, Barcelona, Spain; 30 m long, 0.25 mm i.d., 0.25 μm film thickness). Detector and injector temperatures were both set to 240 °C. The individual fatty acids were identified by comparing their retention times with those of standard fatty acids using the araquidonic acid as internal standard for quantification.

Statistics Analysis. Data obtained from the biochemical and the cation analyses were used as variables and evaluated by one-way analysis of variance (ANOVA) (STATHGRAPHICS Plus 5.1 version software) in order to compare the effect of the partial replacement of NaCl by three different salting treatments on the variables studied. Those cases, which show a significant effect, were compared using Fisher's least significant difference (LSD) procedure.

RESULTS AND DISCUSSION

Mineral Content from Dry-Cured Loins at the End of the Ripening Stage. The residual content of the different cations employed for the four brining treatments in the dry-cured loins is shown in **Table 1**. Taking into account the natural content of minerals in the loin (see **Table 1**), the salt composition at the end of the ripening period inside the loins is reflecting somehow the salt penetration and diffusion through the loin. However, lower concentrations of Ca^{2+} and Mg^{2+} were found in the loins in relation to the proportions of these chloride salts employed as brine. These results are in agreement with those obtained by Aliño et al. (*42*) in pork loin and Martínez-Álvarez et al. (*43*) in cod, who also confirmed the difficulty of divalent cations to penetrate inside the muscle. This could be explained by the fact that Ca^{2+} and Mg^{2+} cations have higher charge density (0.050 and 0.082 units of charge/molecular weight, respectively) that would increase their difficulty to penetrate inside the loin (*44*). Furthemore, these divalent cations were strongly bound to the outermost layers of the muscle proteins, compacting the surface of the meat and so delaying its penetration inside the muscle (*45*). Anyway, the employ of these mixtures of salts not only could reduce considerably the sodium content but also could improve the daily nutritional mineral requirements (*12*).

Table 2. Proteolytic Enzyme Activity (U/g) 10^{-3} in Dry-Cured Loins Submitted to Four Types of Salting Treatments: (I) Control, 100% NaCl; (II) 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$; (III) 45% NaCl, 25% KCl, 20% $CaCl_2$, and 5% $MgCl_2$; and (IV) 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$[a]

enzyme	I	II	III	IV	pooled SE
cathepsin B	0.89^a	1.80^b	1.24^c	2.01^b	0.08
cathepsin B+L	3.10^a	6.22^b	4.15^c	5.59^b	0.18
cathepsin H	0.08	0.03	0.03	0.16	0.05
dipeptidyl peptidase I	4.64^a	1.52^b	1.03^c	1.06^{bc}	0.15
dipeptidyl peptidase II	0.27	0.38	0.31	0.28	0.06
dipeptidyl peptidase III	4.51^a	4.49^a	4.10^a	1.71^b	0.72
dipeptidyl peptidase IV	0.31	0.21	0.27	0.20	0.04
alanyl aminopeptidase	7.66^a	13.17^b	9.92^c	11.05^{bc}	0.30
arginyl aminopeptidase	0.31^a	0.81^c	0.59^b	0.49^b	0.03
leucyl aminopeptidase	3.23^a	7.55^c	4.24^b	6.73^c	0.23
methionyl aminopeptidase	1.63^a	1.03^b	1.16^b	1.64^a	0.10

[a] Means with different superscript letters within a row differ significantly ($p < 0.05$). SE: Standard error.

Effect of the Salting Treatments on Enzyme Activities. The majority of the studies conducted during the last decades have been focused on the effect of the sodium chloride on porcine muscle proteases (*2*, *3*, *46*). Lately, Armenteros et al. (*47*) carried out an in vitro study focused on the effect of alternative chloride salts (KCl, $CaCl_2$, and $MgCl_2$) on the porcine muscle protease activities and concluded that KCl exerted an effect similar to that of NaCl on all of the studied enzymes, while divalent salts ($CaCl_2$ and $MgCl_2$) achieved an inhibitory rate similar to that of NaCl but at much lower concentrations. Nevertheless, the proteolytic activity inside the loins is affected not only by the salt concentrations and its diffusion inside the muscle but also by the water activity (a_w), temperature, pH, and the processing conditions (*26*, *48*). This will have relevant effects on texture, flavor, and appearance of the final product (*47*, *48*).

The endogenous protease activity (cathepsins, dipeptidylpeptidases, and aminopeptidases) remaining in the dry-cured loins submitted to four different salting treatments (treatments I, II, III, and IV) is shown in **Table 2**. The loins salted with treatments II and IV did not show significant differences in cathepsin B and B+L activity, and both were significantly higher than those found in the loins salted traditionally (**Table 2**). This fact could be due to the use of the similar concentrations of monovalent salts (NaCl

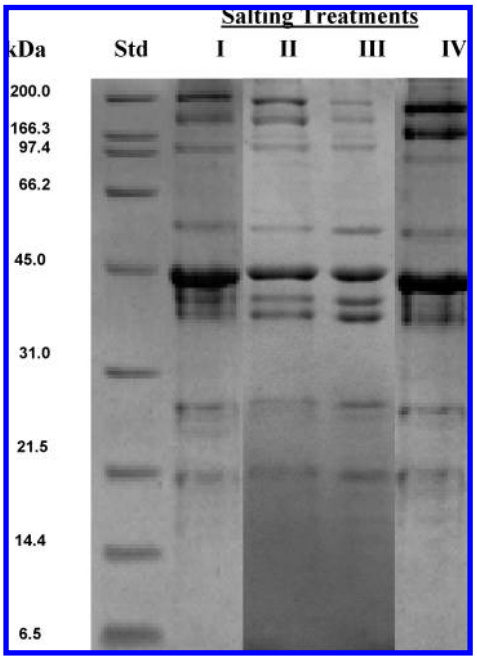

Figure 1. Twelve percent SDS−PAGE gel of myofibrillar proteins in the dry-cured loin submitted to four types of salting treatments. Std: BioRad molecular weight standards. Treatments as described in **Table 1**.

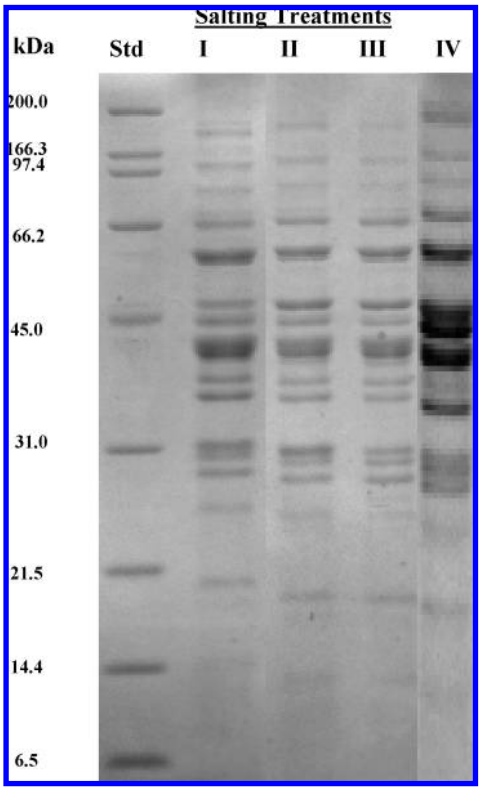

Figure 2. Twelve percent SDS−PAGE gel of sarcoplasmic proteins in the dry-cured loin submitted to four types of salting treatments. Std: BioRad molecular weight standards. Treatments as described in **Table 1**.

and KCl) and divalent salts ($CaCl_2$ as $MgCl_2$) as brine in both treatments. Ca^{2+} and Mg^{2+} cations are capable of binding strongly to the protein polar groups, strengthening protein interaction and hindering the salt penetration (*49*), and consequently, the cathepsin activities inside the loin increased. However, cathepsin activities in the loins salted with treatment III were lower than these, probably due to the higher concentration of divalent salts ($CaCl_2$ and $MgCl_2$) employed in this salting treatment where its inhibitory effect would be preferential. These results pointed out that the use of the same proportions of KCl, $CaCl_2$, and $MgCl_2$ as NaCl substitutes led to the same effect on cathepsin activities (*47*).

In addition, the polypeptides generated by the action of cathepsins are further degraded to smaller peptides and amino acids by exopeptidases, mainly dipeptidylpeptidases and aminopeptidases (*4*). Thus, loins salted with treatments II, III, and IV showed lower DPPI activity than the control loins, whereas DPPIII activity only decreased in the loins submitted to treatment IV, where the NaCl replacement reached values of 70%. DPPII and IV activities were not affected by any of the experimental salting treatments (**Table 2**). On the other hand, all Aminopeptidases activites (AAP, RAP, and LAP) were significantly increased, except MAP activity, which was considerably lowered in the loins submitted to treatments II and III.

Effect of the Salting Treatments on Proteolysis. The electrophoretic profiles of myofibrillar and sarcoplasmic proteins extracted at the end of the ripening stage did not reveal a clear effect of the type of salting treatment on the proteolysis in the dry-cured loins. Indeed, the profiles of myofibrillar (**Figure 1**) and sarcoplasmic (**Figure 2**) proteins from dry-cured loins submitted to the salting treatments II, III, and IV did not present different patterns with regard to the control loins salted traditionally (100% NaCl).

On the other hand, the polypeptides generated during the hydrolysis of meat proteins can be further degraded to smaller peptides and free amino acids mainly by the action of proteolytic enzymes (*4*). Aminopeptidases are the main enzymes involved in the generation of free amino acids through the hydrolysis of amino acids from the N-terminus of peptides and proteins (*2*). Some of these free amino acids are of great importance because they may be further involved in degradation reactions that generate volatile compounds which contribute to flavor development in dry-cured products (*50*). Results of the free amino acids concentrations from dry-cured loins submitted to four different salting treatments are shown in **Table 3**. As can be seen, there was an increase in the total amount of free amino acids in the loins salted with the experimental salting treatments II, III, and IV with respect to the control loins. This increase could be attributed to the highest AAP, RAP, and LAP aminopeptidase activity found in the loins submitted to the experimental salting treatments II, III, and IV, as shown in **Table 2**.

Effect of the Salting Treatments on Free Fatty Acid Content. The free fatty acid composition (FFA) at the end of the aging stage has been employed as an index of the lipolysis phenomena (**Table 4**). The dry-cured loins submitted to the experimental salting treatments II, III, and IV did not significantly promote a release of free saturated fatty acids (SFA) different than the control loins. Additionally, the salting treatments II and IV, in which the concentration of divalent salts had the same proportion, did not strongly affect either the total content of free monounsaturated (MUFA) nor the polyunsaturated (PUFA) fatty acids, indicating that the lipolysis phenomenon was carried out in a very similar way and also similar to those salted traditionally (100% NaCl). However, the loins submitted to treatment III showed a significant decrease in the total amounts of free MUFA and PUFA. This decrease was previously reported in $Mg^{2+}-Na^{+}$ cheese, where the lipolysis phenomena decreased when the NaCl content was replaced with a mixture of $Mg^{2+}-Na^{+}$ salts (*51*). Consequently, high concentrations of divalent cations in brine contributed to a reduction in the lipolysis phenomena, and hence, the total amount of free fatty acids decreased.

Table 3. Free Amino Acids Concentration (mg/100 g loin) in the Dry-Cured Loins Submitted to Four Types of Salting Treatments: (I) Control, 100% NaCl; (II) 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$; (III) 45% NaCl, 25% KCl, 20% $CaCl_2$, and 5% $MgCl_2$; and (IV) 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$[a]

Free amino acids (FAA)	I	II	III	IV
Asp	$7.36^{a} \pm 0.94$	$15.88^{b} \pm 4.13$	$18.01^{b} \pm 3.64$	$14.86^{ab} \pm 4.27$
Glu	$34.86^{a} \pm 4.24$	$52.25^{ab} \pm 8.99$	$71.06^{b} \pm 8.71$	$45.88^{ab} \pm 9.75$
Ser	$41.34^{a} \pm 2.33$	$65.96^{b} \pm 12.06$	$66.38^{b} \pm 7.76$	$54.69^{ab} \pm 6.84$
Asn	$15.93^{a} \pm 1.20$	$27.79^{b} \pm 4.54$	$29.03^{b} \pm 3.24$	$20.79^{ab} \pm 2.67$
Gly	$32.13^{a} \pm 1.57$	$45.70^{b} \pm 7.21$	$45.70^{b} \pm 5.14$	$39.78^{ab} \pm 4.30$
Gln	$34.39^{a} \pm 1.95$	$76.67^{c} \pm 10.45$	$63.21^{bc} \pm 4.21$	$43.00^{ab} \pm 6.22$
Ala	$70.94^{a} \pm 2.37$	$99.83^{b} \pm 12.17$	$96.00^{b} \pm 11.54$	$82.08^{ab} \pm 7.32$
Arg	$36.41^{a} \pm 2.69$	$63.52^{b} \pm 12.98$	$71.51^{b} \pm 12.18$	$51.04^{ab} \pm 6.24$
β-Ala	3.55 ± 0.30	3.18 ± 0.34	3.25 ± 0.39	2.33 ± 0.19
Pro	$35.92^{a} \pm 1.30$	$50.24 \pm {}^{b}7.43$	$50.74^{b} \pm 7.38$	$39.63^{ab} \pm 6.94$
Tyr	49.63 ± 2.76	55.79 ± 8.56	57.23 ± 6.55	55.33 ± 3.03
His	$17.91^{a} \pm 1.34$	$36.02^{b} \pm 7.28$	$36.47^{b} \pm 6.69$	$25.79^{ab} \pm 4.74$
Thr	$36.65^{a} \pm 1.78$	$48.83^{b} \pm 6.77$	$50.17^{b} \pm 5.67$	$48.68^{ab} \pm 5.74$
Val	$54.56^{a} \pm 1.65$	$69.88^{ab} \pm 9.14$	$79.77^{b} \pm 11.63$	$65.62^{ab} \pm 5.58$
Met	$32.78^{a} \pm 2.00$	$43.93^{b} \pm 4.45$	$51.61^{b} \pm 4.90$	$45.55^{ab} \pm 2.19$
Ile	$49.88^{a} \pm 3.26$	$71.76^{b} \pm 8.63$	$79.93^{b} \pm 10.13$	$64.27^{ab} \pm 2.69$
Leu	$92.36^{a} \pm 5.93$	$131.46^{b} \pm 16.83$	$140.76^{b} \pm 19.26$	$119.63^{ab} \pm 4.68$
Phe	$55.34^{a} \pm 4.53$	$76.28^{b} \pm 8.05$	$82.20^{b} \pm 8.65$	$71.68^{ab} \pm 1.64$
Lys	$47.94^{a} \pm 4.90$	$94.01^{bc} \pm 11.84$	$104.31^{c} \pm 14.74$	$62.45^{ab} \pm 10.87$
total FAA	$749.88^{a} \pm 4.86$	$1128.99^{b} \pm 2.99$	$1197.33^{b} \pm 7.29$	$953.08^{ab} \pm 5.82$

[a] Results are expressed as means of the three replicates ± SE. Means in the same row with different superscript letters are significantly different ($p < 0.05\%$).

Table 4. Free Fatty Acids Concentration (mg/100 g fat) in the Dry-Cured Loins Salted with the Four Different Salting Treatments: (I) Control, 100% NaCl; (II) 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$; (III) 45% NaCl, 25% KCl, 20% $CaCl_2$, and 5% $MgCl_2$; and (IV) 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$[a]

Free fatty acids (FFA)	I	II	III	IV
C14:0	$2.88^{a} \pm 0.42$	$2.01^{ab} \pm 0.05$	$1.53^{b} \pm 0.20$	$2.36^{ab} \pm 0.56$
C16:0	95.30 ± 9.44	113.14 ± 8.42	73.22 ± 10.02	105.66 ± 17.56
C18:0	$59.49^{ab} \pm 6.92$	$82.54^{b} \pm 4.67$	$54.75^{a} \pm 5.34$	$71.06^{ab} \pm 11.19$
SFA	$157.67^{ab} \pm 16.15$	$197.70^{b} \pm 12.66$	$129.49^{a} \pm 15.35$	$179.08^{ab} \pm 29.11$
C16:1	8.52 ± 1.21	6.87 ± 0.26	5.38 ± 1.04	9.82 ± 1.70
C18:1	$136.81^{a} \pm 16.37$	$107.55^{ab} \pm 5.29$	$71.18^{b} \pm 9.62$	$155.27^{a} \pm 26.61$
C20:1	$2.25^{ab} \pm 0.34$	$1.87^{ab} \pm 0.08$	$1.28^{b} \pm 0.09$	$2.65^{a} \pm 0.59$
MUFA	$147.58^{a} \pm 17.85$	$116.29^{ab} \pm 5.12$	$77.84^{b} \pm 10.75$	$167.74^{a} \pm 28.83$
C18:2	145.13 ± 12.30	152.04 ± 15.85	109.47 ± 17.05	172.00 ± 48.93
C18:3	$3.53^{ab} \pm 0.69$	$3.19^{ab} \pm 0.25$	$2.45^{b} \pm 0.48$	$5.51^{a} \pm 0.87$
C20:2	$2.06^{a} \pm 0.34$	$2.48^{ab} \pm 0.32$	$1.75^{a} \pm 0.23$	$3.87^{b} \pm 0.99$
C20:3	$4.88^{a} \pm 0.40$	$4.34^{ab} \pm 0.59$	$3.33^{b} \pm 0.50$	$6.40^{c} \pm 0.58$
C20:4	$42.98^{ab} \pm 3.81$	$40.10^{ab} \pm 6.24$	$30.51^{b} \pm 3.02$	$57.77^{a} \pm 8.70$
C22:4	$2.10^{ab} \pm 0.61$	$1.49^{ab} \pm 0.24$	$0.64^{b} \pm 0.09$	$2.79^{a} \pm 0.37$
PUFA	203.47 ± 17.62	203.64 ± 23.41	148.17 ± 20.03	248.34 ± 58.26
total FFA	508.72 ± 47.58	517.62 ± 40.87	355.49 ± 44.36	595.71 ± 115.22

[a] Results are expressed as means of the three replicates ± SE. Means in the same row with different letters are significantly different ($p < 0.05\%$). SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.

Effect of the Salting Treatments on Sensory Analysis. Table 5 shows the mean scores for color, aroma, texture, taste, and overall quality traits from the dry-cured loins submitted to four different salting treatments. The loins submitted to the salting treatment II, with NaCl substitution up to 45%, did not show significant differences ($p < 0.05$) with respect to color, texture, taste, and overall quality traits than the control loins, and it was even preferred in relation to aroma trait by the assessors at the 99% confidence level. These results are in agreement with those reported for replacements up to 50% of NaCl by other chloride salts in fermented sausages (*17*) and dry-cured loin (*11*).

On the other hand, nonsignificant differences ($p < 0.05$) were found in relation to the color attribute between the control loins and those salted with the treatments III and IV. However, the aroma attribute was preferred by the assessors in the control loins against those submitted to the treatment III, whereas the loins salted with the treatment IV did not reveal significant differences. This fact could be probably due to the highest levels of $CaCl_2$ and $MgCl_2$ employed as brine in the treatment III, which promoted an increase in the proteolysis phenomena generating off-flavors in the final product. In relation to the texture attribute, the assessors did not find significant differences ($p < 0.05$) between the control loins and those salted with treatments II and III, whereas the loins salted with treatment IV received lower scores. Finally, both control loins and those submitted to treatment II were preferred by the assessors with respect to taste and overall quality traits than those submitted to treatments III and IV. This could be mainly attributed to the use of adequate concentrations of KCl that together with the small amounts of divalent salts, particularly $MgCl_2$, avoid the appearance of off-flavors in the final product. In fact, several authors (*19*, *52*) observed these phenomena when using $MgCl_2$ as curing agent. Consequently, treatment II could be chosen as the best way to reduce the NaCl content without affecting the product acceptability.

Table 5. Sensory Analysis (Paired Comparison Test) at the End of the Ripening Stage of the Dry-Cured Loins Submitted to Four Types of Salting Treatments: (I) Control, 100% NaCl; (II) 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$; (III) 45% NaCl, 25% KCl, 20% $CaCl_2$, and 5% $MgCl_2$; and (IV) 30% NaCl, 50% KCl, 15% $CaCl_2$, and 5% $MgCl_2$

sensory traits	I	II	III	IV	*P* values[a]
color	27[b]	21			n.s.
	25		23		n.s.
	19			29	n.s.
aroma	16	32			<0.05
	37		11		<0.001
	30			18	n.s.
texture	22	26			n.s.
	28		20		n.s.
	33			15	<0.01
taste	25	23			n.s.
	32		16		<0.05
	36			12	<0.001
overall quality	25	23			n.s.
	35		13		<0.001
	35			13	<0.001

[a] Significance levels: n.s. ($p < 0.05\%$). [b] Number of assessors that preferred each batch (total number equal to 96).

In summary, our study indicates that the partial substitution of NaCl by a combination of $MgCl_2$ and $CaCl_2$ in addition to KCl in the brine salting mixture produced a higher proteolysis in the loins that was reflected in a larger content of free amino acids at the end of the ripening stage. Furthermore, the sensory analysis clearly demonstrated that treatment II did not show any significant differences to the control loins and was preferred by the assessors in relation to the aroma attribute. So, this formulation could be successfully used to reduce the sodium content in meat products without affecting the biochemical and sensory properties.

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