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Physicochemical properties and microbiology of dry-cured loins obtained by partial sodium replacement with potassium, calcium and magnesium

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ABSTRACT

The partial replacement of sodium chloride by other chloride salts has been proposed as a possible strategy to reduce the sodium content of cured meat products.

The aim of this study was to evaluate the effect brought about by partial replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ on physicochemical and microbiological parameters of dry-cured pork loin after the curing and drying process.

The replacement of around 70% NaCl significantly increased the hardness and chewiness of dry-cured loins but it is possible to obtain low sodium dry-cured loin, up to 45% substitution by potassium (25%), calcium (15%) and magnesium (5%), with no significant effects either on the physicochemical characteristics or the microbial counts compared with the traditional product with the usual amounts of sodium chloride.

1. Introduction

According to reports (SACN, 2003; WHO, 2003), a significant sector of the population is sensitive to sodium in the diet that exerts a significant increase in blood pressure (BP) with linked risks of heart disease and strokes. This has promoted the tendency to reduce sodium intake, to lower blood pressure levels and confer significant health benefits by contributing to a decreased risk of cardiovascular disease (Matthews & Strong, 2005). For these reasons, consumer demand for a variety of low-salt products, especially meat products has increased (Ruusunen & Puolanne, 2005).

Sodium reduction in meat products is possible but difficult to achieve due to the important properties of NaCl, especially in the meat industry. In fact, NaCl is an essential ingredient in processed meat products, contributing to the water holding capacity, colour, fat binding properties, flavor and texture (Toldrá, 2002). Moreover, salt decreases water activity (a_w) and this significantly affects the shelf-life (Sofos, 1984; Wirth, 1989).

Different strategies have been attempted to reduce sodium content in meat products (Terrell, 1983), mainly by replacing the NaCl with other chloride salts such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), or calcium chloride ($CaCl_2$) (Toldrá & Barat, 2009). KCl is probably the most common salt substitute used in low or reduced-salt/sodium meat products (Gelabert, Gou, Guerrero, & Arnau, 2003; Hand, Terrell, & Smith, 1982a; Ibáñez, Quintanilla, Cid, Astiasarán, & Bello, 1996; Ibáñez, Quintanilla, Cid, Astiasarán, & Bello, 1997; Ibáñez et al., 1995; Riera, Martínez, Salcedo, Juncosa, & Sellart, 1996; Ruusunen & Puolanne, 2005) as it is associated with decreased BP (Geleijnse, Witteman, Bak, den Breeijen, & Grobbee 1994). Hand, Terrell, and Smith (1982b) suggested that partial (50%) or complete (100%) replacement of NaCl with KCl or LiCl for curing bone-in hams can be achieved without significant reduction in cured colour. Keeton (1984), using four curing formulations based on total and partial replacement of NaCl with KCl in country-style hams, concluded that aspects as firmness and colour were not different at the end of the aging period. Gou, Guerrero, Gelabert, and Arnau (1996) observed that colour was not affected by the substitution of NaCl with glycine, potassium lactate, or KCl in dry-cured loins whereas high potassium lactate levels presented colour problems in fermented sausages. Aliño, Grau, Toldrá, et al. (2009) studied the replacement of NaCl by KCl in Spanish dry-cured loin. They stated that, although no significant differences in water activity, chloride and water contents, colour parameters and microbial counts were observed, replacement of around 70% increased significantly the hardness and chewiness.

Calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) have also been used in meat products. An inverse association of magnesium intake and other dietary factors with BP has been established (Jee et al., 2002; Joffres, Reed, & Yano, 1987; Mizushima, Cappuccio, Nichols, & Elliott, 1998). Indeed, calcium intake may slightly decrease BP (Geleijnse et al., 1994). Low calcium intake is associated with osteoporosis, hypertension and colon cancer. Heaney and Barger-Lux (1991) suggested that processed meat products

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could provide an important opportunity for calcium supplementation since such products are consumed by people of all ages. Therefore, several studies have concentrated on investigating other chloride salts such as calcium and magnesium in some processed meat products. However there are few experiments on Spanish dry-cured loin. Seman, Olson, and Mandigo (1980) studied the partial replacement of NaCl by $MgCl_2$ or KCl in bologna and found no sensory defects at low levels. Seperich and Ashoor (1983) experimented with replacing NaCl with $CaCl_2$ in bologna found no effects on microbial growth or texture. Gimeno, Astiasarán, and Bello (1998) replaced NaCl with KCl, $MgCl_2$ and $CaCl_2$ in dry fermented sausages and were able to reduce the NaCl content by about 50%. However sodium replaced sausages lacked a salty taste. These authors studied the influence of partial replacement of NaCl with KCl and $CaCl_2$ on texture, colour and microbial evolution of dry fermented sausages and stated that the product could be considered acceptable (Gimeno, Astiasarán, & Bello, 1999, 2001). In sectioned and formed hams, Collins (1997) stated that using a 70% NaCl/30% KCl or 70% NaCl/30% $MgCl_2$ mixtures made no difference in terms of flavor, tenderness and overall acceptability compared to hams made with 100% salt. However, formulations using 30% $CaCl_2$ had lower pH, moisture content, yield and residual nitrite. Terrell, Childers, and Kayfus (1982) replaced NaCl with a number of other chloride salts and found that replacement of NaCl with any chloride salt, except $CaCl_2$, significantly decreased moisture loss in raw and cooked beef clod muscles.

The goal of this study was to evaluate the effect of partial replacement of NaCl with a mixture of KCl, $CaCl_2$, and $MgCl_2$, on physicochemical and microbiological parameters of dry-cured pork loin after curing and drying, through instrumental measures.

2. Materials and methods

Twelve fresh loins (*Longissimus dorsi*) with an average weight of 2.8 ± 0.2 kg and a mean pH value measured at 24 h post-mortem of 5.74 ± 0.01, were selected in a local slaughterhouse. All the loins were frozen at −40 °C and stored for at least 30 days at −20 °C. Then, the frozen loins were thawed at 3 °C for 5 days. The raw material was characterized previously (Aliño, Grau, Toldrá, et al., 2009). Loins were divided into four groups of three loins and salt was rubbed onto the surface with mixtures of NaCl, KCl, $CaCl_2$ and $MgCl_2$ salts (Table 1). NaCl and KCl proportions in the mixture were chosen in accordance with the results obtained previously (Aliño, Grau, Toldrá, et al., 2009) and $CaCl_2$ and $MgCl_2$ were added.

The salting process was carried out with a thermodynamic control, by placing a known amount of salt on the product surface and allowing it to dissolve and penetrate (Fuentes, Barat, Fernández-Segovia, & Serra, 2008). The main information needed to characterize the rub salting process, is the amount of salt mixture added to the loin to reach the same salting degree as in commercial dry-cured loins. Salt concentration must be expressed in chloride concentration on dry basis (X^{Cl}), to compare properly all the experimental data, since this value is constant throughout the curing process, and in the final product. The water content of dry-cured loins after curing and drying must also be defined, and should be around the commercial moisture value. These two parameters would help to obtain low sodium dry-cured loin with partially replacement of NaCl by KCl, $CaCl_2$ and $MgCl_2$, with the same water content as commercial dry-cured loins.

The target chloride concentration (X^{Cl}_{target}) was defined as the target X^{Cl} to be reached in the dry-cured loins at the end of the processing stages and x^w_f, the moisture content at the end of the curing process. These two parameters were established in a previous study where five different dry-cured loin commercial brands were analyzed (Aliño, Grau, Toldrá, et al., 2009), being X^{Cl}_{target} = 0.06 g Cl/g dry material and x^w_f = 0.47 g/g (w/w).

Chloride amount, M_{Cl} (g of chloride), added to each loin was calculated from target chloride concentration on a dry basis, X^{Cl}_{target} (g of chloride/g of dry matter), initial weight of the loins, M_0 (g), and initial wet basis water fraction, x^w_0 (g/g), according to Eq. (1).

$$M_{Cl} = X^{Cl}_{target} \cdot M_0 \cdot (1 - x^w_0) \tag{1}$$

Salt amount, M_{salt} (g), was calculated according to the composition of the salt mixture (I, II, III and IV); 150 ppm of KNO_3 and 150 ppm of $NaNO_2$ were added to the mixtures as curing agents (Toldrá, 2006).

After salting, loins were post-salted to allow the salt mixture to penetrate, stuffed into regenerated collagen casings and then dried until they reached the final weight which would correspond to the commercial water content (x^w_f). Final weight was calculated by means of mass balances, from initial weight of the loins (M_0), initial water fraction (x^w_0), final water fraction (x^w_f) and amount of salt added to each loin (M_{salt}). All loins were weighted every two days during the process. Processing conditions throughout the process are shown in Table 2.

2.1. Sampling

In all the treatments, the sampling was carried out at the end of the drying stage once loins reached their final weight and thus the desired water content (x^w_f). Analyses of the loins were carried out using four adjoining slices of 20 mm thickness each cut transversally to the length of the loin and taken from the centre of the loin. One slice was used for microbiological analyses; the second for colour, and physicochemical analyses, the third for ion profile penetration and the last one for textural analyses. Physicochemical analyses were also carried out in the samples used for texture determinations.

2.2. Colour determinations

A slice, 20 mm thick was taken in a transversal cut from the central section of the loin. Measurements were taken shortly after cutting the slice. Reflectance spectra were determined with a UV/VIS Minolta Spectrophotometer model CM-3600d from 400 to 700 nm, at 10 nm intervals, using an integrating sphere. The colour system employed was CIE $L^*a^*b^*$. D-65 illuminant was used with 10° observer angles (Cassens et al., 1995). The illuminant D-65 evaluates colour using typical bright daylight with an overcast sky and colour temperature at 6500 K. Each slice was measured

Table 1
Composition of salt formulation for rub salting.

Formulation	NaCl (%)	KCl (%)	$CaCl_2$ (%)	$MgCl_2$ (%)
I	100	–	–	–
II	55	25	15	5
III	45	25	20	10
IV	30	50	15	5

Table 2
Conditions at the different stages of the dry-curing process.

Stage		Time (days)	Temperature (C°)	Relative humidity (%)
Salting		2	3 ± 1	90
Post-salting	Without casing	6	3 ± 1	90
	With casing	5	3 ± 1	90
Drying	1st period	7	10 ± 1	85
	2nd period	Until final weight	12 ± 1	75

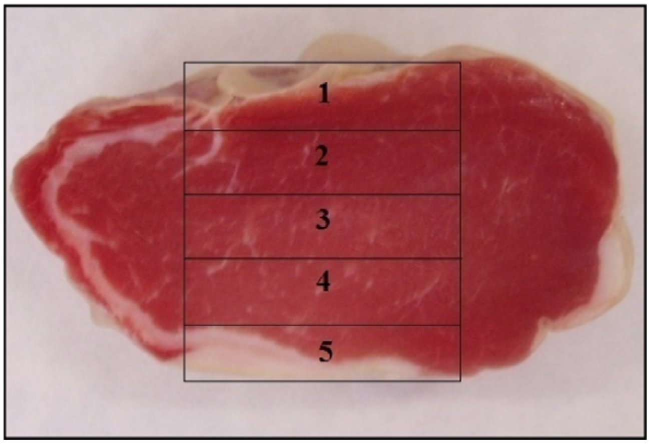

Fig. 1. Samples analyzed from the profile section.

at three locations, outer zone (zone 1), middle (zone 3) and inner zone (zone 5) (Fig. 1). L^*, a^* and b^* values were obtained as the mean of six determinations per zone.

2.3. Texture profile analysis (TPA)

Textural properties were measured by double compression using a Texture Analyzer TA.XT2 (Stable MicroSystems, UK) equipped with a flat-ended cylindrical plunger (SMS-P/75). The force (N) was recorded continuously during compression in a texture profile curve with a load cell of 250 N. Determinations were carried out on three cubes taken (inner, middle and outer) from one slice of each loin. Cubical samples ($20 \times 20 \times 20$ mm.) were compressed axially (20% compression), with a constant cross-head speed of 1 mm/s. The holding time between both compressions was 5 s (Tabilo, Flores, Fiszman, & Toldrá, 1999). Force versus time was recorded and the following parameters calculated: hardness (g), cohesiveness (dimensionless), springiness (dimensionless), adhesiveness and chewiness (g).

2.4. Ion profile penetration

Ion and water profile was determined in a slice 20 mm thick. Five zones were established as shown in Fig. 1. Zone 1, corresponds to the superficial layer, zones 2, 3 and 4 to the inner zones and finally, zone 5 corresponds to the zone in contact with the fat layer of the loin.

2.5. Physicochemical analyses

Analytical determinations were carried on the cubic samples used for TPA analysis and moisture content was determined on slices used for colour analysis. These were immediately cut and analytical determinations carried out on each sample. Moisture content was determined by oven drying to constant weight at 100 °C (ISO Norm R-1442, 1979). Fat content according to ISO Norm R-1443 (1973) using a FOSS Soxtec System 2055 Tecator. The water activity of each sample was determined by using an Aqualab® dew point hygrometer (Decagon Devices Inc., Washington, USA). Chloride was determined after sample homogenization in a known amount of distilled water at 9000 rpm in an ULTRA-TURRAX T25 for 5 min and centrifuged to remove any fine debris present. Afterwards, the solution was filtered and exactly 500 μl was taken and titrated in a Chloride Analyzer equipment (CIBA Corning Mod. 926). In the case of the ion penetration study, moisture content and chloride was also determined in the five samples of the section of the slice (Fig. 1). The same aliquot was used to determine sodium, potassium, calcium and magnesium in each sample by ion chromatography using an ion exchange column (Metrosep C2, 250/4.0, Methrom®, Herisau, Switzerland) in PC-controlled Compact IC 761 equipment (Methrom®, Herisau, Switzerland), with the following parts: built-in double piston pump, electrically operated injection valve and a temperature-stabilized high performance conductivity detector. The mobile phase was tartaric acid–dipicolinic acid (4.0–0.75 mmol/L) at 1.0 ml/min. The separation was monitored using a conductivity detector and software IC Net 2.3 (Methrom® Ltd., Herisau, Switzerland) was used for data collection and processing. The concentration of each cation was determined by interpolation in the corresponding calibration curve. The calibration 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 were means of three determinations.

Sodium, potassium, calcium and magnesium concentration in the meat liquid phase (z^{Na}, z^{K}, z^{Ca} and z^{Mg}, respectively) were estimated from water and sodium, potassium, calcium and magnesium weight fractions (x^{w}, x^{Na}, x^{K}, x^{Ca} and x^{Mg}, respectively) as shown in Eq. (2). All determinations were done in triplicate.

$$z^i = \frac{x^i}{x^i + x^w} \tag{2}$$

2.6. Microbiological analysis

Microbiological analyses were carried out on one of the adjoining slices of the central transversal section in sterile conditions and considering total mesophilic aerobic flora (ISO 4833, 2003), salt-tolerant flora (Tomlinson, 1995), lactic acid bacteria, lactose positive (Tomlinson, 1995), *Enterobacteriaceae* (Tomlinson, 1995), faecal coliforms (Tomlinson, 1995), *Bacillus cereus* (ISO 7932, 2004), *Listeria* spp. (ISO 11290-2, 1998), coagulase-positive staphylococci (ISO 6888-1, 1999), *Clostridium perfringens* and sulfite-reducing clostridium (Tomlinson, 1995), *Salmonella* spp. (ISO 6579, 2002) and *Shigella* spp. (ISO 21567, 2004).

2.7. Statistics

The effect of the salt combination, on several variables was by analysis of variance, one-way ANOVA using the statistical software STATGRAPHICS Plus 5.1®. The variables were: total weight changes, moisture, chloride concentrations on a dry basis, water activity, colour parameters and microbial counts at the end of the curing and drying process. In those cases where the effect was significant ($p < 0.05$) the mean values were compared using Fisher's least significant difference (LSD) procedure.

The influence of the different formulations of salts and moisture on the textural characteristics of the final product were analyzed by multiple regression analysis with the stepwise removal procedure and forward selection method, using the Statgraphics® Plus 5.1 version software (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Influence of different salt combinations on weight changes during the curing process

The total weight changes (ΔM_t^o) were calculated by means of Eq. (3), (M_t^o and ΔM_0^o are the loin weight, at time t and 0, respectively). No significant differences were observed between mean values of total weight changes at the end of processing (after 60 days), being $\Delta M_t^o = 0.42 \pm 0.03$ in treatment I (formulation control), 0.43 ± 0.03, 0.42 ± 0.02 and 0.41 ± 0.03 in treatments II, III and IV, respectively.

These values are in accord with the industrial process where loins are considered to have been fully processed on reaching around 40% weight loss.

$$\Delta M_t^o = \frac{M_t^o - M_0^o}{M_0^o} \quad (3)$$

Similar behaviour between the total weight changes throughout the process was observed for the loins salted with the four formulations, I (control), II, III and IV. Fig. 2(1) shows ΔM_t^o in I and Fig. 2(2, 3 and 4) the relative variation of ΔM_t^o (Eq. (4)) in II, III and IV with regards to the ΔM_t^o of the control batch (ΔM_t^o I) after 40 days of processing.

$$\Delta M_t^o \text{ relative} = \frac{\Delta M_t^o(\text{II, III, IV}) - \Delta M_t^o(\text{I})}{\Delta M_t^o(\text{I})} \quad (4)$$

In addition, the three stages corresponding to salting and post-salting (a) and the two periods of drying, b and c, respectively are shown. It seems that loins salted with formulations II and IV containing 15% $CaCl_2$, 5% $MgCl_2$ and 25% and 50% KCl, respectively, registered a lower loss of weight during the first stages (salting and post-salting). This could be due to the quicker penetration of the mixtures containing KCl that would hinder the exit of water from within the meat. However, concentrations of $CaCl_2$ and $MgCl_2$ up to 20% and 10%, respectively (III) increase weight loss (Fig. 2(3)). This could be due to the lower charge density of K^+ (0.026 units of charge/molecular weight) compared to Ca^{2+} and Mg^{2+} ions (0.050 and 0.082 units of charge/molecular weight, respectively) that would decrease and increase, respectively, their ability to penetrate the muscle (Blesa et al., 2008). Similar results were observed in loin pile salted with low sodium salts (Aliño, Grau, Baigts, & Barat, 2009) and in dry-cured hams using KCl as a substitute for NaCl (Comaposada, Arnau, & Gou, 2007). Moreover, Ca^{2+} and Mg^{2+} cations being the most electronegative of all those assayed, could bind strongly to the protein polar groups, strengthening protein interactions (Xiong & Brekke, 1991) and thus hindering the penetration of salt.

However, similar behaviour was observed during the drying stage (c) for the three formulations in contrast to the control formulation (Fig 2). In fact, no significant differences in the mean values of ΔM_t^o for the four formulations were observed (p-value $\geqslant 0.05$) by the end of the process.

3.2. Characterization of the salting process

The x^w (w/w), a_w and X^{Cl} (dry basis) mean values at the end of the process in the four batches and in commercial loins analyzed previously (Aliño, Grau, Fuentes, & Barat, 2009; Aliño, Grau, Toldrá, et al., 2009) are shown in Table 3. As can be observed, x^w and X^{Cl} were very similar to commercial values no significant differences were observed between the mean X^{Cl} from one formulation to another, including the commercial ones (p-value $\geqslant 0.05$). Indeed, there were no significant differences (p-value $\geqslant 0.05$) between the a_w values of commercial dry-cured loins and the a_w values of loins salted with formulations II, III and IV even though there was a statistical significant difference with the loins salted with the control formulation. The difference in a_w values of low sodium dry-cured loin and the control formulation could be due to the fact that NaCl decreases a_w more than KCl. In fact, the values of parameter "B", characteristic of every electrolyte in the Pitzer–Bromley model to predict water activity in aqueous solutions, are $B = 0.0574$ and 0.024, for NaCl and KCl, respectively. Even though a_w values between controls and commercial dry-cured loin were

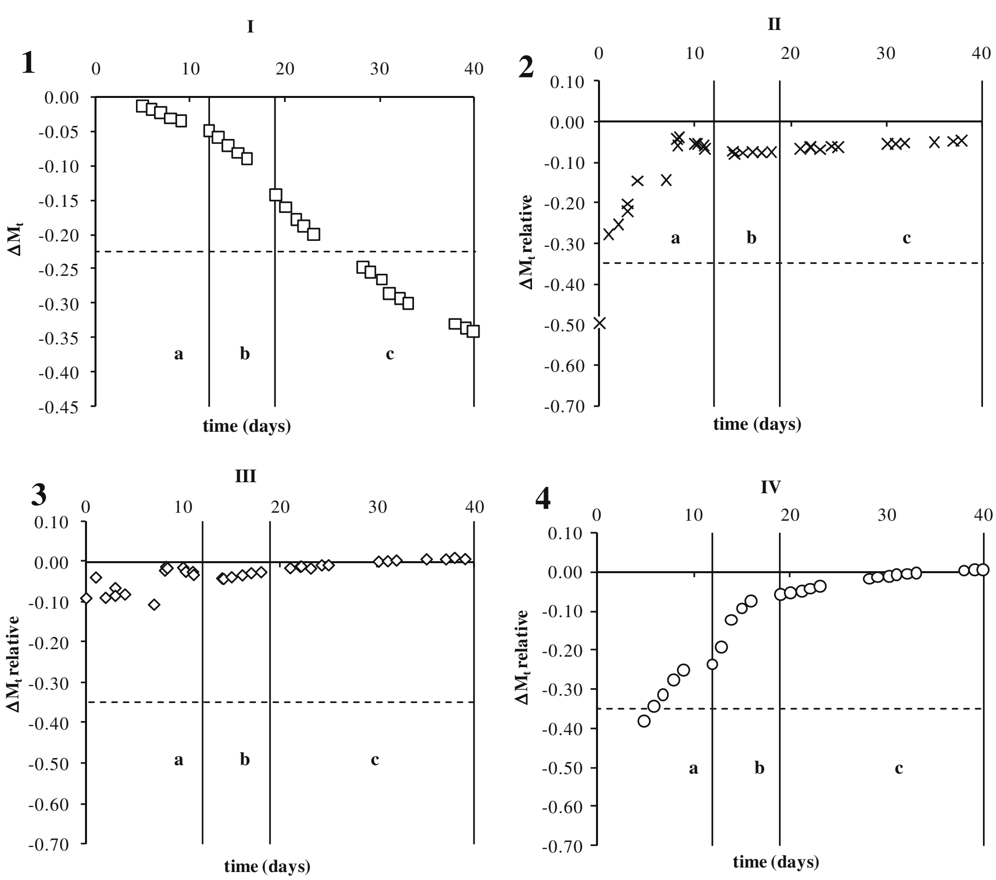

Fig. 2. Total loin weight changes in I, ΔM_t^o I, (1) and the relative variation of ΔM_t^o in II, III and IV (2, 3 and 3, respectively) with regards to ΔM_t^o I (Eq. (4)).

Table 3
Moisture (x^w, w/w), chloride concentration on a dry basis (X^{Cl}) and water activity (a_w) of dry-cured loins rub salted with the different salt formulations and of commercial dry-cured loins (Aliño, Grau, Toldrá, et al., 2009; Aliño, Grau, Fuentes, et al., 2009).

Formulation	x^w ± SD	X^{Cl} ± SD	a_w ± SD
I	0.48 ± 0.06^a	0.056 ± 0.007^a	0.92 ± 0.01^a
II	0.50 ± 0.02^a	0.054 ± 0.005^a	0.937 ± 0.004^c
III	0.51 ± 0.03^a	0.057 ± 0.003^a	0.940 ± 0.004^c
IV	0.51 ± 0.02^a	0.054 ± 0.003^a	0.923 ± 0.007^{ab}
Commercial	0.47 ± 0.02^a	0.056 ± 0.007^a	0.932 ± 0.003^{bc}

Means in a column with different letters are significantly different at $p < 0.05$.

significantly different, they could be acceptable (a_w (I) = 0.92 ± 0.01 and aw (commercial) = 0.932 ± 0.003). These differences could be due to differences in the raw material, the curing salts or the processing conditions.

3.3. Texture analysis

A Stepwise Multiple Regression analysis with forward selection of significant variables ($p \leqslant 0.05$) was performed to evaluate the influence of water content on a dry basis (X^w) and of salt formulation on texture. Salt formulations (II, III and IV) were considered as "dummy variables" (Hardy, 1993), so as to determine the influence of the studied salt formulation with regards to formulation control. Table 4 shows the final fitted models obtained by multiple regression analysis ($p < 0.01$). Moisture content had a significant effect on hardness and chewiness ($p < 0.01$) Fig. 3a and b shows the relationship between hardness and chewiness, respectively, and X^w for all dry-cured samples. As can be observed, hardness and chewiness decreased with X^w. For a X^w between 1.4 and 1.6 hardness and chewiness remained practically unchanged while for $X^w \leqslant 1.2$ the hardness increased substantially. The same results were obtained in dry-cured loin salted with mixtures of NaCl and KCl (Aliño, Grau, Toldrá, et al., 2009). Moisture significantly affected cohesiveness and springiness although the correlation of the fitted model was low; suggesting it also depends on other meat properties. Moreover, X^w did not have a significant effect on adhesiveness, over the range studied. Formulation IV, with 70% of NaCl substitution, 50% by KCl, 15% by $CaCl_2$ and 5% by $MgCl_2$ had a significant effect on all the texture parameters except cohesiveness. It seems that replacement up to 70% NaCl by KCl, $CaCl_2$ and $MgCl_2$ significantly increases hardness and chewiness of dry-cured loins. This is in agreement with previous results where replacement of 70% NaCl by KCl caused significant effects on hardness and chewiness (Aliño, Grau, Toldrá, et al., 2009).

3.4. Colour

The colour of dry-cured loin is mainly due to the presence of heme pigments, primarily nitrosylmyoglobin and metmyoglobin, and partially to the presence of chili pepper that contains carotenoids, although their contribution to the overall colour is not well defined (Campus, Flores, Martínez, & Toldrá, 2008). Fig. 4A shows a three dimensional representation of a^*, b^* and L^* for each salt treatment. No significant differences were observed in the mean values of a^*: redness, b^*: yellowness, C^*: chroma and h^*: hue between the three zones analyzed for each loin and between the loins salted with the four formulations ($p > 0.05$). However, mean values of lightness (L^*) in loins salted with formulation III were lower than in loins salted with I, II and IV ($p < 0.05$). This could be due to the higher calcium and magnesium concentrations in formulation III, 20% and 10%, respectively, compared to 15% and 5% in formulations II and IV. Moreover, while formulations II and III have the same potassium concentration (25%), formulation IV has 50% potassium. Fig. 4B shows the relationship between h^* and the ratio a^*/b^* with percentage of sodium in the mixture (I: 100%; II: 55%; III: 45% and IV: 30%). As can be observed, h^* increased with sodium replacement whereas a^*/b^* decreased when amount of sodium was reduced. However, these differences were not significant, neither for h^* nor for a^*/b^* ($p > 0.05$).

Table 4
Estimated parameters of the mathematical model ($p < 0.01$) for hardness (g), chewiness (g), cohesiveness, springiness and adhesiveness ($Y = a + b{\cdot}\mathrm{II}{\cdot}X^w + c{\cdot}\mathrm{III}{\cdot}X^w + d{\cdot}\mathrm{IV}{\cdot}X^w + e{\cdot}X^w + f{\cdot}(X^w)^2$).

Y	Constant a	II·X^w b	III·X^w c	IV·X^w d	X^w e	$(X^w)^2$ f	R^2 (%)	Standard error
Hardness	33755.7	–	–	248.004*	−43195.2*	13972.7*	86	460.48
Chewiness	21744.2	–	–	204.735*	−27655.9*	8869.96*	85	319.75
Cohesiveness	0.88	–	−0.02*	–	–	−0.06*	30	0.05
Springiness	0.91	–	–	0.05*	–	−0.08*	23	0.08
Adhesiveness	−14	–	–	−16*	–	–	10	22

* Variables with coefficient in the same line are statistically significant at $p < 0.01$.

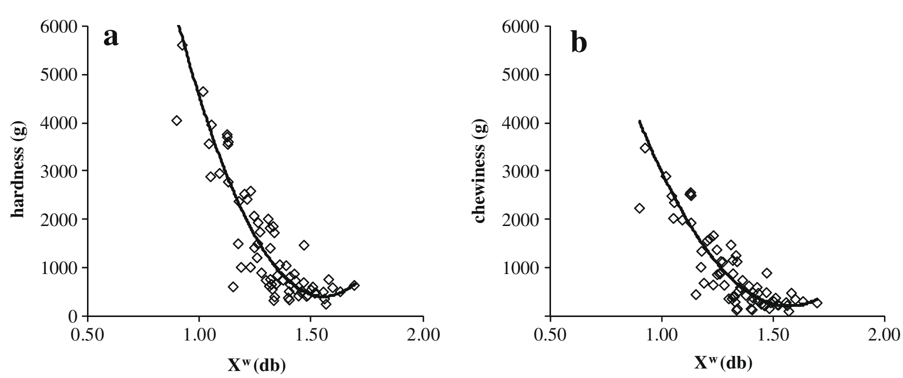

Fig. 3. Experimental and predicted hardness and chewiness versus water content on a dry basis (X^w, db) in dry-cured loins (a and b, respectively).

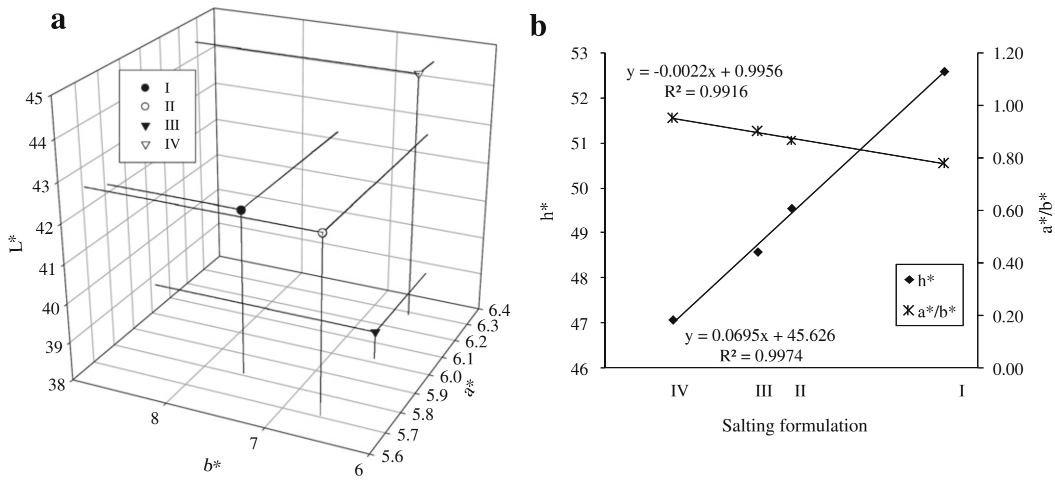

Fig. 4. Three-dimensional representation of a^*, b^* and L^* for each salt treatment (A) and the relationship between h^* and a^*/b^* with regards to the salt formulation.

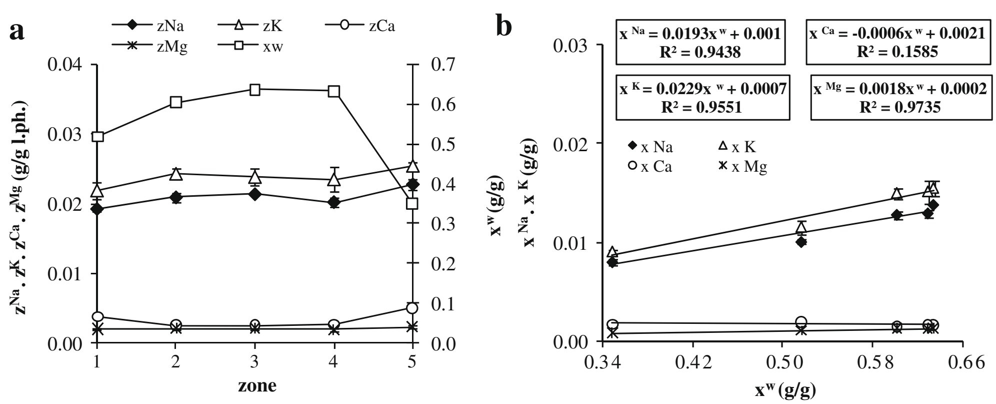

Fig. 5. Water weight fraction (x^w, w/w), Na^+, K^+, Ca^{2+} and Mg^{2+} concentration in the meat liquid phase (z^{Na}, z^K, z^{Ca} and z^{Mg}, respectively) throughout the loin profile (a), and linear correlation between sodium, potassium, calcium and magnesium weight fractions (x^{Na}, x^K, x^{Ca} and x^{Mg}, respectively) with x^w (b) in dry-cured loins salted with formulation III.

Replacing NaCl with other chloride salts such as potassium chloride (KCl), magnesium chloride ($MgCl_2$), or calcium chloride ($CaCl_2$) may affect the sensory properties of meat products. In fact, KCl, the most used substitute, imparts a bitter taste at high concentrations, and consequently, its use is restricted (Leak, Kemp, Fox, & Langlois, 1987). Indeed, the maximum level of NaCl that could be substituted in fermented sausages (Gelabert et al., 2003) and dry-cured loins (Gou et al., 1996) without imparting undesirable flavor and texture attributes was 40%. More recently, Armenteros, Aristoy, Barat, and Toldrá (2009a) have reported substitutions of up to 50% by KCl in dry-cured loin without affecting sensory properties. The results of the sensory analysis of dry-cured loins salted with formulations II, III and IV revealed that loins salted with treatment II (55% NaCl, 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$) were not significantly different from the loins salted traditionally (100% NaCl), and were preferred by the assessors in relation to the aroma attribute. So, this treatment could be successfully used for sodium reduction (Armenteros, Aristoy, Barat, & Toldrá, 2009b).

3.5. Ion profile penetration

Fig. 5a shows cation concentration in the loin liquid phase (z^{Na}, z^K, z^{Ca} and z^{Mg}) and loin water weight fraction (x^w, w/w) throughout the dry-cured loins salted with formulation III. As can be observed, sodium, potassium and magnesium concentrations in the liquid phase were uniform ($p > 0.05$) throughout the loin profile so that an equilibrium concentration was reached at the end of the dry-curing process. However, calcium concentrations were higher in zones of lower water content (zones 1, superficial lean layer, and 5, in contact with the fat layer) these values being significantly different from calcium concentrations in the inner zones ($p < 0.05$). In fact, calcium binds more strongly to the outermost layers of the muscle proteins, compacting the surface of the meat (Aliño, Grau, Baigts, et al., 2009; Iyengar & Sen, 1970). Therefore, the amount of calcium left in the liquid phase in the dry zones should be similar to the amount in the inner zones of the loin of higher water content and thus an equilibrium concentration should be reached. The same patterns were observed in loins salted with II and IV.

A linear and positive relationship between cation weight fraction (x^{Na}, x^K and x^{Mg}) and x^w was observed in loins salted with the three formulations. Fig. 5b shows this relationship for III. The same pattern was observed in treatments II and IV. On the contrary, calcium weight fraction had a negative relationship with water content (Fig. 5b) which confirms the presence of calcium bound to proteins in the dried layers of the loin.

Fig. 6 shows mass cation ratios of Na/Ca and Na/Mg (a) and of K/Ca and K/Mg (b) in loins salted with formulation II. The same pattern was observed in formulations III and IV. These ratios were higher in the inner zones, 2, 3 and 4, than in the superficial zones

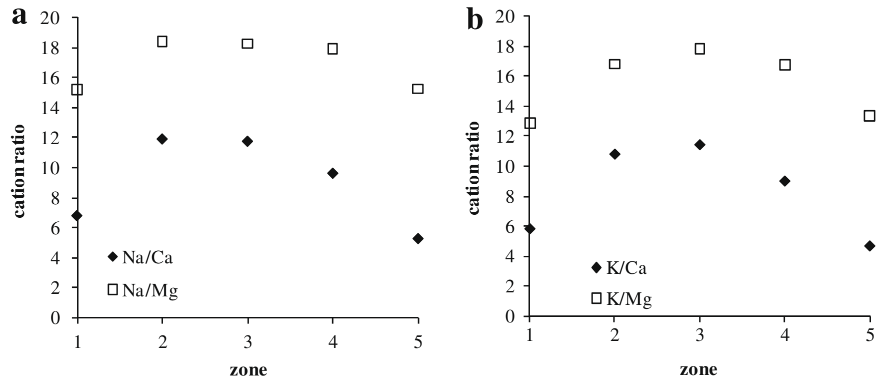

Fig. 6. Mass cation ratios, Na/Ca and Na/Mg (a) and K/Ca and K/Mg (b) throughout the loin profile in dry-cured loins salted with formulation II.

1 and 5 ($p < 0.05$), indicating greater penetration of sodium and potassium in the inner muscles than of calcium and magnesium.

3.6. Microbiological analyses

Microbiological counts (aerobic mesophiles, salt-tolerant flora and lactic acid bacteria) are shown in Table 5. There were no significant differences ($p > 0.05$) between the different salt formulations. The lack of significant differences for aerobic mesophiles and lactic acid bacteria in different salt formulations has been reported in Cheddar cheese (Reddy & Marth, 1995a, 1995b) and fermented sausages (Ibáñez et al., 1995). Terrell et al. (1982) found no significant differences between 2.5% NaCl or equivalent ionic strengths of KCl, $MgCl_2$ and $CaCl_2$ in total aerobic plate counts in pork sausage refrigerated for 12 days. On the other hand, in the case of dry-cured ham, the type of salt mixture used in the salting process can affect the level of these bacteria (Blesa et al., 2008). This behaviour was also observed previously where the effect of partial replacement of sodium chloride with potassium chloride was studied (Aliño, Grau, Toldrá, et al., 2009).

The replacement of NaCl by other salts appears to reduce the quantity of salt-tolerant flora. Specifically, the loins with salt formulation III (45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$) had the least halotolerant microorganisms however the differences were not significant. Studies with different levels of NaCl and KCl suggested lower levels of halotolerant microorganisms when NaCl levels are below 50% (Aliño, Grau, Toldrá, et al., 2009).

Desirable bacteria, such as salt-tolerant bacteria and lactic acid bacteria, were predominant. Yamanaka, Akimoto, Sameshima, Arihara, and Itoh (2005) indicated that NaCl induced selective multiplication of salt-tolerant bacteria and lactic acid bacteria, and suppressed the growth of coliforms. In this study, the salt mixtures used appeared to do the same. The counts of these microorganisms were lower than observed by others (Yamanaka et al., 2005; Campus et al., 2008).

The lactose positive *Enterobacteriaceae* and faecal coliforms showed levels lower than 3 cfu/g, for all loins, thus, no influence of the salt mixture was observed. Similar results were obtained for other salt mixtures (Aliño, Grau, Toldrá, et al., 2009) and in dry-cured hams during the post-salting stage (Blesa et al., 2008).

Coagulase-positive staphylococci and *B. cereus* were present in 33% of the samples treated with 100% NaCl; the highest levels were 1.3×10^3 and 4.8×10^2 cfu/g, respectively. But, when the salt mixtures were used, the prevalence of these microorganisms increased. The incidence observed in these cases was between 80% and 100% for coagulase-positive staphylococci and 50–70 for *B. cereus*. The highest levels were 5×10^3 cfu/g and 4×10^3 cfu for *Staphylococcus aureus* and *B. cereus*, respectively.

Yamanaka et al. (2005) reported that before curing, only gram-negative bacteria (*Vibrio, Acinetobacter, Pseudomonas* and *Enterobacteriaceae*) are present, but during curing, the number of viable gram-positive bacteria (*Micrococcus, Staphylococcus* and *Pediococcus*) increases and the number of gram-negative bacteria decreases. Nevertheless, the genus *Staphylococcus* has a strong tolerance to high concentrations of NaCl (more than 10%) compared with *Micrococcus*, which explains the presence of staphylococci at the end of the curing process (Molina, Silla, Flores, & Monzó, 1989; Silla, Molina, Flores, & Silvestre, 1989; Yamanaka et al., 2005). Nevertheless, these values were lower than 10^5 cfu/g which is the limit for producing food poisoning (Dinges, Orwin, & Schlievert, 2000).

C. perfringens, sulfite-reducing clostridium, *Salmonella* spp. and *Shigella* spp. were not detected in any samples independent of the salt mixture used. Strong, Foster, and Duncan (1970) reported that KCl appears to inhibit growth of *C. perfringens* more than NaCl. Barbut, Tanaka, and Maurer (1986) concluded that NaCl was better than KCl or $MgCl_2$ for inhibiting *Clostridium botulinum* toxin production in turkey frankfurters, $MgCl_2$ being the least inhibitory and that the replacement of half of the NaCl with KCl or $MgCl_2$ generally reduced the time for toxin production to occur compared to KCl or $MgCl_2$ alone.

No significant differences ($p < 0.05$) were observed in the counts of pathogenic microorganisms in loins salted with the four mixtures. *Listeria* spp. was detected at a prevalence of 17% in all samples analyzed for each formulation. The highest level detected was 2.15×10^3 cfu/g. Boziaris, Skandamis, Anastasiadi, and Nychas (2007) reported that equimolar concentrations of NaCl or KCl exerted similar inhibitory effects on *Listeria monocytogenes* in terms of lag phase duration, growth or death rate and that NaCl can be replaced by KCl without risking microbiological safety, with regard to these microorganisms. Epidemiological data indicates that food

Table 5
Mean values (±standard deviation) of microbiological analyses (log cfu/g) of aerobic mesophiles, salt-tolerant flora and lactic acid bacteria.

Formulation	Aerobic mesophiles n.s.	Salt-tolerant flora n.s.	Lactic acid bacteria n.s.
I	3.29 ± 1.06^a	3.13 ± 1.01^a	2.68 ± 0.71^a
II	2.77 ± 0.22^a	2.68 ± 0.70^a	2.22 ± 0.90^a
III	3.02 ± 0.60^a	2.20 ± 0.42^a	2.66 ± 1.06^a
IV	2.91 ± 0.14^a	2.96 ± 0.34^a	2.81 ± 1.03^a

Means in a column with different letters are significantly different ($^*p \leqslant 0.05$; n.s.: non significant).

involved in listeriosis outbreaks are those in which the *Listeria monocytogenes* organism has multiplied and in general has reached levels significantly above 1000 cfu/g, although in many European countries, a concentration over 100 cfu/g (Risk Assessment Drafting Group, 2004) is considered a risk.

The results indicate that NaCl can be replaced with salt mixtures, in dry-cured loins, with no risks for safety. In other studies no differences in the microbial counts were found between products salted with different salt formulations (Aliño, Grau, Toldrá, et al., 2009; Blesa et al., 2008; Ibáñez et al., 1995; Reddy & Marth, 1995a, 1995b; Seperich & Ashoor, 1983; Terrell et al., 1982).

However, studies show the difficulty in predicting the antimicrobial action of salt mixtures because they depend on numerous factors such as temperature, pH, the solutes present in the media, the raw material or the strain of microorganism (Gimeno et al., 1998, 2001; Ibáñez et al., 1995, 1996; Sofos, 1983).

4. Conclusion

The presence of potassium in the salt mixture increased water loss whereas calcium and magnesium induced the opposite effect during the salting and post-salting stages, although these differences were not observed during the drying stage. The replacement of around 70% NaCl by KCl, $CaCl_2$ and $MgCl_2$ significantly increased hardness and chewiness of dry-cured loins. However, no significant differences were observed in the mean values of chloride content, moisture and colour among the loins salted with the four formulations. Potassium penetrated more easily than sodium, calcium and magnesium in the muscle. Calcium had more difficulty penetrating the muscle and remained bound to proteins in the outer layers of the loin.

The replacement of NaCl by KCl, $CaCl_2$ and $MgCl_2$ appeared to reduce, insignificantly, the quantity of salt-tolerant flora and to increase the prevalence of Coagulase-positive staphylococci and *B. cereus*. However, the microbial counts among the loins salted with the different formulations, indicated that NaCl can be partially replaced with mixtures of KCl, $CaCl_2$ and $MgCl_2$ with no risks for safety in dry-cured loins.

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