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# Effect of the partial NaCl substitution by other chloride salts on the volatile profile during the ripening of dry-cured lacón

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**SUMMARY:** The influence of three salting treatments (treatment II: 50% NaCl-50% KCl; III: 45% NaCl-25% KCl-20% $CaCl_2$-10% $MgCl_2$; IV: 30% NaCl-50% KCl-15% $CaCl_2$-5% $MgCl_2$) on the formation of volatile compounds throughout the process was studied and compared to those of a control "lacón" (treatment I: 100% NaCl). There was an intense formation of volatile compounds throughout the processing, particularly during the dry-ripening stage. The most abundant chemical family in all the formulations, in the final product was hydrocarbons followed by aldehydes. The total volatile compound release was more intense in the control "lacóns" (1164 AU×$10^6$·$g^{-1}$dry matter) than in "lacóns" from formulations II, III and IV (817–891 AU×$10^6$·$g^{-1}$dry matter). The "lacóns" from formulation I showed the highest amounts of aldehydes. The "lacóns" from formulations I and II presented the highest amounts of hydrocarbons. The main conclusion is that the replacement of NaCl produces changes in the volatile profile and could be affect the aroma of "lacón".

***KEYWORDS:*** *Calcium chloride; Dry-cured "lacón"; Magnesium chloride; Potassium chloride; Volatile compounds*

**RESUMEN:** ***Efecto del reemplazo parcial de NaCl por otras sales en el perfil de volátiles durante la maduración del lacón crudo-curado***. Se estudió la influencia de tres tratamientos de salado (tratamiento II: 50 % NaCl-50 % KCl; III: 45 % NaCl-25 % KCl-20 % $CaCl_2$-10 % $MgCl_2$; IV: 30 % NaCl-50 % KCl-15 % $CaCl_2$-5 % $MgCl_2$) en la formación de compuestos volátiles durante la elaboración de lacón, en comparación con un control (tratamiento I: 100 % NaCl). Hubo una intensa formación de compuestos volátiles durante el procesado, principalmente durante la fase de secado-maduración. La familia química más abundante en el producto final fueron los hidrocarbonos, seguidos por los aldehídos. La liberación de volátiles fue más intensa en los lacones control (1164 AU×$10^6$·$g^{-1}$ materia seca) que en los otros lacones (817–891 AU×$10^6$· $g^{-1}$ materia seca). Los lacones de la formulación I mostraron las mayores cantidades de aldehídos, y los lacones de las formulaciones I y II presentaron los mayores contenidos de hidrocarburos. La principal conclusión es que el reemplazo de NaCl produce cambios en los compuestos volátiles y por lo tanto podrían afectar al aroma del lacón.

***PALABRAS CLAVE:*** *Cloruro cálcico; Cloruro magnésico; Cloruro potásico; Compuestos volátiles; Lacón crudo-curado*

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## 1. INTRODUCTION

Dry-cured "lacón" is a traditional cured meat product made in the north-west of Spain from the fore leg of the pig which is cut at the shoulder blade-humerus joint, following very similar manufacturing processes to those used in the production of dry-cured ham as described by Purriños *et al*., (2011a). In Galicia (NW Spain), this product has been awarded Geographically Protected Identity (G.P.I.) (Official Journal of the European Communities, 2001).

Salt is an essential ingredient in dry-cured "lacón" due to its contribution to the water-holding capacity, prevention of microbial growth, and reduction in water activity which facilitate the solubilization of certain proteins and confer a typical salty taste (Lorenzo *et al*., 2008; Purriños *et al*., 2011b; Purriños *et al*., 2013). Moreover, the salt affects some chemical and biochemical reactions such as proteolysis, lipolysis and lipid oxidation, which contribute to the development of texture and typical flavor of dry-cured "lacón" (Garrido *et al*., 2012; Purriños *et al*., 2012).

In recent years different studies have begun to show that meat consumption is being more and more influenced by health and nutritional considerations. The mean daily sodium intake of the European population ranges from about 3 to 5 g (8 to 11 g NaCl) (EFSA, 2005). On a general basis, it has been established that the consumption of more than 6 g NaCl/day/person is associated with aging and increase in blood pressure. Therefore, limiting dietary sodium intake should be achieved by restricting daily salt (sodium chloride) consumption to less than 5 g per day (WHO/FAO, 2003). In the case of Spain, in 2008 the Spanish Food Safety and Nutrition Agency started a salt reduction plan with certain specific goals which would enable its intake to go down from the current value of 9.7 g/day to an intake of less than 8.0 g/day by 2014.

Due to the increased knowledge about the links between sodium intake and coronary heart diseases, consumers' demand for low-salt meat products with the same quality as conventional ones has increased. To this regards, the partial substitution of NaCl by a mixture of salts (potassium, calcium, and magnesium salts) appears to be the best alternative to reduce the sodium content in dry-cured "lacón" (Lorenzo *et al*., 2015). However, the effect of the partial NaCl replacement by other chloride salts on the volatile profile of dry-cured "lacón" is unknown.

The aim of this work was to determine the influence of partial NaCl replacement by a mixture of KCl, $CaCl_2$ and $MgCl_2$ on the generation and release of volatile compounds to the headspace of dry-cured "lacón" for their possible contribution to the flavor of this product.

## 2. MATERIAL AND METHODS

### 2.1. Samples

Fifty-two fresh "lacón" pieces with an average weight of 4.61±0.47 kg each were obtained from a local slaughterhouse in the area of Ourense (Spain). Four of the raw pieces were sampled and analyzed in order to characterize the raw material. The remaining forty-eight "lacón"raw pieces were submitted to the traditional "lacón" processing (Purriños *et al*., 2011a). The "lacón" samples were randomly divided into four batches with twelve "lacóns" in each batch. "Lacóns" from the first batch were salted with the traditional NaCl (100% NaCl, treatment I) and were used as the control of the volatile compounds, whereas the other batches were salted in the same way but with partial replacements of NaCl by other salts. So, the second batch was salted with 50% NaCl and 50% KCl (treatment II); the third batch with 45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$ (treatment III); and the fourth batch with 30% NaCl, 50% KCl, 15% $CaCl_2$ and 5% $MgCl_2$ (treatment IV). The salting stage was carried out between 2 and 5 °C and the relative humidity (HR) between 80 and 90% for a total of 5 days. After the salting stage the pieces were taken from the heap, brushed, washed, and transferred to a post-salting room where they stayed for 14 days at 2–5 °C and around 85–90% HR. After the post-salting stage the pieces were transferred to a room at 12 °C and 74–78% HR where a drying-ripening process took place for 84 days. Samples were taken after the salting, post-salting, and drying-ripening stages. In each sampling point, a total of 4 "lacón" samples from each batch were analyzed. The "Lacón" pieces were skinned, deboned, and the *triceps brachii* muscle was extracted. The samples were vacuum packed and stored at −30 °C for no longer than four weeks until analysis.

### 2.2. Chemical composition and pH values

Moisture, fat and protein contents were determined according to the Association of Official Analytical Chemists (2005) in triplicate. The moisture content was determined by drying in an oven at 105 °C±2 °C; the nitrogen content was determined by the Kjeldahl method and the protein content was estimated by multiplying the nitrogen content by 6.25; the fat content was determined by the Soxhlet method using petroleum ether as solvent. The pH of the samples was measured using a digital pH meter (model 710 A+, Thermo Orion, Cambridgeshire, UK) equipped with a penetration probe.

### 2.3. Mineral composition

The quantification of mineral elements (Na, K, Ca and Mg) was performed by inductively coupled plasma-optical emission spectroscopy (ICP-OES),

according to the procedure described by Lorenzo *et al.* (2015). The final value for each element was obtained by calculating the average of three determinations.

### 2.4. Volatile compound profile

The extraction of the volatile compounds was performed using solid-phase micro-extraction (SPME). A SPME device (Supelco, Bellefonte, PA, USA) containing a fused-silica fiber (10 mm length) coated with a 50/30 µm thickness of DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was used for HS-SPME extraction.

The muscle samples were ground with a commercial grinder, a 1 g portion was weighed into a 24 mL vial and the vial was screw-capped with a laminated Teflon rubber disk. The fiber was inserted into the sample vial through the septum and then exposed to the headspace. The extractions were carried out in an oven to ensure a homogeneous temperature for sample and headspace. The fiber was conditioned prior to analysis by heating it in a gas chromatograph injection port at 270 °C for 60 min, following the manufacturer specifications. Extraction was performed at 35 °C for 30 min. Before extraction, the samples were equilibrated for 15 min at the temperature used for extraction. Once sampling was finished, the fiber was drawn into a needle and transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system.

A gas chromatograph 6890N (Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a mass detector 5973N (Agilent Technologies Spain, S.L., Madrid, Spain) was used with a DB-624 capillary column (J&W scientific: 30 m, 0.25 mm id, 1.4 µm film thickness). The SPME fiber was desorbed and maintained in the injection port at 260 °C for 8 min. The sample was injected in the splitless mode. Helium was used as carrier gas with a linear velocity of 40 $cm \cdot s^{-1}$. The temperature programme was isothermal for 10 min at 40 °C, raised to 200 °C at a rate of 5 $°C \cdot min^{-1}$, and then raised to 250 °C at a rate of 20 $°C \cdot min^{-1}$, and held for 5 min: total run time 49.5 min. Injector and detector temperatures were both set at 260 °C. The mass spectra was obtained using a mass selective detector working in electronic impact at 70 eV, with a multiplier voltage of 1953 V and collecting data at a rate of 6.34 $scans \cdot s^{-1}$ over the range m/z 40-300.

The compounds were identified comparing their mass spectra with those contained in the NIST05 (National Institute of Standards and Technology, Gaithersburg) library, and/or by comparing their mass spectra and retention time with standards (Supelco, Bellefonte, PA, USA), and/or by calculation of retention index relative to a series of standard alkanes ($C_5$–$C_{14}$) (for calculating linear retention index, Supelco 44585-U, Bellefonte, PA, USA) and matching them with data reported in the literature. The results are expressed as AU (area units)$\times 10^6 \cdot g^{-1}$ of dry matter.

### 2.5. Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics 19 software (IBM, Corp, 2010). The normality was assessed using the Kolmogorov-Smirnov test, and the Levene's homogeneity of variance test was applied to examine the equality of variances. After verification of normal distribution and constant variance of data, significant differences were determined using one-way analysis of variance (ANOVA). A Duncan's test was performed to compare the mean values for processing time (0, 5, 19 and 103 days) and partial sodium replacement at a significance level of $P<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of the salting treatments on physicochemical properties and mineral content

The results of chemical composition and pH values at the end of dry-cured "lacón" submitted to four different salting treatments are shown in Table 1. Moisture content was significantly ($P<0.05$) affected by NaCl replacement by other salts, since "lacóns" submitted to formulations IV presented the highest values. These differences could be due to the quicker penetration of the salt mixtures containing KCl that would hinder the exit of water from the inside of the meat (Aliño *et al*., 2009). This finding is in agreement with those reported by Wu *et al.* (2014) who observed significantly ($P<0.05$) higher moisture contents in bacon samples salted with 30% NaCl and 70% KCl compared with samples salted with 100% NaCl. However, Armenteros *et al*. (2012a) did not find significant differences among salting formulations on the moisture content of dry-cured ham. On the other hand, the protein and intramuscular fat contents did not show significant differences among treatments.

Regarding pH values, the NaCl replacement by other salts induced significant ($P<0.01$) differences among treatments. In this study the "lacóns" salted with lower NaCl concentrations (formulations III and IV) presented the lowest values of pH, while the highest values were obtained in "lacóns" from formulation II. Conflicting data are available in the literature about KCl, $MgCl_2$, and $CaCl_2$ inclusion in dry-cured meat products regarding this aspect. To this regards, Gimeno *et al*. (1999) studied the effect of a mixture of NaCl (10 $g \cdot kg^{-1}$), KCl (5.52 $g \cdot kg^{-1}$), $MgCl_2$ (2.35 $g \cdot kg^{-1}$), and $CaCl_2$ (4.64 $g \cdot kg^{-1}$) to partially substitute NaCl and reported a greater pH decrease in the reduced NaCl formulation compared to the traditional one. However, Zanardi

Table 1. Chemical composition and pH values of dry-cured lacón at the end of processing (mean ± standard deviation of four replicates)

| | Salt formulations | | | | SEM | Sign. |
|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | | |
| Moisture (%) | 55.28±3.83$^{ab}$ | 53.21±2.92$^{a}$ | 58.87±0.87$^{bc}$ | 61.66±0.28$^{c}$ | 1.12 | * |
| Protein (% of dry matter) | 33.28±2.77 | 36.76±4.07 | 31.03±2.91 | 32.09±3.66 | 0.94 | n.s. |
| Fat (% of dry matter) | 9.82±2.52 | 7.65±2.55 | 6.19±1.57 | 7.50±2.85 | 0.63 | n.s. |
| pH | 5.96±0.08$^{ab}$ | 6.08±0.09$^{b}$ | 5.83±0.05$^{a}$ | 5.92±0.05$^{a}$ | 0.03 | ** |

Salt formulations: treatment I: control, 100% NaCl; treatment II: 50% NaCl and 50% KCl; treatment III: 45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$; treatment IV: 30% NaCl, 50% KCl, 15% $CaCl_2$ and 5% $MgCl_2$
$^{a–c}$ Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P$<0.05). Sign.: Significance; n.s.: not significant; * ($P$<0.05); *** ($P$<0.001).

*et al.*, (2010) noticed that the salt mixture (NaCl 13.5 g·kg$^{-1}$, KCl 4.2 g·kg$^{-1}$, $CaCl_2$ 2.4 g·kg$^{-1}$, and $MgCl_2$ 2.4 g·kg$^{-1}$) did not affect the pH evolution throughout the processing.

Table 2 shows the mineral content of dry-cured "lacón" submitted to four different salting treatments. With regards to the natural content of minerals in the "lacón", the salt composition at the end of the ripening period inside the "lacón" is somehow reflected in the salt penetration and diffusion through the "lacón". A significant reduction ($P$<0.001) in the Na content was achieved through the partial substitution of NaCl by the mixture of chloride salts employed during their production. In our study, the "lacón" samples submitted to formulation I presented the highest amount of sodium (2446.6 mg·100 g$^{-1}$), which was similar to a normal level for "lacón" with these characteristics (Lorenzo *et al.*, (2003). The partial substitution of sodium chloride by the mixture of chloride salts significantly ($P$<0.001) reduced the sodium content and increased potassium, calcium and magnesium contents. However, lower concentrations of $Ca^{+2}$ and $Mg^{+2}$ were found in the "lacón" in relation to the proportions of these chloride salts employed during their production. These findings are in agreement with those obtained by Aliño *et al.* (2009) and Armenteros *et al.* (2009) who also observed 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 "lacón" (Blesa *et al.*, 2008).

### 3.2. Effect of the salting treatments on volatile compounds

The SPME technique is not normally used for absolute quantifications, but when exactly the same extraction methodology is applied, this technique allows for comparing relative amounts among samples. A total of 31 volatile compounds were detected in the headspace of "lacón" at the end of the dry-ripening process. The compounds were grouped by chemicals and their linear retention index (Table 3), comprising 3 acids, 4 alcohols, 4 aldehydes, 2 esters, 15 hydrocarbons, 2 ketones and 1 furan. At the end of the process the volatile compound profile maintained the relationship hydrocarbons>aldehydes>alcohols>acids>ketones>furan>esters. Lorenzo *et al.* (2014) also reported that hydrocarbons were the most abundant compounds in a previous study of dry-cured "lacón". However, other studies found that the most abundant group of volatile compounds

Table 2. Mineral composition (ppm) of dry-cured lacón at the end of processing (mean ± standard deviation of four replicates)

| | Salt formulations | | | | | |
|---|---|---|---|---|---|---|
| ppm | *I* | *II* | *III* | *IV* | SEM | Sign. |
| Na | 2446.60±108.46$^{c}$ | 1483.91±118.55$^{b}$ | 738.21±20.34$^{a}$ | 586.35±109.39$^{a}$ | 189.50 | *** |
| K | 565.11±22.22$^{a}$ | 1915.42±78.12$^{d}$ | 876.08±80.46$^{b}$ | 1668.94±99.21$^{c}$ | 127.61 | *** |
| Ca | 9.35±1.27$^{a}$ | 9.31±0.59$^{a}$ | 42.87±10.48$^{b}$ | 33.73±11.08$^{b}$ | 4.19 | *** |
| Mg | 35.04±5.35$^{a}$ | 31.89±3.35$^{a}$ | 42.73±2.14$^{b}$ | 36.97±3.70$^{b}$ | 1.64 | * |

Salt formulations: treatment I: control, 100% NaCl; treatment II: 50% NaCl and 50% KCl; treatment III: 45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$; treatment IV: 30% NaCl, 50% KCl, 15% $CaCl_2$ and 5% $MgCl_2$
$^{a–d}$ Mean values in the same row (corresponding to the same parameter) not followed by a common letter differ significantly ($P$<0.05). Sign.: significance: n.s.: not significant; * ($P$<0.05);***($P$<0.01).

TABLE 3. Volatile compounds (AU×$10^6$·$g^{-1}$dry matter) in the headspace of dry-cured "lacón" salted with different salt formulations at the end of processing. Data are the average values of four replicates

| | | | Salt formulations | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **LRI** | **R** | ***I*** | ***II*** | ***III*** | ***IV*** | **SEM** | **Sign.** |
| Acetic acid | 720 | *ms,lri* | 2.47 | 1.81 | 1.88 | 1.31 | 0.37 | ns |
| Butanoic acid | 883 | *ms,lri* | $15.58^{b}$ | $20.92^{b}$ | $22.53^{b}$ | $5.16^{a}$ | 2.94 | *** |
| Nonanoic acid | 1370 | *ms,lri* | $0.57^{b}$ | $0.00^{a}$ | $0.67^{b}$ | $0.00^{a}$ | 0.10 | *** |
| *Total acids* | | | *$19.64^{b}$* | *$22.01^{b}$* | *$24.87^{b}$* | *$5.78^{a}$* | *3.18* | * |
| 1-Pentanol | 834 | *ms, lri* | $21.35^{b}$ | $5.59^{a}$ | $14.83^{b}$ | $23.77^{b}$ | 2.71 | * |
| 1-Hexanol | 925 | *ms, lri* | $7.27^{b}$ | $0.00^{a}$ | $20.89^{c}$ | $16.63^{c}$ | 2.59 | *** |
| 1-Octen-3-ol | 1095 | *ms, lri* | $24.32^{ab}$ | $16.61^{a}$ | $32.84^{bc}$ | $41.94^{c}$ | 3.36 | ** |
| Benzyl alcohol | 1107 | *ms,lri* | 15.84 | 12.55 | 13.29 | 11.66 | 0.85 | ns |
| *Total alcohols* | | | *$64.80^{b}$* | *$35.82^{a}$* | *$63.94^{b}$* | *$93.99^{c}$* | *6.44* | *** |
| Pentanal | 736 | *ms,lri, s* | $15.64^{a}$ | $0.00^{b}$ | $13.84^{a}$ | $12.05^{a}$ | 2.34 | *** |
| Hexanal | 823 | *ms,lri, s* | $608.55^{c}$ | $40.97^{a}$ | $297.10^{b}$ | $274.90^{b}$ | 59.87 | *** |
| Heptanal | 933 | *ms,lri, s* | $8.32^{ab}$ | $6.46^{a}$ | $12.31^{b}$ | $16.20^{c}$ | 1.29 | ** |
| Decanal | 1347 | *ms,lri, s* | $3.92^{c}$ | $2.60^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 0.53 | *** |
| *Total aldehydes* | | | *$626.52^{c}$* | *$49.38^{a}$* | *$318.64^{b}$* | *$299.13^{b}$* | *61.48* | *** |
| Ethanol, 2-(2-butoxyethoxy)-, acetate | 1419 | *ms,lri* | $1.69^{b}$ | $1.27^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 0.25 | ** |
| 1,2,3-Propanetriol, diacetate | 1514 | *ms* | $1.10^{b}$ | $0.87^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 0.17 | ** |
| *Total esters* | | | *$2.79^{b}$* | *$1.85^{b}$* | *$0.00^{a}$* | *$0.00^{a}$* | *0.39* | *** |
| Pentane, 2,3,4-trimethyl- | 660 | *ms* | 3.81 | 5.01 | 2.66 | 2.07 | 0.51 | ns |
| Heptane | 700 | *ms,lri, s* | 2.71 | 2.26 | 2.40 | 0.63 | 0.39 | ns |
| Pentane, 2,3,3-trimethyl- | 706 | *ms,lri* | $12.23^{b}$ | $9.38^{b}$ | $3.24^{a}$ | $3.99^{a}$ | 1.25 | ** |
| Octane | 800 | *ms,lri, s* | 14.1 | 27.82 | 14.79 | 16.56 | 2.45 | ns |
| Heptane, 3-methylene- | 807 | *ms,lri* | $2.63^{b}$ | $3.42^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 0.52 | *** |
| Hexane, 3-ethyl- | 860 | *ms* | $1.30^{ab}$ | $1.82^{b}$ | $0.00^{a}$ | $1.93^{b}$ | 0.32 | ns |
| Heptane, 2,2,4-trimethyl- | 904 | *ms,lri* | $7.29^{b}$ | $7.37^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 1.20 | *** |
| Heptane, 2,5,5-trimethyl- | 924 | *ms,lri* | $5.42^{b}$ | $6.73^{b}$ | $0.00^{a}$ | $0.00^{a}$ | 0.89 | *** |
| Heptane, 2,2,4,6,6-pentamethyl- | 998 | *ms,lri* | $569.66^{b}$ | $627.46^{b}$ | $376.47^{a}$ | $428.29^{a}$ | 37.99 | * |
| 3-Ethyl-3-methylheptane | 1003 | *ms* | $1.73^{b}$ | $2.14^{b}$ | $1.64^{b}$ | $0.00^{a}$ | 0.27 | ** |
| Decane, 2,6,7-trimethyl- | 1134 | *ms* | $48.14^{b}$ | $30.57^{ab}$ | $37.37^{b}$ | $0.00^{a}$ | 7.54 | * |
| Dodecane | 1200 | *ms,lri, s* | 3.97 | 3.51 | 2.68 | 2.45 | 0.28 | ns |
| Undecane, 5-methyl- | 1207 | *ms,lri* | $5.63^{c}$ | $4.77^{bc}$ | $4.01^{ab}$ | $3.00^{a}$ | 0.40 | * |
| Decane, 5-methyl-6-methylene- | 1265 | *ms,lri* | 3.16 | 3.95 | 3.08 | 1.98 | 0.46 | ns |
| Tridecane | 1300 | *ms,lri, s* | 1.39 | 1.42 | 2.04 | 1.83 | 0.13 | ns |
| *Total hydrocarbons* | | | *$650.12^{b}$* | *$698.82^{b}$* | *$432.59^{a}$* | *$459.53^{a}$* | *40.89* | * |
| 2-Butanone, 3-hydroxy- | 765 | *ms,lri* | $15.87^{c}$ | $7.76^{ab}$ | $4.02^{a}$ | $11.86^{b}$ | 1.52 | * |
| 2-Heptanone | 940 | *ms,lri* | $3.73^{a}$ | $2.73^{a}$ | $5.61^{b}$ | $3.49^{a}$ | 0.33 | ** |

TABLE 3 *(continued)*

| | | | Salt formulations | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **LRI** | **R** | ***I*** | ***II*** | ***III*** | ***IV*** | **SEM** | **Sign.** |
| *Total ketones* | | | *20.43*$^{c}$ | *10.95*$^{ab}$ | *6.42*$^{a}$ | *15.35*$^{b}$ | *1.74* | ** |
| Furan, 2-pentyl- | 1009 | *ms,lri* | 4.06 | 3.80 | 6.05 | 5.80 | 0.53 | ns |
| *Total compounds* | | | *1164.38*$^{b}$ | *817.24*$^{a}$ | *873.03*$^{a}$ | *891.36*$^{a}$ | *53.31* | * |

Salt formulations: *I*: (100% NaCl), *II*: (50% NaCl and 50% KCl), *III*: (45% NaCl, 25% KCl, 20% $CaCl_2$ and 10% $MgCl_2$), *IV*: (30% NaCl, 50% KCl, 15% $CaCl_2$ and 5% $MgCl_2$). Sign.: significance; ns: not significant; *$P$<0.05; **$P$<0.01; ***$P$<0.001. $^{a\text{-}c}$ Means in the same row not followed by a common superscript letter differ significantly ($P$<0.05; Duncan test); S.E.M.: Standard error of the mean; AU: area units resulting of counting the total ion chromatogram (TIC) for each compound; LRI: linear retention index calculated for DB-624 capillary column (J&W scientific: 30 m×0.25 mm id, 1.4 µm film thickness) installed on a gas chromatograph equipped with a mass selective detector; R: Reliability of identification; LRI: volatiles identified by comparing their LRI with those reported in the literature (Lorenzo and Fonseca, 2014; Lorenzo and Domínguez, 2014; Domínguez *et al*., 2014); ms: mass spectrum agreed with mass database (NIST05); s: mass spectrum and retention time identical to an authentic standard.

were alcohols (Wu *et al*., 2015), esters (Bermúdez *et al*., 2015; Gómez and Lorenzo, 2013; Lorenzo, 2014; Lorenzo and Fonseca, 2014) or aldehydes (Armenteros *et al*., 2012b; Purriños *et al*., 2011b; Purriños *et al*., 2012). These different results could be explained by the volatile extraction methods used, since the purge and trap extraction and the SPME technique offer potentially different profiles of volatile compounds (Bermúdez *et al*., 2015). In addition, there are many factors that affect SPME fiber performance, such as the choice of stationary phase and the extraction conditions.

The changes in the most relevant volatile compounds during "lacón" processing are shown in Figure 1. The production and release of the volatile compounds increased throughout the dry-cured "lacón" processing from 82 AU×$10^6$·$g^{-1}$ dry matter in fresh meat to 1164, 817, 873 and 891 AU×$10^6$·$g^{-1}$ DM at the end of dry-ripening of "lacóns" from formulations I, II, III and IV, respectively. Our results are in agreement with other studies with different meat products, such as "cecina" (Lorenzo, 2014), "lacón"(Lorenzo and Fonseca, 2014; Purriños *et al*., 2012), dry-cured ham (Armenteros *et al*., 2012b; Bermúdez *et al*., 2015), dry-cured loin (Lorenzo and Carballo, 2015) and bacon (Wu *et al*., 2015). The production of volatile compounds was particularly high during the dry-ripening stage. This fact could be due to the increase in temperature and reduction in moisture in the course of the process (Lorenzo and Fonseca, 2014).

As mentioned above, hydrocarbons were the most abundant chemical group at the end of the ripening process in dry-cured "lacón". They represent 55, 49 and 51% of the total volatile compounds in "lacóns" from formulations I, III and IV, respectively, and 85% of the total volatile compounds in "lacóns" from formulation II. In this study, heptane, 2,2,4,6,6-pentamethyl, was the most abundant hydrocarbon in all the batches (representing about 90% of total hydrocarbons), followed by decane, 2,6,7-trimethyl and octane. These results agree with Bermúdez *et al*. (2015) who reported that the heptane, 2,2,4,6,6-pentamethyl was the most important aliphatic hydrocarbons in dry-cured hams. Aliphatic hydrocarbons with less than ten carbons atom arise mainly from lipid oxidation (Ruíz *et al*., 2002), while those with longer chains could be accumulated in the fat depots of the animal, probably from feeding.

An increase in the total amount of hydrocarbons was observed during the process, from initial values of 72 AU×106·$g^{-1}$ DM in fresh meat to 650, 698, 432 and 459 AU×$10^6$ ·$g^{-1}$ DM at the end of the dry-ripening of samples from formulations I, II, III and IV, respectively (Figure. 1). In the salting and post-salting stages, the contents of hydrocarbons decreased and subsequently increased. This tendency was previously described in "lacón" (Purriños *et al.*, 2012). On the contrary, Armenteros *et al*.(2012b) reported that hydrocarbons increased during the post-salting stage and decreased in the final stage of the process. In this study, the production of hydrocarbons was not affected during the salting and post-salting stages by the partial replacement of NaCl content by other chloride salts, but their amounts increased at different rates up to the end of the dry-ripening stage. Furthermore, the total content of hydrocarbons was significantly higher ($P$<0.05) in cured "lacóns" from formulations I and II than those from III and IV. This fact was mainly due to the significantly higher values ($P$<0.05) of heptane, 2,2,4,6,6-pentamethyl, but also due to higher values ($P$<0.001) of pentane, 2,3,3-trimethyl, heptane, 3-methylene, heptane, 2,2,4-trimethyl and heptane, 2,2,5-trimethyl in formulations I and II than in the other ones. In this case, NaCl could have contributed to an increased release of these compounds. On the contrary, Armenteros *et al*. (2012b)

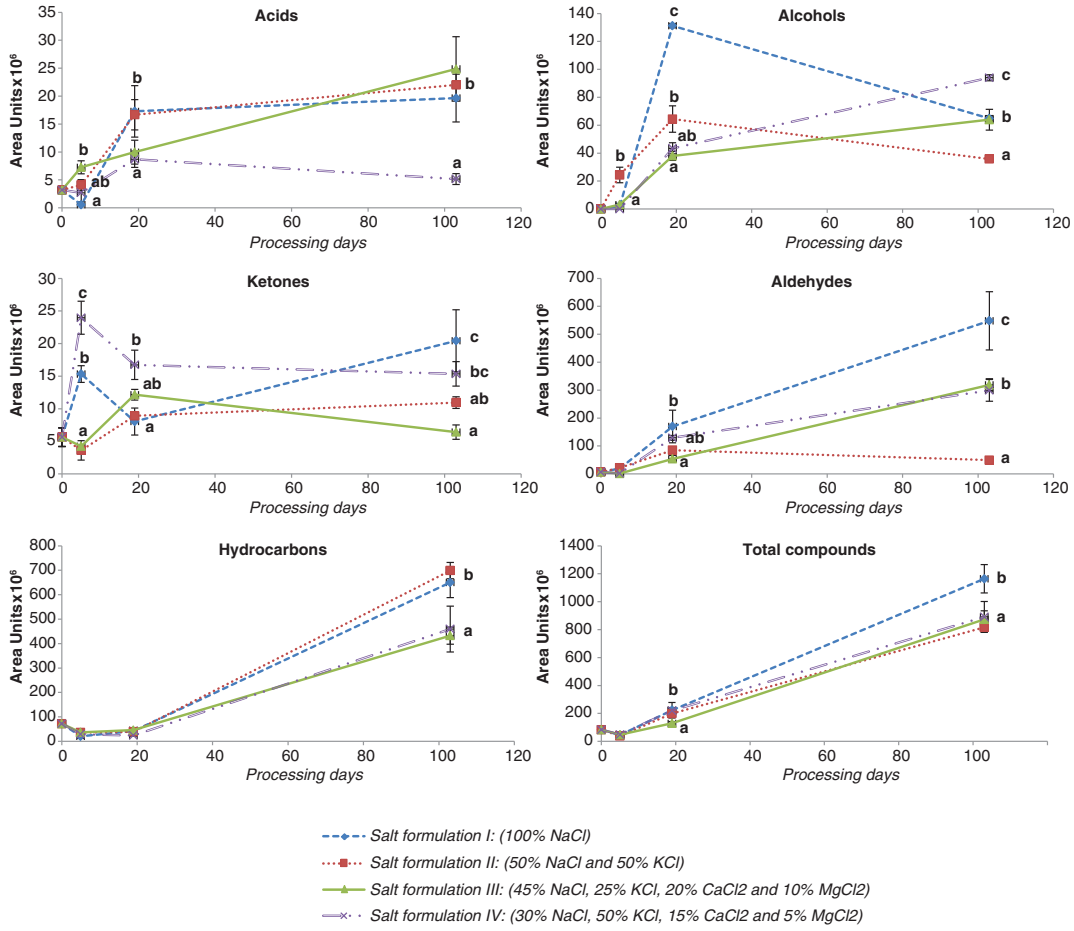


FIGURE 1. Evolution of volatile compounds throughtout dry-cured "lacón" processing using different salting formulations. Error bars indicate the standard error for each treatment. Different letters indicate significant differences among formulations ($P$<0.05). Plotted values are the means of four replicates.

found higher quantities of branched hydrocarbons in hams with the replacement of NaCl than in the control batch (salted with 100% NaCl). Despite the high content of these compounds, they probably had a low impact on flavor due to their relatively high odor thresholds (Domínguez and Lorenzo, 2014).

In the present study, aldehydes represented about 35–50% of the total volatile compounds in the "lacóns" from batches I, III and IV and 5.9% of the total volatile compounds in "lacóns" from batch III. Regarding the aldehydes, hexanal, derived from the *n*-6 fatty acids such as linoleic and arachidonic acids was the most abundant (Węsierska *et al.*, 2013). It represented between 80% and 95% of total aldehydes. These results agree with those reported by Lorenzo *et al.* (2014) and Lorenzo and Fonseca (2014) who observed that hexanal was the most abundant aldehyde in dry cured "lacón" and it was considered a good indicator of the lipid oxidation in this meat product. Moreover, hexanal was also the most important aldehyde in dry-cured bacon (Wu *et al.*, 2015), "cecina" (Lorenzo, 2014), ham (Armenteros *et al.*, 2012b; Bermúdez *et al.*, 2015) and dry-ripened "chorizo" (Gómez and Lorenzo, 2013). The high levels of straight chain aldehydes, such as pentanal, hexanal, heptanal and decanal, indicated that lipid oxidation occurred actively during the dry-curing process and played a very important role in the formation of headspace volatiles by cured meat products (Wu *et al.*, 2015).

The amount of aldehydes increased from initial values of 6.45 AU×$10^6$·$g^{-1}$ DM in the raw pieces to 626, 318 and 299 AU×$10^6$·$g^{-1}$ DM at the end of dry-ripening of "lacóns" submitted to formulations I, III and IV, respectively. These results were previously reported by Armenteros *et al.* (2012b) and Lorenzo and Carballo (2015) who noticed that total aldehydes increased during the process of dry-cured meat products. However, in "lacóns" from formulation II, the values of aldehydes increased until the end of the

post-salting stage (85.15 AU×$10^6$·$g^{-1}$ DM) and then decreased in the final stage (49.38 AU×$10^6$·$g^{-1}$ DM). These findings are in agreement with those reported by Lorenzo *et al*. (2014), Lorenzo (2014) and Lorenzo and Fonseca (2014) who noticed a decreased during the final stages. The decrease in aldehydes in "lacóns" submitted to formulation II at the end of the process could be the reflection of their participation in other chemical reactions yielding other volatile or non-volatile compounds (Armenteros *et al*., 2012b).

On the other hand, hexanal showed differences ($P$<0.001) among the batches. The "lacóns" from formulation I had the highest values (608.55 AU×$10^6$·$g^{-1}$ DM) and the lowest were presented by the "lacóns" submitted to formulation II (40.97 AU×$10^6$·$g^{-1}$ DM). The other 2 batches presented intermediated values (297.10 and 274.90 AU×$10^6$·$g^{-1}$ DM for the "lacóns" from formulations III and IV, respectively). Pentanal was found in the samples submitted to formulations I, III and IV (values between 12.05 and 15.64 AU×$10^6$·$g^{-1}$ DM), while the highest values of heptanal were observed in the "lacóns" submitted to formulation IV (16.20 AU×$10^6$·$g^{-1}$ DM) in comparison with the other batches (between 6.46 and 12.31 AU×$10^6$·$g^{-1}$ DM). The formulations I and II also presented low values of decanal.

Armenteros *et al*. (2012b) and Purriños *et al*. (2012) also found that dry-cured products with high contents of NaCl showed the highest amounts of hexanal. According to these authors, the higher values of hexanal in "lacóns" submitted to formulation I might be related with the fact that NaCl could have exerted a pro-oxidant effect. This result was also reported by Du and Ahn (2005) who noticed that NaCl (less than 2%) was a pro-oxidant in meat and meat products. Another possible explanation could be that the replacement of NaCl by other salts affects the degree of proteolysis in ripened meats as NaCl inhibits muscle proteases (Garrido *et al*., 2012), and a significant reduction in such ions in the meat systems generally leads to a more intense proteolysis (Estévez, 2011). A large production of free amino acids and peptides in "lacóns" from formulations II, III and IV could have contributed to inhibiting lipid oxidation and, hence, the formation of lipid derived volatiles such aldehydes. On the contrary, Andrés *et al.* (2007) did not observe variations in the hexanal contents among dry-cured hams with different salt contents. Aldehydes are known as the major contributors to the unique flavor of dry-cured products due to their low odor thresholds (Bermúdez *et al*., 2015).

Alcohols were the third chemical family after the ripening period, they represented between 4 and 10% of the total volatile compounds, and four different alcohols were identified: 1-pentanol, 1-hexanol, 1-octen-3-ol and benzyl alcohol. These compounds have also been detected in other dry-cured meat products (Bermúdez *et al*., 2015; Lorenzo *et al*., 2014; Purriños *et al*., 2012). Among the alcohols, in all the batches, 1-octen-3-ol was the most abundant and represented about 40% of the total alcohols. Our results agree with those reported by Bermúdez *et al*. (2015), Lorenzo *et al.* 2014 and Lorenzo and Fonseca (2014), who found that the most abundant alcohol at the end of the dry-ripened stage was 1-octen-3-ol.

The formation and release of alcohols was affected from the beginning by the processing time, as well as by the formulation. In fresh meat alcohols were not detected, and they increased during cold stages. After the post-salting stage the values of alcohols were 131.2, 64.4, 37.9 and 43.4 AU×$10^6$·$g^{-1}$ DM in "lacóns" from formulation I, II, III and IV, respectively. Then, total alcohols of the samples from formulations I and II gradually declined to the end of the process, while in samples submitted to formulations III and IV increased to the end of the process (Figure. 1). The amount of total alcohols was significantly ($P$<0.05) higher in "lacóns" from formulation IV (93.9 AU×$10^6$·$g^{-1}$ DM), followed by "lacóns" submitted to formulations I and III (64.8 and 63.9 AU×$10^6$·$g^{-1}$ DM) and finally the "lacóns" from formulation II (35.8 AU×$10^6$·$g^{-1}$ DM).

The samples submitted to formulation IV presented the higher values of 1-octen-3-ol, 1-pentanol and 1-hexanol than the samples from formulations I and II. The intermediate values were observed in samples from formulation III. Our results are in agreement with Armenteros *et al*. (2012b), who found the highest amounts in the samples with replacement of NaCl. This result probably was due to a more intense chemical reduction of corresponding aldehydes by microbial enzymes in "lacóns" submitted to treatment IV. NaCl is known to have antimicrobial activity, and a partial replacement could explain a more intense microbial growth and a higher production of these compounds. It is in agreement with reported by Lorenzo *et al*. (2015) in a previous study, who found that lacóns from formulations III and IV presented the highest counts of total viable counts, salt tolerant flora and yeasts. In contrast, Purriños *et al*. (2012) found that the "lacóns" with high salt content had higher amount of alcohols than in "lacóns" with reduced salt content.

Only three acids were detected through the manufacture process of dry-cured "lacón". Butanoic acid was the most abundant at the end of the process representing about 80–90% of total acids. These findings are in agreement with the study of Bermúdez *et al*. (2015) in dry-cured hams. However, other authors found that acetic acid was the main acid detected in dry-cured products (Lorenzo *et al*., 2014; Wu *et al*., 2015). During cold stages, the generation of acids was affected by partial replacement of NaCl by other salts. After post-salting stage, the batches I and II presented higher ($P$<0.05) values of acids (17.3 and

16.7 AU×$10^6$·$g^{-1}$ DM, respectively) than batches III and IV (9.9 and 8.7 AU×$10^6$·$g^{-1}$ DM, respectively). Then, the evolution of acids was different depending on the batches; in the samples submitted to formulations I, II and III the acid content increased to the final of dry-ripened process. However, the acid content of the "lacóns" from batch II decreased at the end of the process (Figure. 1) and is in agreement with those reported by Lorenzo and Fonseca (2014) who also found that the acids content decreased after post-salting stage. At the end of the final stage, were not found differences among samples submitted to formulations I, II and III (19.6, 22.0 and 24.9 AU×$10^6$·$g^{-1}$ DM, respectively), while the samples from batch IV showed significantly ($P$<0.05) lower values (5.78 AU×$10^6$·$g^{-1}$ DM) than the other batches. These differences were mainly related to the differences in butanoic acid content (15.6, 20.9, 22.5 and 5.78 AU×$10^6$·$g^{-1}$ DM for samples from formulations I, II, III and IV, respectively).

In this study, two ketones were detected in the headspace of "lacón". The most abundant ketone was 2-butanone, 3-hydroxy representing about 70–75% of total ketones. In contrast, Bermúdez *et al.* (2015) and Lorenzo and Fonseca(2014)reported that 2-heptanone was the most abundant ketone in dry-cured ham and dry-cured "lacón", respectively, while Wu *et al.*(2015) noticed that 2-butanone and 2-butanedione were the predominant ketones in bacon. At the end of the processing, "lacóns" submitted to formulation I had significantly ($P$<0.05) higher levels of ketones than those from the other formulations. "Lacóns" submitted to formulation III presented the highest values of 2-heptanone (5.61 AU×$10^6$·$g^{-1}$ DM), while the values of 2-butanone, 3-hydroxy were significantly ($P$<0.05) higher in the "lacóns" from treatment I than from the other formulations. Our results are in agreement with those reported by Armenteros *et al.* (2012b), who found the highest values of 2-butanone, 3-hydroxy in control dry-cured hams (salted with 100% NaCl) than in dry-cured hams with salt replacement.

Only furan, 2-pentyl, was detected after the dry-ripening process (values between 3.8 and 6.1 AU×$10^6$·$g^{-1}$ DM) and it did not show differences among treatments. Furan, 2-pentyl, has been found in other dry-cured meat products manufactured from whole pieces (Bermúdez *et al.*, 2015; Lorenzo *et al.*, 2014; Lorenzo and Carballo, 2015). This furan is a non-carboxylic compound derived from linoleic acid and other *n*-6 fatty acids, with a relatively low threshold and vegetable aromatic note (Fay and Brevard, 2005).

Finally, esters were only detected in samples from formulations I and II, but their amounts were very low (2.79 and 1.85 AU×$10^6$·$g^{-1}$ DM in the "lacóns" submitted to formulations I and II, respectively), and they represented less than 0.02% of the total volatile compounds. Esters have low olfaction threshold values. However, taking into account that the samples have very low values of these compounds, it can be considered that they do not contribute to the aroma of "lacón".

In addition, in a previous study (Lorenzo *et al.*, 2015), the sensory analysis showed that the panellists gave the highest scores of intensity odor to "lacóns" salted with 100% NaCl (formulation I) and the lowest values were observed in "lacóns" salted with 50% NaCl and 50% KCl (formulation II). As discussed above, the odor of "lacóns" not only depends on the amount of volatile compounds, but also for their threshold. However, the "lacóns" from formulation I, which had the highest amount of total volatile compounds also presented the highest scores of odor intensity according to Lorenzo *et al.* (2015), while the "lacóns" from formulation II had the lowest values of total volatile compounds and odor intensity (Lorenzo *et al.*, 2015). Therefore, in this case it seems that the amount of total volatile compounds was directly related to odor intensity.

## 4. CONCLUSIONS

The formation of the volatile compounds significantly increased during the dry-curing process, particularly during the dry-ripening stage. The replacement of NaCl by other salts influenced the formation of the majority of volatile compounds. At the end of processing, the control samples (salted with 100% of NaCl) showed the highest values of total volatile compounds. This fact is mainly due to higher values of hexanal and total aldehydes in this batch than in the other ones. Therefore, NaCl acts as a pro-oxidizing and solubilizing agent, increasing the relative levels of volatile compounds from lipid oxidation, among others. The results obtained in this study indicate that partial NaCl replacement by other chloride salts has an impact on the formation of volatile compounds in dry-cured "lacón". Finally, the odor intensity was directly related to the amount of total volatile compounds.

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