JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Effect of the Partial Replacement of Sodium Chloride by Other Salts on the Formation of Volatile Compounds during Ripening of Dry-Cured Ham

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ABSTRACT: The effect of the partial NaCl replacement by other salts (potassium, calcium, and magnesium chloride) on the formation of volatile compounds through the processing of dry-cured ham was studied using solid-phase microextraction (SPME). Three salt formulations were considered, namely, I (100% NaCl), II (50% NaCl and 50% KCl), and III (55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$). There was an intense formation of volatile compounds throughout the processing of dry-cured hams, particularly during the "hot-cellar" stage. The differences between treatments were found to be more remarkable at the end of the curing process. Hams from formulations I and II had significantly higher amounts of lipid-derived volatiles such as hexanal than hams from formulation III, whereas the latter had significantly higher amounts of Strecker aldehydes and alcohols. Plausible mechanisms by which salt replacement may affect the generation of volatile compounds include the influence of such replacement on lipid oxidation and proteolysis phenomena. The potential influence of the volatiles profile on the aroma of the products is also addressed in the present paper.

KEYWORDS: *dry-cured ham, sodium replacement, potassium chloride, magnesium chloride, calcium chloride, lipid-derived volatiles*

INTRODUCTION

Dry-cured ham is a meat product traditionally produced in the "Mediterranean region" with high levels of acceptance among Spanish consumers due to its sensory features, in particular, the aroma and flavor attributes.[1−3] Nevertheless, it is regarded as a nonrecommended product for hypertensive persons[4] owing to its high sodium chloride content (>5%). Several physiological studies, including the DASH-sodium study (dietary patterns, sodium intake, and blood pressure study) and the WHO-Cardiac study, support the association between excessive sodium chloride (NaCl) intake and the development of cardiovascular diseases such as hypertension, coronary heart disease, and stroke. The impact of these studies on consumers may explain the current tendency to reduce the daily sodium intake.[5,6] Consequently, the meat industry is demanding strategies to decrease sodium content in dry-cured ham to obtain more healthful products. However, sodium chloride reduction is highly challenging because NaCl plays an important role in this product. In particular, NaCl contributes to microbial stability through the reduction of water activity (a_w), improves protein solubilization, and affects the proteolysis, lipolysis, and lipid oxidation phenomena during product processing, improving the product texture and contributing directly to the flavor.[7]

During the past decade, several approaches have been focused on the reduction of the total sodium content in dry-cured ham, but some of these actions have led to serious technological problems. NaCl modulates the activity of muscle proteases, and the reduction of such salts explains the intense action of muscle endopeptidase and exopeptidase enzymes. This intense proteolysis causes, in turn, defective textures such as excessive softness in the final product and unpleasant flavors due to excessive content of low molecular weight nitrogen compounds (peptides and free amino acids).[8,9] Moreover, the decrease of Na^+ content also seems to have a slight promoting effect on the lipolysis phenomena. [10] 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 ham. Lately, Armenteros et al.[11] have reported substitution of NaCl up to 45% of a mixture of chloride salts (KCl, $CaCl_2$, and $MgCl_2$) without affecting the proteolytic phenomena in dry-cured ham. Nevertheless, the sensory attributes were significantly affected by the use of low-salts mixtures, particularly when the NaCl content was partially replaced by $CaCl_2$ and $MgCl_2$ salts.

On the other hand, the study of dry-cured ham flavor is very interesting for understanding the pathways leading to the formation of odor compounds during the dry-curing process. The dry-cured flavor is the result of enzymatic reactions (proteolysis and lipolysis) and also of chemical processes (lipid oxidation, Maillard and Strecker reactions) that take place throughout ham ripening.[12,13] Proteolysis influences flavor development due to the formation of free amino acids and other low molecular weight compounds such as peptides. Free amino acids are involved in flavor development as they are precursors of many odorants. The main routes for the generation of volatile compounds from amino acids are

Received: April 3, 2012
Revised: July 15, 2012
Accepted: July 17, 2012
Published: July 17, 2012

dx.doi.org/10.1021/jf3013772 | J. Agric. Food Chem. 2012, 60, 7607−7615

Maillard and Strecker reactions.[14] Some amino acid-derived Strecker aldehydes have been recognized as the most odor-active compounds in dry-cured hams, and they contribute to the overall flavor in this product.[15] Nevertheless, the most abundant volatile compounds from dry-cured ham arise from lipid oxidation.[16]

Few studies have reported the effect of NaCl on the generation of volatile compounds in dry-cured ham.[17] It is known, however, that sodium chloride acts as a pro-oxidant agent because salt may activate a component in the lean meat, which results in a change in the oxidation characteristics of the meat product.[18] This effect has been shown to be dependent on the amount of NaCl added to the meat product.[19,20] However, the effect of the partial NaCl replacement by other chloride salts on the volatiles profile of dry-cured ham is unknown.

Consequently, the aim of this work was to determine the influence of the 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 hams for their possible contribution to the flavor of this product.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents used for the present work were purchased from Panreac (Panreac Química, S.A., Barcelona, Spain), Merck (Darmstadt, Germany), and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

Processing of the Hams. The raw material, salting formulations, and processing conditions used in the manufacture of dry-cured hams were equivalent to those used by Armenteros et al.[11] in a previous work. Briefly, 48 fresh hams from Landrace × Large White pigs were selected in a local slaughterhouse within the pH range from 5.5 to 6.0. Three of the hams were used as a control of the raw material. The remaining 45 hams were randomly divided into three groups and submitted to the salting process. Salt formulations were chosen according to the results obtained in previous works,[21,22] as aforementioned. Hams from the first group (n = 13) were salted with the traditional NaCl content (100% NaCl, formulation I). The second group (n = 16) was salted using a mixture of NaCl and KCl at 50% (formulation II), and the third group (n = 16) was salted with 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$ (formulation III). In all cases, 200 ppm of KNO_3 and 100 ppm of $NaNO_2$ were included in the salt formulations and subsequently applied by kneading and rubbing on the surface of the hams, as curing agents.

The salting stage was carried out at 3 ± 1 °C and 90% relative humidity for a total of 12 days. After salting, hams were brushed to remove the remaining salt from their surfaces, transferred to the postsalting chamber, and kept below 4 ± 1 °C and 75−85% relative humidity during 50 days in the case of hams salted with formulation I (100% NaC) and for 80 days for formulations II and III as described Blesa et al.[23]

During the last stage, corresponding to the ripening period (~5 months), hams were placed in air-conditioned chambers and subjected to different temperature and relative humidity cycles to accelerate the drying process. The temperature was increased from 4 to 22 °C at 0.15 °C per day during 150 days, whereas relative humidity was progressively reduced to 65%, followed by a cellar phase at 18 ± 1 °C (see Figure 1). The weight loss of each ham was measured and recorded throughout the process, and the process was finished when the total weight loss of the ham reached 32−34% of the initial weight, which is in the range of typical values achieved in the meat industry.[12] The total length of the curing process was approximately 9 months.

Sampling. Samples (50 g) from biceps femoris muscles were taken and kept at −80 °C until analyzed. The sampling process was carried out on raw material and after 20 and 50 days of postsalting stages for formulations I, II, and III, at 80 days for formulations II and III, and finally at 270 days for the three formulations (I, II, and III). The ham sample numbers are as follows: n = 3 for raw material; n = 13 for formulation I (3 were sampled and analyzed at day 20, 3 at day 50, and the remaining at day 270); n = 16 for formulations II and II (3 were sampled and analyzed at day 20, 3 at day 50, 3 at day 80, and the remaining at day 270).

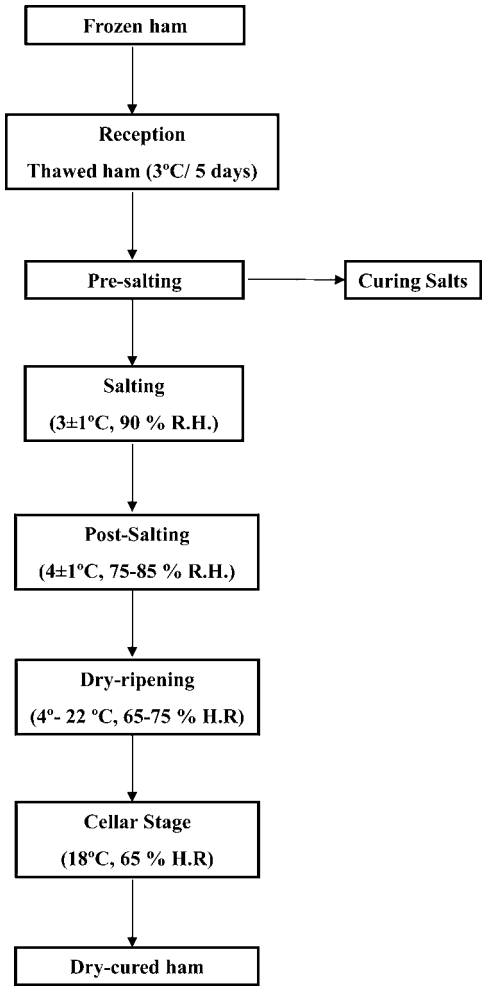

Figure 1. Process flow diagram for dry-cured hams submitted to salting formulations I, II, and III.

Physicochemical Analysis. Moisture content was determined by oven-drying to constant weight at 175 °C using a moisture halogen analyzer (Mettler Toledo AG, Switzerland), whereas fat and total protein were determined using AOAC methods.[24,25] Salt contents were analyzed in the biceps femoris muscle at the end of the ripening period by ion chromatography following the method described by Gómez et al.,[26] with slight modifications.

Analysis of Volatile Compounds. Volatile compounds were extracted from the headspace (HS) of the ham samples by using a solid-phase microextraction (SPME) fiber (Supelco, Bellefonte, PA, USA) coated with divinylbenzene−carboxen−poly(dimethylsiloxane) (DVB/CAR/PDMS) 50/30 μm and subsequently analyzed by gas chromatography (Hewlett-Packard 5890 series II, Palo Alto, CA, USA) coupled to mass spectrometry (Mass Selective Detector Hewlett-Packard HP-5973A) (GC-MS). The extraction procedure was performed as follows: Samples (1 g) of minced ham were placed in 4 mL glass vials and sealed with a silicon cap. During extraction, samples were immersed in a temperature-controlled water bath at 37 °C for 30 min. After extraction, the SPME fiber was immediately transferred to the injector of the chromatograph, which was in splitless mode at 280 °C. Volatiles were separated using a 5% phenyl−95% dimethylpolysiloxane column (Restek, CA, USA) (30 m, 25 mm i.d., 1 mm film thickness). The carrier gas was helium at 18.5 psi of column head pressure, resulting in a flow of 1.6 mL min^{-1} at 40 °C. The SPME fiber was desorbed and maintained in the injection port at 220 °C

during the whole chromatographic run. The temperature program was isothermal for 10 min at 40 °C and then raised at the rate of 7 °C min^{-1} to 250 °C and held for 5 min. The GC-MS transfer line temperature was 270 °C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV and a multiplier voltage of 1.650 V, collecting data at a rate of 1 scan s^{-1} over a range of m/z 40−300. Volatile compounds were either positively identified by comparing their linear retention indexes (LRI) with those from standard compounds (Sigma-Aldrich, Steinheim, Germany) or tentatively identified by comparing their mass spectra with those contained in the Wiley library and by comparison of their LRI with those reported in the scientific literature.[27,28] Results are given in area units (AU).

Statistical Analyses. Data obtained from volatile analyses (per each experimental point and formulation) were used as variables and evaluated by one-way analysis of variance (ANOVA) to study the effect of the partial replacement of NaCl by two different salting formulations on the volatiles profile of hams at different processing stages. Tukey's test was performed when ANOVA revealed significant ($p < 0.05$) differences between formulations. SPSS (v. 12.0) software was used to carry out all statistical tests.

RESULTS AND DISCUSSION

Chemical Composition of Biceps Femoris from Raw and Dry-Cured Hams. The chemical composition of muscles biceps femoris from raw and dry-cured hams submitted to salting formulations I, II, and III is shown in Table 1. No significant differences were found between experimental salting formulations with respect to the values of moisture, fat, and protein. The moisture values were already reported in Armenteros et al.[11] The values for protein and the lipid contents were within the normal range for this type of product.[29]

Table 1. Chemical Composition (Grams per 100 g, Mean ± Standard Deviation) of Biceps Femoris of Raw and Dry-Cured Hams[a]

	moisture	protein	fat
raw meat	72.31a ± 0.77	23.67c ± 1.38	2.96c ± 0.93
I[b]	62.84b ± 0.00	31.05a ± 2.72	4.49b ± 0.36
II	62.18b ± 0.00	40.67a ± 4.25	4.33b ± 0.99
III	64.96b ± 0.00	29.32b ± 1.27	6.49a ± 1.71

[a]Means with different letters in the same row are significantly different ($p < 0.05$). [b]Salting formulations: I, control, 100% NaCl; II, 50% NaCl and 50% KCl; III, 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$.

On the other hand, salt contents were analyzed at the end of the ripening period in the biceps femoris muscles, reaching values ranging from 2.5 to 3.0% of the total weight of ham. The results obtained were similar to that previously observed by Armenteros et al.[11]

Influence of Processing Time and Salting Conditions on the Volatiles Profile. Figures 2−4 show the volatile compound evolution throughout the manufacture of dry-cured hams. The production and release of the volatile compounds increased throughout the dry-cured ham processing, particularly during the "hot" or "cellar stages" (drying−ripening), due to the gradual increase in temperature. Overall, salt formulations did not affect the evolution of the volatile compounds during the "cold stages" (salting and post salting) as only few significant differences were found. On the contrary, at the end of the process, volatiles generation was drastically affected by the salt formulations, as can be seen in Table 2. A total of 65 volatile compounds were detected at the end of the process, with aldehydes (16) such as hexanal, nonanal, and 2- and 3-methylbutanal being the major group of volatile compounds found in the three formulations studied. The other chemical groups identified were linear and branched hydrocarbons (6), acids (9), alcohols (13), ketones (10), and esters (11) (see Table 2).

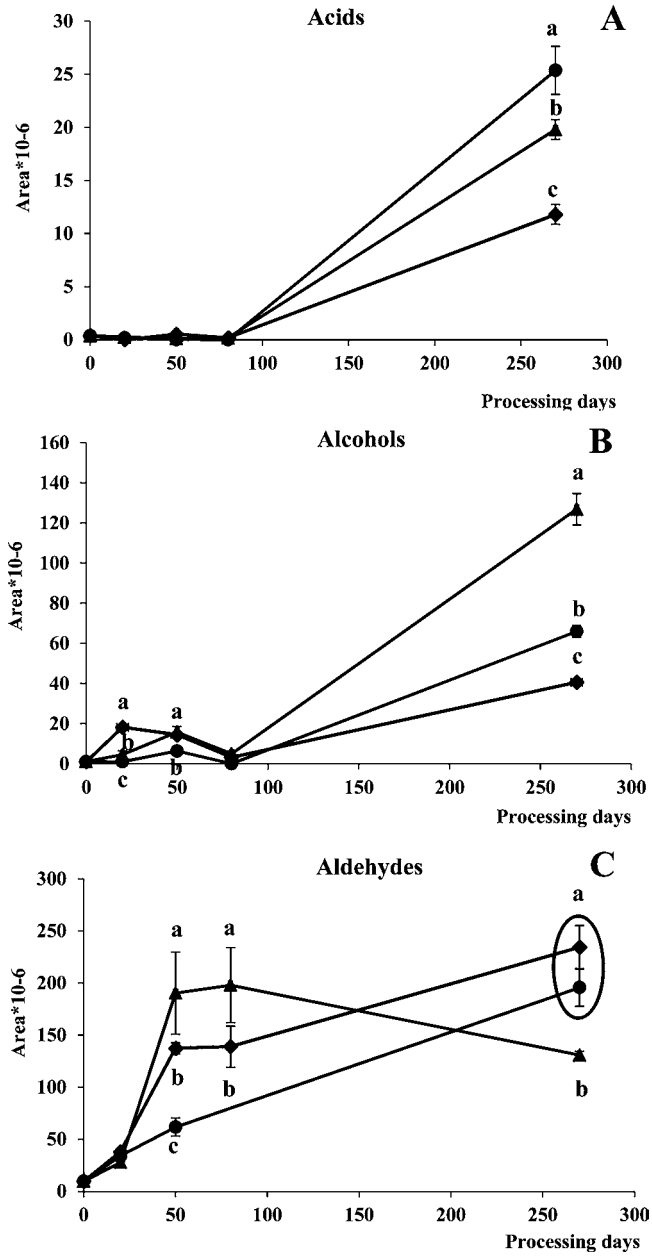

Figure 2. Evolution of the acids (A), alcohols (B), and aldehydes (C) in the muscle biceps femoris throughout the dry-cured ham processing using three different salting formulations: (●) formulation I, 100% NaCl (control); (◆) formulation II, 50% NaCl and 50% KCl; (▲) formulation III, 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$. Labeled error bars indicate the standard error for each treatment. Different letters indicate significant differences among formulations ($p < 0.001\%$).

The formation of these volatile compounds is related to the intense degradation processes that take place in proteins and lipids during the processing of dry-cured ham.[12] The major chemical volatile compounds found in our study are in agreement with those that, using other techniques for the

dx.doi.org/10.1021/jf3013772 | J. Agric. Food Chem. 2012, 60, 7607−7615

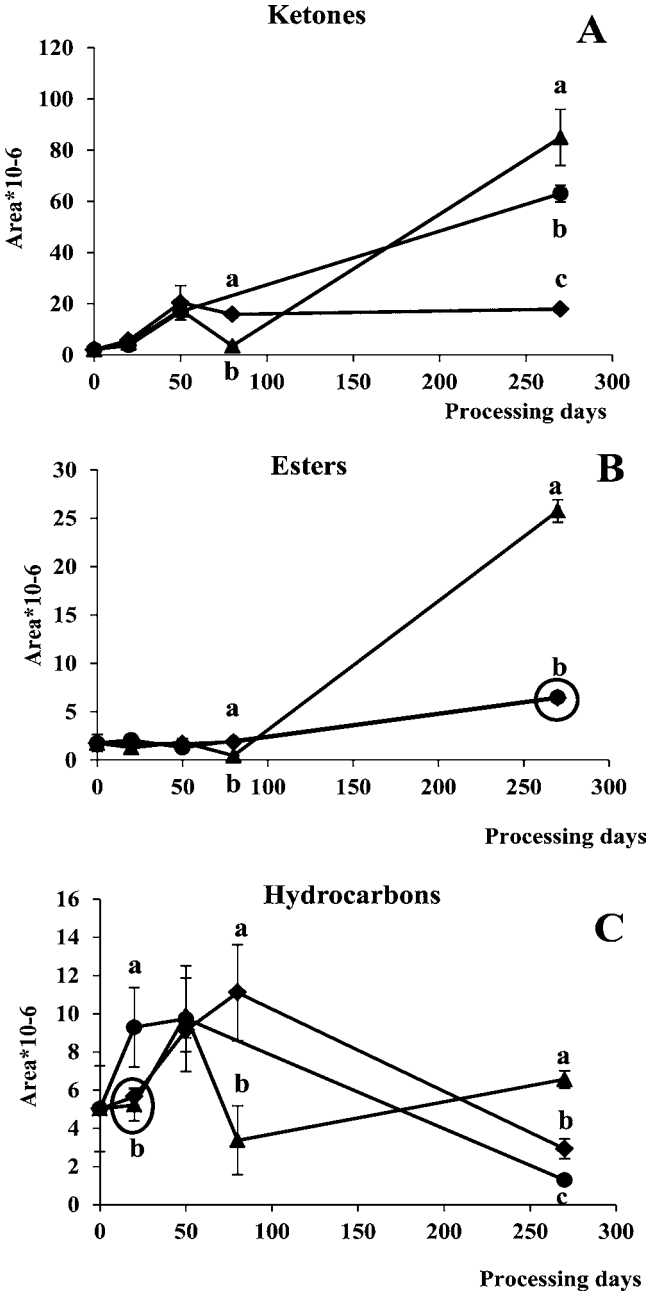

Figure 3. Evolution of hydrocarbons (A), esters (B), and ketones (C) in the muscle biceps femoris throughout the dry-cured ham processing using three different salting formulations: (●) formulation I, 100% NaCl (control); (◆) formulation II, 50% NaCl and 50% KCl; (▲) formulation III, 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$. Labeled error bars indicate the standard error for each treatment. Different letters indicate significant differences among formulations ($p < 0.001\%$).

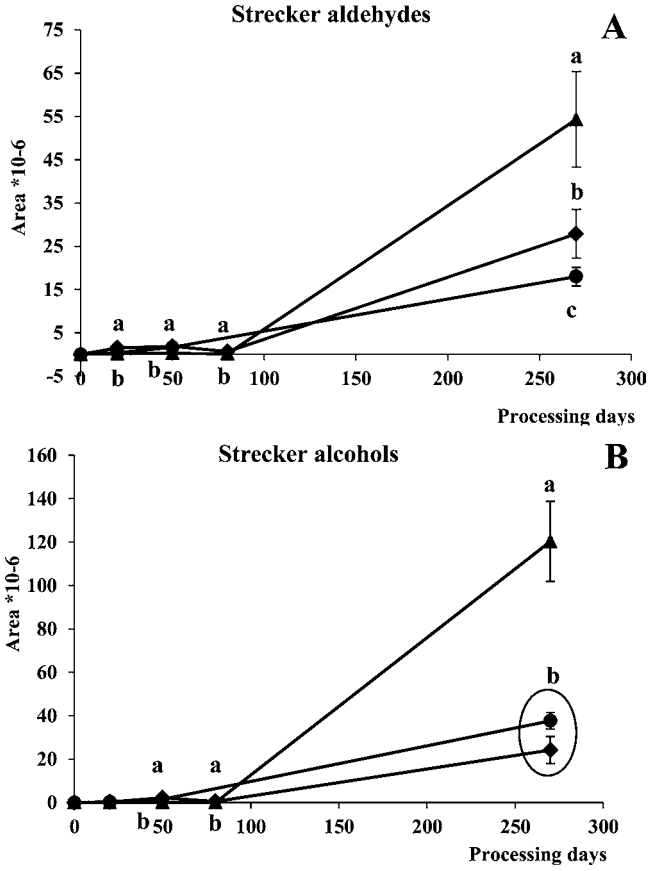

Figure 4. Evolution of Strecker aldehydes (A) and Strecker alcohols (B) in the muscle biceps femoris throughout the dry-cured ham processing using three different salting formulations: (●) formulation I, 100% NaCl (control); (◆) formulation II, 50% NaCl and 50% KCl; (▲) formulation III, 55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$. Labeled error bars indicate the standard error for each treatment. Different letters indicate significant differences among formulations ($p < 0.001\%$).

analysis of volatile compounds, were reported in Iberian dry-cured ham[30] and dry-cured hams from other pig breeds.[31]

Specifically, the generation of carboxylic acids was not affected during the "cold stages" by the partial replacement of NaCl content by other chloride salts, but their amounts increased at a different rate up to the end of the "cellar stage" (see Figure 2A). Thus, the total content of carboxylic acids at the end of the curing process was significantly higher in control hams (100% NaCl) than in hams subjected to formulation II. 2-Methyl- and 3-methylbutanoic acids were the most abundant in control hams. These carboxylic acids may have a positive effect on the impact on aroma due to conversion into fruity esters.[32]

The formation and release of alcohols was affected from the beginning by the processing time, as well as by the salting formulations applied (see Figure 2B). As aforementioned, the differences between formulations increased by the end of the process. The percentage of total alcohols, without considering the branched ones, was considerably higher in hams from formulation III than in hams from formulations I and II (see Table 2). Heptan-1-ol and oct-1-en-3-ol are present in very high quantities in hams from formulation I (100% NaCl). In this case, NaCl may have contributed to an increased release of these two compounds by a salting-out effect[19] as observed by Flores et al.[31] in Serrano dry-cured ham and by Garcia et al.[32] in Iberian dry-cured ham. On the other hand, significantly higher amounts of total branched alcohols were found in dry-cured hams from formulation III than in the others. This fact was probably due to a more intense chemical reduction of the corresponding branched aldehydes by microbial enzymes in hams from this treatment.[33] NaCl is known to have antimicrobial activity, and a partial replacement would explain a more intense microbial growth and a higher production of branched alcohols originating from microbial degradation of the

dx.doi.org/10.1021/jf3013772 | J. Agric. Food Chem. 2012, 60, 7607−7615

Table 2. Volatile Compounds (AU × 10^6) in the Headspace of Dry-Cured Hams Salted with Different Salt Formulations at the End of Processing[a]

	LRI[b]	rel[c]	formulation I	formulation II	formulation III	SEM[d]	*p* value[e]
acids							
acetic acid	667	MS+LRI	3.21	3.20	3.57	0.44	<0.001
2-methylpropanoic acid	720	MS+lri	0.00b	0.00b	0.78a	0.09	<0.001
propanoic acid	691	MS+LRI	0.57a	0.00b	0.00b	0.07	<0.001
butanoic acid	789	MS+LRI	1.48a	0.40c	0.97b	0.13	0.001
pentanoic acid	900	MS+lri	0.00b	1.93a	1.76a	0.09	<0.001
3- methylbutanoic acid	850	MS+lri	10.15a	5.20b	3.51b	0.25	<0.001
2-methylbutanoic acid	845	MS+lri	5.56a	0.62b	1.44b	1.10	<0.001
hexanoic acid	980	MS+lri	1.21a	0.25b	0.09b	0.57	<0.001
heptanoic acid	1093	MS+lri	1.22b	0.85b	7.44a	0.77	<0.001
total			25.36a	11.80c	19.79b	2.27	<0.001
alcohols							
propan-1-ol	541	MS+LRI	2.23a	0.00c	0.21b	0.25	<0.001
2-methyl-3-buten-2-ol	613	MS+lri	0.00b	0.55a	0.00b	0.07	<0.001
1-methylpropan-2-ol	550	MS+LRI	11.96a	5.22b	14.14a	1.16	0.001
pent-1-en-3-ol	688	MS+lri	2.79a	1.86ab	1.11b	0.23	0.003
3-methylbutan-1-ol	742	MS+lri	11.79b	2.25c	36.83a	3.67	<0.001
2-methylbutan-1-ol	748	MS+lri	2.90b	0.41b	14.96a	1.67	<0.001
2-ethylbutan-1-ol	755	MS+lri	0.00b	0.00b	0.26a	0.03	<0.001
pentan-1-ol	767	MS+LRI	2.63a	2.60a	0.45b	0.32	0.001
hexan-1-ol	870	MS+LRI	0.79a	0.00b	0.64a	0.10	<0.001
heptan-1-ol	938	MS+LRI	8.17a	2.62b	0.35c	0.84	<0.001
oct-1-en-3-ol	985	MS+LRI	11.95a	8.93a	3.20b	1.02	<0.001
2-ethylhexan-1-ol	1033	MS+LRI	11.00b	16.30b	54.33a	4.92	<0.001
octan-1-ol	1083	MS+LRI	0.00b	0.00b	0.39a	0.05	<0.001
total Strecker alcohols			37.65b	24.19b	120.26a	10.61	<0.001
total			65.94b	40.65c	126.87a	9.21	<0.001
aldehydes							
2-methylpropanal	555	MS+LRI	0.88b	1.58a	0.76b	0.13	0.008
butanol	657	MS+LRI	0.16b	0.22a	0.00c	0.03	<0.001
3-methylbutanal	660	MS+LRI	10.22b	16.15b	36.00a	2.93	<0.001
2-methylbutanal	666	MS+LRI	5.24b	8.78b	14.20a	1.09	<0.001
pentanal	702	MS+LRI	6.29b	8.69a	1.88c	0.77	<0.001
hexanal	802	MS+LRI	143.53a	168.23a	47.44b	15.00	<0.001
heptanal	905	MS+LRI	6.65a	7.06a	3.11b	0.59	0.003
3-(methylthio)propanal	915	MS+LRI	0.30a	0.00c	0.15b	0.03	<0.001
hept-(*Z*)-2-enal	961	MS+LRI	0.23b	0.40a	0.15b	0.03	<0.001
benzaldehyde	969	MS+LRI	0.37b	0.33b	0.62a	0.04	0.005
octanol	1081	MS+LRI	5.48	5.90	5.39	0.28	0.744
benzeneacetaldehyde	1093	MS+LRI	0.98b	1.21b	2.55a	0.21	<0.001
nonanal	1111	MS+LRI	13.35	14.87	16.32	0.85	0.390
non-(*E*)-2-enal	1164	MS+LRI	0.29	0.35	0.28	0.02	0.495
decanal	1210	MS+LRI	0.37	0.49	0.53	0.04	0.166
dodecanal	1426	MS+LRI	1.69a	0.26b	1.46a	0.17	<0.001
total Strecker aldehydes			17.99b	27.86b	54.28a	4.05	<0.001
total			195.69b	234.32a	130.83b	13.54	0.001
ketones							
butanodi-2,3-enone	600	MS+lri	0.99a	0.44b	0.29b	0.08	<0.001
butan-2-one	623	MS+LRI	1.76a	0.19b	0.37b	0.19	<0.001
pentan-2-one	695	MS+LRI	5.12a	1.18c	3.26b	0.44	<0.001
3-hydroxybutan-2-one	720	MS+LRI	14.87a	8.72b	3.03c	1.28	<0.001
hexan-2-one	792	MS+LRI	38.25b	2.65c	64.63a	6.95	<0.001
heptan-2-one	894	MS+LRI	0.00b	0.00b	10.96a	1.28	<0.001
dihydro-2(3*H*)-furanone	980	MS+lri	0.31b	0.36b	0.76a	0.05	<0.001
octan-3-one	990	MS+LRI	0.43b	4.42a	0.55b	0.50	<0.001
octan-2-one	994	MS+LRI	0.39a	0.00b	0.50a	0.06	<0.001
nonan-2-one	1097	MS+LRI	0.86a	0.00c	0.57b	0.09	<0.001
total			62.99a	17.90b	84.93a	7.66	<0.001

dx.doi.org/10.1021/jf3013772 | *J. Agric. Food Chem.* 2012, 60, 7607−7615

Table 2. continued

	LRI[b]	rel[c]	formulation I	formulation II	formulation III	SEM[d]	p value[e]
esters							
acetic acid methyl ester	518	MS+lri	0.00b	0.00b	0.10a	0.01	<0.001
acetic acid ethyl ester	616	MS+lri	2.50b	2.69b	8.02a	0.69	<0.001
2-methylpropanoic acid ester	619	MS+lri	1.99a	0.21a	0.00c	0.23	<0.001
2-methylethylpropanoic acid ester	730	MS+lri	0.55a	0.00b	0.67a	0.08	<0.001
2-methylethylbutanoic acid ester	775	MS+lri	0.73b	0.16c	1.29a	0.12	<0.001
3-methylethylbutanoic acid ester	778	MS+lri	1.66a	0.60b	2.32a	0.21	<0.001
hexanoic acid ethyl ester	1000	MS+lri	0.80b	1.54b	4.45a	0.43	<0.001
heptanoic acid methyl ester	1010	MS+lri	0.23b	0.00c	0.45a	0.05	<0.001
heptanoic acid ethyl ester	1095	MS+lri	0.57b	1.16b	9.13a	1.00	<0.001
2-ethylhexyl acetic acid ester	1120	MS+lri	0.00b	0.25a	0.00b	0.03	<0.001
octanoic acid ethyl ester	1176	MS+lri	0.05b	0.28a	0.23a	0.03	<0.001
total			6.49b	6.41b	25.76a	2.24	<0.001
hydrocarbons							
pentane	520	MS+LRI	0.50b	1.58a	0.38b	0.13	<0.001
2-methylpentane	571	MS+lri	0.22c	0.62b	0.94a	0.09	<0.001
3-methylpentane	580	MS+lri	0.00c	0.62b	3.37a	0.40	<0.001
methylbenzene	715	MS+lri	0.00b	0.00b	1.12a	0.14	<0.001
oct-2-ene	796	MS+lri	0.31ab	0.42a	0.25b	0.03	0.010
dodecane	820	MS+LRI	0.33	0.44	0.52	0.04	0.256
total			1.30c	2.93b	6.57a	0.58	<0.001

[a]In the same row, means with different letters significantly differed in ANOVA test. [b]Linear retention index. [c]Reliability of identification. LRI, volatiles identified by comparing their LRI with standard compounds; lri, volatiles tentatively identified by comparing their LRI with those reported in the literature.[27,28] [d]Standard error of the mean. [e]Statistical significance in ANOVA test.

respective branched aldehydes. This hypothesis requires, however, further confirmation.

The production of aldehydes increased significantly during the "cold stages" of the process in the three formulations studied, but especially in formulation III. At the end of the dry-ripening stage the trend changed and aldehyde concentrations were higher in hams from formulations I and II (see Figure 2C). The decrease of aldehydes in hams from formulation III at the end of the process could be the reflection of their participation in other chemical reactions yielding other volatile or nonvolatile compounds.[34] In particular, the implication of the reactive carbonyls in the Strecker degradation of free amino acids would explain the significant loss of aldehydes in hams from formulation III and the significantly higher amount of Strecker aldehydes in these samples by the end of the process. The Strecker degradation would be promoted in these samples as a result of a more intense proteolysis and, hence, a larger release of amino acids in hams from formulation III as explained below. Variations in the lipid oxidation-derived compounds during processing of the present products are in agreement with previous works carried out in Iberian dry-cured hams.[34,35]

Hexanal was the most abundant straight-chain aliphatic aldehyde detected in dry-cured hams from the three formulations. This compound is considered to be the main volatile derived from oxidation of n-6 fatty acids and also may impart unpleasant, rancid, and pungent flavor notes to dry-cured meat products.[36] In our study the level of hexanal was significantly higher in the dry-cured hams from formulations I and II than in those from formulation III. The presence of NaCl could have exerted a pro-oxidant effect in the dry-cured hams submitted to formulations I and II, promoting lipid oxidation reactions confirmed by the high concentration of hexanal found in these samples. Hexanal has been related to the development of rancid flavors, although the presence of a great variety of other volatile or even nonvolatile compounds could hide these rancid notes. With regard to this, Armenteros et al.[11] observed in a recent study in which similar hams were sensory evaluated, that hams from formulation III attained significantly lower scores in flavor than the other two formulations (I and II) despite the lower hexanal content in the former. Divalent cations could produce metallic, astringent, and irritative sensations, and this is probably why the acceptability of hams submitted to formulation III was lower.[37] The replacement of NaCl by other salts is known to affect the degree of proteolysis in ripened meats as NaCl inhibits muscle proteases, and a significant reduction of such ions in the meat systems generally leads to a more intense proteolysis.[22,38] A larger production of free amino acids and peptides in hams from formulation III could have contributed to inhibit lipid peroxidation and, hence, the formation of lipid-derived volatiles such as hexanal. In addition, the lower levels of carbonyl compounds, particularly hexanal, found in the dry-cured hams submitted to formulation III could be the consequence of the reaction between these compounds with amino acids from proteolysis to form Maillard and Strecker compounds[13] (methyl-branched aldehydes and alcohols). In fact, 2- and 3- methylbutanal were the most abundant Strecker aldehydes in hams submitted to formulation III (see further details about these compounds below).

On the other hand, the amount of ketones increased in hams up to 50 days of the postsalting stage. Beyond this point the evolution depended on the formulation applied as in hams from formulation II the amount remained stable, whereas an intense increase up to the end of the ripening process was observed in hams from formulations I and III (see Figure 3A). At the end of the processing, control hams (formulation I) and those salted with formulation III showed significantly higher levels of ketones than those from formulation II. The most abundant

ketones were hexan-2-one and 3-hydroxybutan-2-one. The amounts of hexan-2-one and heptan-2-one were significantly higher in dry-cured hams salted with formulation III, whereas octan-3-one was more abundant in hams salted with formulation II. The amount of 3-hydroxybutan-2-one was significantly higher in the control hams. Previously published studies[32,39] reported a slight influence of salt on ketone formation in meat products. These compounds are considered to be contributors to the overall aroma of hams[16] due to their low olfaction threshold.

Esters were not generated during the "cold stages" in any of the three formulations. However, by the end of the process a significant ester production was observed in hams from formulation III (see Figure 3B). No differences were observed for the total amount of ester compounds between control hams and those salted with formulation II. The low NaCl concentration used as brine in formulation III may increase ester production by activating the esterases and/or by decreasing the formation of ethanol.[32] Higher contents of esters in dry-cured hams have been related to higher amounts of alcohols, because esters are formed by esterification of carboxylic acids and alcohols.

The aliphatic hydrocarbons content increased up to the end of post-salting ("cold stages") in hams from formulations I and II and then decreased. Formulation III had a different trend as shown in Figure 3C. At the end of the process, five aliphatic hydrocarbons were identified in the dry-cured hams from the three formulations studied, whereas one aromatic hydrocarbon, methylbenzene, was identified in only formulation III. Linear hydrocarbons, such as pentane and oct-2-ene, were more abundant in the dry-cured hams from formulation II. However, the group of branched hydrocarbons, 2- and 3-methylpentane, were found at higher quantities in dry-cured hams from formulation III. This fact could be attributed to the composition of salting formulation III, which produced a higher release of free fatty acids as reported elsewhere,[40] and also to the presence of molds, which can produce higher amounts of branched hydrocarbons as secondary degradation products from triglycerides.[41]

Finally, Strecker alcohols and aldehydes derived from branched amino acids (valine, isoleucine, and leucine) through Maillard reactions are abundant and common volatile components of dry-cured hams.[30] No significant increase of these compounds and no significant differences among salting formulations were observed during the salting and postsalting periods (see Figure 4), probably due to the low temperatures in these "cold stages". The amounts of Strecker aldehydes and alcohols increased after the postsalting stage, along with the increase of temperature during the subsequent "hot stages". These compounds could be used as indicators of the ripening time (see Figure 4A), and these results are in agreement with those found by Ruiz et al.[30] for lengthily aged Iberian dry-cured ham. The progress of the formation of Strecker aldehydes was expected because these reactions require the presence of free amino acids and dicarbonyl compounds, which also have been formed in high amounts in the last "hot stages" of the curing process[42] due to an increase of the exopeptidase activity (aminopeptidases and dipeptyl peptidases).

In the Strecker reaction, amino acids are decarboxylated and deaminated, forming aldehydes, which can also react further, yielding a wide variety of aromatic compounds. The high amount of Strecker aldehydes in dry-cured products could be related with the high acceptability of these products, because these compounds have very low threshold values and add pleasant aroma notes. 2- and 3-methylbutanal are the main products of the Strecker degradation, which are formed from the degradation of free isoleucine and leucine, respectively. These compounds have been abundantly isolated in dry-cured ham and linked to long ripening process, as aforementioned.

NaCl is known to exert a clear inhibitory effect on the protease activity during the processing of dry-cured meats.[7] Thus, the lower NaCl content in formulation III could have led to a higher muscle protease activity,[22] a more intense amino acid generation, and, subsequently, a higher amount of these branched compounds in hams from this formulation (see Figure 4). Assessing proteolysis by means of total free amino acids, Armenteros et al.[22] reported a decreased proteolysis in dry-cured loins in which NaCl was replaced by other sodium salts. However, this effect was not found by the same authors[11] in dry-cured hams submitted to salting treatments similar to those applied to the present samples. Nevertheless, total free amino acids may not be an accurate measurement of the extent of proteolysis in lengthily ripened products as these compounds are involved in further reactions, such as Maillard reaction and Strecker degradation and, therefore, they are eventually lost to a certain extent. In fact, the greater amount of Strecker aldehydes in hams from formulation III necessarily involves a more intense consumption of free amino acids in these samples. This point could explain the lack of significant effect of salt replacement on proteolysis found by Armenteros et al.[11] in dry-cured hams.

Because of their low thresholds values and pleasant "cured" flavors, Strecker aldehydes are known to contribute positively to the dry-cured ham flavor.[43] Therefore, the marked increase of 2- and 3-methylbutanal content during the ripening stage in hams from formulation III could be reflected in the flavor development, in this case associated with pleasant aroma notes. However, in a previous study, hams salted with formulation III showed lower acceptability with respect to the flavor attribute.[11] Nevertheless, this lower acceptability may not be directly linked to the aroma volatiles but to other sensory attributes such as taste, negatively affected by the presence of the replacement ions.

In conclusion, the partial NaCl replacement by other salts has a great influence on the formation of volatile compounds throughout the processing of dry-cured hams. There was an intense formation and release of volatile compounds during dry-cured ham processing, particularly during the "hot stages" (drying−ripening). The most pronounced differences were detected at the end of the curing process. Thus, salting formulation III (55% NaCl, 25% KCl, 15% $CaCl_2$, and 5% $MgCl_2$) led to a more intense production and release of the most abundant volatile compounds studied, except hexanal and some other minor compounds. Considerable differences were also detected between hams salted with formulations I and II. The results obtained indicate that it is necessary to establish an adequate partial NaCl (%) replacement by other chloride salts to guarantee the quality and acceptability of the final product in terms of flavor and odor.

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Funding

This work was supported by Project AGL2004-05064-C02-01 from the Ministry of Education and Science (Madrid, Spain) and FEDER funds. Co-financing was provided by the Ministry of Economy and Competitiveness through Project AGL2010-15134 and the European Community through the Marie Curie Reintegration (ERG) Fellowship PERG-GA-2009-248959 - Pox-MEAT. M.A. thanks the University of Extremadura for a postdoctoral grant (Human Resources Recruitment Program "D-action"). M.E. thanks the Spanish Ministry of Science and Innovation for the contract through the Ramón y Cajal (RYC-2009-03901) program.

Notes

The authors declare no competing financial interest.

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