STRECKER ALDEHYDES IN DRY-CURED HAMS AS AFFECTED BY PARTIAL REPLACEMENT OF SODIUM BY POTASSIUM, CALCIUM AND MAGNESIUM

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Abstract— **The partial replacement of sodium by other ions has been proposed as a strategy to reduce sodium content in dry-cured meats. The influence of such replacement on the formation of volatile compounds with impact on the aroma of the final product is ignored. The present study was aimed to evaluate the formation of Strecker aldehydes in dry-cured hams salted using different chloride salts. Forty-five fresh hams were randomly divided into three groups: Hams from Formulation I (n=13) were salted by rubbing and kneading with 100 % NaCl, hams from Formulation II (n=16) were salted using a mixture of NaCl and KCl at 50 % while hams from Formulation III (n=16) were salted with 55 % NaCl, 25 % KCl, 15 % $CaCl_2$ and 5 % $MgCl_2$.**

The results showed that Strecker aldehydes increased throughout the ripening process. Among all Strecker aldehydes, 3-methyl butanal and 2-methyl butanal were the most abundant. Dry-cured hams salted with the Formulation III showed the highest proportion of 2- and 3-methyl butanal and the total amount of Strecker aldehydes. Partial replacement of NaCl by other chloride salts is known to lead to a more intense proteolytic rate during the ripening process. A larger amount of free amino acids in these hams, including leucine and isoleucine, would enable the formation of the corresponding Strecker aldehydes and hence, explain the present results. Consequently, the partial substitution of NaCl by other chloride salts could influence quality of dry-cured hams by promoting the formation of specific key-odorants.

Keywords— **sodium replacement, volatile compounds, dry-cured hams.**

I. INTRODUCTION

Dry-cured ham is a meat product traditionally manufactured in the Mediterranean countries with high level of acceptance among consumers due to their sensory features, in particular those concerning to the aroma and flavour. Nevertheless, its high sodium chloride content (> 5 %) makes it a non-recommended product for hypertensive persons (*1*). For these reasons, meat industry is demanding strategies to decrease sodium content in dry-cured ham in order to obtain more healthy products. Several approaches focused on the reduction of sodium content in dry-cured meats and fermented sausages have been made (*2*). However, a reduction in the total amount of salt added led to an excessive proteolysis and a slight promoting effect on the lipolysis phenomena. Additionally, the partial Na^+ replacement by a mixture of chloride salts (KCl, $CaCl_2$ and $MgCl_2$) may generate bitter taste, off-flavours or metallic and astringent sensations in the final product (*3*).

The study of ham flavour is very interesting for understanding the pathways leading to the formation of odour compounds during dry-curing process. The flavour of dry-cured hams is due to compounds derived from enzymatic reactions (proteolysis and lipolysis) and chemical reactions (lipid oxidation, Strecker and Maillard reactions) that take place throughout ham ripening. Proteolysis influences flavour development due to the formation of free amino acids and other low-molecular weight compounds like peptides. Free amino acids take part indirectly in flavour development as they are precursors of many odorants. The main routes for generation of volatile compounds from amino acids are Maillard and Strecker reactions (*4*).

Volatiles arising from the Maillard reaction are branched short-chain aldehydes and their corresponding alcohols. In the Strecker reaction, amino acids are decarboxilated and deaminated, forming aldehydes, which can also react further, providing a wide variety of aromatic compounds. The high proportion of Strecker aldehydes in dry-cured products could be related with the high acceptability of these products, since these compounds has very low threshold values and add pleasant aroma notes. 2- and 3- Methylbutanal are the main products of the Strecker degradation, which come from amino acids isoleucine and leucine, respectively. These compounds have been abundantly isolated in dry-cured ham and linked to long ripening process. Because of their low threshold values and pleasant "cured" flavours, these compounds contribute positively to the dry-cured ham flavour (*5*).

The flavour perception in dry-cured ham is largely influenced on salt concentration. In this regard, several studies reported the effect of NaCl on the generation of volatile compounds in dry-cured ham. However, the effect of the partial NaCl replacement by other chloride salts on the formation of odor-compounds in dry-cured ham is unknown.

Consequently, the aim of this work was to determine the influence of the partial NaCl replacement by other salts on the formation and release of Strecker aldehydes in dry-cured ham.

II. MATERIALS AND METHODS

A. Processing of the hams

Forty-eight fresh hams with an average weight of 10.7 ± 0.5 kg were randomly selected in a local slaughterhouse among those having a final pH (24 h) within 5.5 and 6.0. All the hams were frozen in an industrial freezer at -40 ºC and stored for 30 days at -20 º C. Frozen hams were thawed in a cold chamber at 3 ºC for 5 days, similarly to the industrial process. Three of the hams were used as a control of the raw material. The remaining 42 hams were randomly divided into three groups (one with 13 hams and two with 16 hams, each) and subjected to the salting process. Thus, 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). This process included a previous manipulation in which the respective salting mixture was mixed with 200 ppm of KNO_3 and 100 ppm of $NaNO_2$ and applied by kneading and rubbing out on the surface of the hams, as curing agents. Afterwards, each piece of ham was placed in individual trays and covered the free-of-skin part with the respective salt formulation. Finally, all the hams were transferred to the salting chamber where remained at 3 ± 1ºC and 90 % of air relative humidity for a total of 12 days. After salting, hams were post-salted at 4.5 ºC and a relative humidity between 75-85%. At the end of the post-salting stage, the hams were taken to the last processing stage (dry-ripening) where temperature was progressively increases from 6 to 20 ºC and relative humedity reduced from 80 to 65 %. The process was finished when total weight loss reached 32-34 % of the initial weight, which is in the range of typical values achieved in the meat industry.

B. Sampling

Samples (50g) from *Biceps femoris* muscles were taken and kept at -80ºC until analysed. In all cases, 3 hams per formulation and sampling days (0, 20, 50, 80 and 270 days) were used.

C. Analysis of volatile compounds

Volatile compounds were extracted by using the solidphase microextraction (SPME) (Supelco Bellefonte, PA) fibre coated divinylbenzene-carboxen-poly(dimethylxilosane) (DVB/CAR/PDMS) 50/30µm and subsequently analysed by gas chromatography coupled to mass spectrometry (GC-MS) (gas chromatograph Hewlett-Packer 5890 series II coupled to mass selective detector Hewlett-Packard HP-5973A). The extraction procedure was performed as follows: samples (1 gram) of minced ham (raw meat, 20, 50, 80 days of post-salting and 270 days of processing) for each formulation was placed in 4-mL glass vials and sealed with a silicon cap. Before extraction, samples were pre-conditioned in a temperature-controlled water bath at 37 ºC for 30 min. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph, which was in splitless mode at 280 ºC. Volatiles were separated according to Estévez et al. (2005) (*6*). Subsequently, 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 (*7*). Results are given in area units (AU).

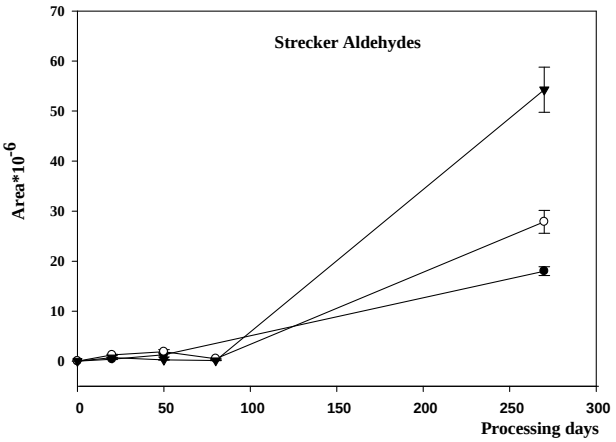
D. Statistic analysis

Data obtained from volatiles analysis (per each experimental point and formulation) were used as variables and evaluated by one-way Analysis of Variance (ANOVA) in order to compare the effect of the partial replacement of NaCl by two different salting formulations on the volatiles profile of the three formulations. Tukey's test was performed when ANOVA revealed significant ($p< 0.05$) differences between formulations. SPSS (v. 12.0) software (1998) was used to carry out all statistic test.

III. RESULTS AND DISCUSSION

Figure 1 show total Strecker aldehydes evolution throughout the ripening of dry-cured hams elaborated with three different salting formulations (I, II and III).

Figure 1. Evolution of Strecker aldehydes throughout the dry-curing process.



Salting formulations: (●) Formulation I, 100 % NaCl (control), (○) Formulation II, 50 % NaCl- 50 % KCl (▼) Formulation III, 55 % NaCl, 25 % KCl, 15 % $CaCl_2$ and 5 % $MgCl_2$.

During the first three sampling days (20, 50 and 80 days) Strecker aldehydes did not increase and no significant differences between salting formulations was observed (see Fig. 1). This fact is most likely due to the low temperatures during the first two "cold phases" (salting and post-salting) that probably led to low volatile compounds formation. The relative amounts of the Strecker aldehydes increased steadily after the post-salting stage, along with the increase of temperature during the subsequent "hot" stages (drying-ripening stage). The increase on the Strecker aldehydes during the "hot" phases has been previously reported in Iberian dry-cured ham and it was successfully associated to a rise in the temperature during the last stages of the process (drying-ripening stage) (*8*). The effect of the ripening time was reflected on the largest levels of Strecker aldehydes found in final products at the end of the process.

The progress of the formation of Strecker aldehydes during the processing of hams was expected since these reactions requires the presence of free amino acids and dicarbonyl compounds and these favorable circumstances take place during the last "hot" phases. Additionally, the increase of free amino acid content during the "hot" stages (drying-ripening) is related to an increase on exopeptidase activities (aminopeptidases and dipeptyl peptidases). Muscle protease activities (cathepsines, aminopeptidases and dipeptidyl peptidases) are higher at the beginning of the dry-cured process than at the end due to salt diffusion and also the processing conditions (water activity, pH and temperature) reduced their activities throughout the process. NaCl is known to have an inhibitory effect on muscle protease activities, particularly cathepsines and other proteases such as alanyl-aminopeptidase (AAP). However, its partial replacement by other salts (KCl, $CaCl_2$ and $MgCl_2$) could due to a less intense inhibitory effect on muscle protease activities, which could explain the largest proteolysis found in meat products with reduced levels of NaCl (*9*).

On the other hand, at the end of the process the relative amount of Strecker aldehydes (270 days) was higher in the dry-cured hams subjected to formulation III than those salted with the formulations I and II (Table 1). 2- and 3- methylbutanal were the most abundant Strecker aldehydes in hams submitted to formulation III (see Table 1). These compounds reached the largest levels at the last stage of the process and therefore they could be used as indicators of the ripening time (see Fig 1). These results are in agreement with the results found by Ruiz et al. (1999) (*8*) who reported that 2- and 3- methylbutanal were the most abundant branched aldehydes in the more aged Iberian dry-cured Ham.

In a previous study, dry-cured loins treated with a brine enriched in $CaCl_2$ and $MgCl_2$ (formulation III) underwent an intense proteolysis phenomena leading to the formation of a large amount of amino acids (*9*). This fact could explain the highest levels of 2- and 3-methybutanal found in the dry-cured hams salted with the formulation III. As aforementioned, the formation of Strecker aldehydes from amino acids requires a previous proteolytic degradation of the proteins so that free amines react with dicarbonyls from Maillard reaction and/or lipid oxidation. According to our hypothesis, a more intense proteolytic activity induced by replacing sodium chloride by other chloride salts ($CaCl_2$ and $MgCl_2$) would enhance the effective reaction between free amino acids and carbonyl compounds.

The marked increase in 2- and 3- methylbutanal content during the ripening stage could be reflected on the flavour development. The results found in our study pointed out that the flavour of dry-cured hams submitted to formulation III could be associated with pleasant arome notes. Nevertheless, Armenteros et al (*submitted*) recently observed that those hams salted with the formulation III showed lower acceptability by the

assessor with respect to the flavour attribute than those submitted to formulation I and II.

Table 1. Strecker aldehyde volatile content at the end of the curing process
Means with different letter superscript in the same line significantly differed in ANOVA test.
[1] Reliability of Identification; LRI: volatiles identified comparing their LRI with standard compounds; lri: volatiles tentatively identified by comparing their LRI with those reported in the literature.
[2] Standard error of the mean
[3] Statistical significance in ANOVA test

Aldehydes	Rel[1]	FI	FII	FIII	SEM[2]	p-value[3]
2-methylpropanal	MS+LRI	0.88^{b}	1.58^{a}	0.76^{b}	0.13	0.008
3-methylbutanal	MS+LRI	10.22^{b}	16.15^{b}	36.00^{a}	2.93	<0.001
2-methylbutanal	MS+LRI	5.24^{b}	8.78^{b}	14.20^{a}	1.09	<0.001
3-(methylthio)-propanal	MS+LRI	0.30^{a}	0.00c	0.15^{b}	0.03	<0.001
benzaldehyde	MS+LRI	0.37^{b}	0.33^{b}	0.62^{a}	0.04	0.005
benzeacetaldehyde	MS+LRI	0.98^{b}	1.21^{b}	2.55^{a}	0.21	<0.001
Total Strecker aldehydes		17.99^{b}	28.05^{b}	54.28^{a}	4.43	<0.001

Little information about the effect of salt reduction on the formation of Strecker aldehydes in dry- cured ham is available in the scientific literature. Thus, a scarce influence of the amount of salt added on branched aldehydes content was found in bacon, whereas in fermented sausages were observed a higher formation of 2- and 3-methylbutanal when used 3.5 % of salt with respect to 1.5 % for its elaboration. Nevertheless, the formation of Strecker aldehydes in fermented sausages should have mainly a microbial origin of 2- and 3- methybutanal could be related to its influence on the microbial metabolism (*10*).

IV. CONCLUSIONS

Consequently, the partial NaCl replacement by other salts has a greater importance on the formation of Strecker aldehydes. There was an intense formation of Strecker aldehydes, particularly 2-and 3- methylbutanl in hams salted with the formulation III at the end of the curing process (~ 9 months). However the differences among dry-cured hams salted with formulation I and II are reduced. The abundance of 2- and 3- methylbutanal could be used as indicators of the ripening time and therefore as flavour development indicators.

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