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Headspace concentration of selected dry-cured aroma compounds in model systems as affected by curing agents

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Abstract

The effect of curing agents (sodium chloride, nitrate, nitrite, glucose, and ascorbic acid) on the headspace concentration of six volatile compounds (2-methyl-butanal, 3-methyl-butanal, 2-pentanone, hexanal, methional, and octanal) has been studied. These volatile compounds were selected based on their presence and contribution to the flavour of typical Spanish dry-cured meat products. The release of volatile compounds to the headspace has been studied by using solid-phase microextraction (SPME) and gas chromatography analysis. The main effect was produced by sodium chloride, because it produced a salting-out effect on all the volatile compounds studied. Furthermore, the presence of nitrite, nitrate, and ascorbic acid in the solution also produced an increase in the headspace concentration of the volatile compounds while glucose decreased the headspace concentration of octanal and hexanal. The changes in the relative proportions of volatile compounds due to the effect of curing agents should be taken into account as they may change the flavour perception of dry-cured meat products.

Keywords: Aroma; Dry-cured products; Curing agents; Headspace; SPME

1. Introduction

The dry-curing process is a traditional process where the curing ingredients are added for meat stabilization at the beginning of the process. In general, the dry-curing process consists of several stages such as salting, post-salting, and ripening that could be as long as several months to 1–2 years (Flores, 1997). During the ripening stage many biochemical reactions, proteolysis, and lipolysis, take place and produce the precursors of flavour compounds (Toldrá & Flores, 1998). In addition, the main ingredients used during the dry-curing process, salt, nitrate and/or nitrite, ascorbic acid, and glucose, affect the product quality due to their own effect (Pearson & Tauber, 1984) and to the control of the muscle enzyme system (Toldrá & Flores, 1998).

The effect of curing agents on the muscle enzymatic system, proteolytic and lipolytic, has been widely studied. Several authors studied the effect of salt on the proteolysis (Flores, Aristoy, & Toldrá, 1997a; Rosell & Toldrá, 1996) and lipolysis (Andrés, Cava, Martin, Ventanas, & Ruiz, 2005; Coutron-Gambotti, Gandemer, Rousset, Maestrini, & Casabianca, 1999; Motilva & Toldrá, 1993; Zanardi, Ghidini, Battaglia, & Chizzolini, 2004) occurred during the dry-curing process. Cathepsins and aminopeptidases, except aminopeptidase B, are inhibited by salt, especially at high concentrations (Flores et al., 1997a; Rico, Toldrá, & Flores, 1991; Toldrá, Rico, & Flores, 1992). Taking advantage of this, Toldrá, Flores, and Sanz (1997) proposed that the addition of an excess of salt may be an easy way to prevent texture defects for those hams showing an excess of initial cathepsin activity. Nitrite is unique and multifunctional ingredient in the meat-curing system. It imparts the characteristic pink color to the cured meat, provides oxidative stability to meat by preventing

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lipid oxidation (Ramarathnam, 1998; Shahidi, Rubin, & Wood, 1987; Yun, Shahidi, Rubin, & Diosady, 1987) and has an antimicrobial effect which is important in preventing the outgrowth of *Clostridium botulinum* and the formation of deadly toxin (Hauschild, Hilsheimer, Jarvis, & Raymond, 1982; Pierson & Smoot, 1982). On the contrary, nitrates and nitrites do not present discernible effects on the proteolytic enzyme activities (Toldrá et al., 1997). Ascorbic acid exerts a slight inhibitory effect on cathepsin H, *m*-calpain, and leucyl aminopeptidasa (Flores et al., 1997a; Rico et al., 1991; Rosell & Toldrá, 1996) and produce a slight inhibition on lipases and esterases (Motilva & Toldrá, 1993). Finally, glucose do not produce any significant effect on the muscle enzyme system (Toldrá & Flores, 1998).

On the other hand, the aroma perception in meat products depends on the concentration and odour threshold of volatile compounds and on their interactions with other food components that will affect its gas phase concentration (Guichard, 2002). In recent years, a considerable amount of research has been focused on the study of volatile compounds in the headspace of dry-cured meat products (Flores, Grimm, Toldrá, & Spanier, 1997b; Gianelli, Flores, & Toldrá, 2002; Ruiz, Cava, Ventanas, & Jensen, 1998) including studies of the odour activity of the volatile compounds being hydrogen sulfide, methanethiol, 3-methyl-butanal, hexanal, and 2-methyl-3-furanthiol several of the compounds with the highest odor activity (Carrapiso, Ventanas, & Garcia, 2002a; Carrapiso, Jurado, & Timon, 2002b). In addition, several studies reported the effect of curing agents mainly salt, nitrate, and nitrite, on the generation of volatile compounds in dry-cured meat products such as Iberian dry-cured ham (Andrés, Cava, Ventanas, Thovar, & Ruiz, 2004) and dry-cured sausages (Olesen, Meyer, & Stahnke, 2004). In cured meat products, the presence of curing agents (salt, nitrate, nitrite, glucose, and ascorbic acid) that are added at the beginning of the dry-curing process (Flores, 1997) can affect the proportion of the volatile compounds in the headspace. However, the effect of these curing agents on the headspace concentration of the volatile compounds responsible of dry-cured aroma is unknown. So that, the objective of this work was to determine the influence of curing agents on the liberation of the flavour compounds to the headspace for a better comprehension of their possible role in flavour release and perception in dry-cured meat products.

2. Materials and methods

2.1. Samples

The aroma compounds 2-methyl-butanal, 3-methyl-butanal, hexanal, octanal, 3-(methylthio)propanal (methional), and 2-pentanone were obtained from Fluka Chemika (Buchs, Switzerland). The selection of six flavour compounds was based on their presence and contribution to the flavour of typical Spanish dry-cured meat products. Hexanal, 2-methyl-butanal, 3-methyl-butanal, and 2-pentanone were selected because of their high proportion in the headspace of Serrano dry-cured ham (Flores et al., 1997b; Gianelli et al., 2002) whereas octanal and methional were selected due to their odor activity in the aroma of Iberian dry-cured ham (Carrapiso et al., 2002a). Because it is not possible to study the hundred of compounds present in the headspace of a ham (Flores et al., 1997b), we selected them considering the previous remarks and also, they are representative from different chemical groups (aldehydes, ketones, sulphide compounds) and chemical structures (linear or branched).

A stock solution containing 50,000 mg/kg of each aroma compound was prepared in ethanol. The aroma compounds were added in triplicate to a solution of 50 mM phosphate buffer, pH 6.0 resulting in a final concentration of 2 mg/kg for 2-methyl-butanal and 3-methyl-butanal, 1 mg/kg for hexanal, octanal, and 2-pentanone and 5 mg/kg for methional. The concentration of the volatile compounds was selected based on the partition coefficient fiber/air and the area obtained on the analysis of the volatile compounds present on the headspace of dry-cured ham under the same conditions (using the fiber CAR/PDMS 75 μm, 30 min for adsorption and 30 °C) (Gianelli et al., 2002).

All of the volatile compounds were simultaneously present in the solution used for the experiments.

2.2. Effect of curing agents

The curing agents, were solubilized in a solution of 50 mM phosphate buffer, pH 6.0 with a final concentrations of sodium chloride of 0, 20, 40, 60, and 80 g/l; glucose 0, 0.5, and 1 g/l; potassium nitrate, 0, 100, 200, and 400 mg/l; sodium nitrite, 0, 50, and 100 mg/l; and ascorbic acid 0, 250, and 500 mg/l. The flavour compounds were added at the appropriate concentration indicated above into the solution containing the respective concentration of curing agent individually studied. The solutions were stored during 15 h at 30 °C in absence of light to allow equilibration.

2.3. Analysis of volatile compounds

Five millilitres of solution containing the mixture of volatile compounds and curing agent was placed in a 10 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco). The quantity of aroma compound present on the headspace of protein and control vials was determined using solid-phase micro extraction (SPME) and gas chromatography analysis using optimized conditions (Gianelli, Flores, & Toldrá, 2005). The 75 μm carboxen/poly(dimethylsiloxane) (CAR/PDMS) fiber (Supelco, Bellafonte, PA) was then exposed to the headspace of the solution without stirring for sampling the aroma compounds at 30 °C. After 30 min of adsorption, the aroma compounds were desorbed by inserting the fiber into the GC injection

port of a gas chromatograph set at 220 °C for 5 min in splitless mode. The split valve was opened 1 min after injection. The fiber was heated on 220 °C for an additional 10 min to avoid an analyte carry-over between samples. The linearity of detection for each aroma compound under these conditions was confirmed within the range of 1–10 mg/kg.

2.4. GC analysis

An 8000 CE Instrument gas chromatograph (Rodano, Milan, Italy) equipped with a flame ionization detector (FID) was used. The aroma compounds were separated in a DB-624 capillary column (J&W Scientific, 60 m, 0.32 mm i.d., film thickness 1.8 μm). Helium was used as carrier gas with a linear velocity of 20.4 cm/s. The fiber was placed in the injector, and the GC oven temperature was started at 38 °C and held for 6 min; then the temperature was increased to 105 °C at a rate of 6 °C/min, then raised to 220 °C at the rate of 15 °C/min, and held for 5 min. The detector temperature was set at 240 °C.

The results are expressed as a percentage of the free volatile compound (ratio of peak areas) found without any curing agent in the solution. All experiments were carried out in triplicate.

2.5. Statistical analysis

The effect of curing agents individually was studied by analysis of variance (ANOVA) using Statgraphics plus v 2.0. The means were compared using Fisher's least significance difference (LSD) procedure ($p < 0.05$).

3. Results and discussion

The characterization of dry-cured ham includes the study of volatile compounds responsible of its unique aroma that includes a large number of products such as, aldehydes, sulfur-containing compounds, ketones, esters, and alcohols (Carrapiso et al., 2002a; Flores et al., 1997b). The extraction of the six volatile compounds analysed in the present study was done through SPME technique previously optimized in order to be able to quantify the changes in volatile concentrations. So that, the linearity of detection of each compound under the conditions selected, fiber and time of exposure, was checked. The results are shown in Table 1 where all the compounds show high regression coefficients indicating a linear response and an absence of competition phenomenon among them.

Table 1
Linearity of detection of each aroma compounds using the 75 μm CAR/PDMS fiber exposed for 30 min to the headspace

	Slope	Regression coefficient
3-Methyl-butanal	190.52	0.980
2-Methyl-butanal	26.27	0.998
2-Pentanone	435.20	0.992
Hexanal	274.90	0.963
Octanal	49.75	0.976
Methional	0.34	0.830

The range of concentrations of curing agents used in meat products is variable and depends on the country and the type of meat product (Flores, 1997). The volatile compound headspace concentration depended on the volatile compound and curing agent assayed. The main effect was produced by sodium chloride, because it produced a significant salting out effect on all the volatile compounds assayed being the highest effect on octanal (Fig. 1). Also, 80 g/l sodium chloride increased more than twice the headspace concentration of all the aldehydes except methional and the ketone, 2-pentanone. This effect was expected because salts are often added to aqueous samples to increase the concentrations of the aroma compounds in the vapor phase (Guichard, 2002). In these sense, Poll and Flink (1984), studied the effect of sodium chloride addition on the volatile headspace concentration of apple juice. These authors demonstrated that the degree of headspace enrichment resulting from salt addition depends on the volatile compound studied. They found that the headspace concentration increase more than 4 times for alcohols, between 1.75 and 3.50 times for aldehydes and less than 1.75 times for esters.

Furthermore, the effect of sodium nitrite and potassium nitrate on the headspace concentration of the volatile compounds is shown in Fig. 2. Nitrite produced a significant release of octanal, hexanal, and methional while nitrate (data not shown) produced the same effect although it was not significant. There are not reports about the effect of nitrate and nitrite on the headspace concentration of volatile compounds in aqueous solutions. However, the effect of nitrate has been assayed in sausage models due to their effect on volatile generation during dry-fermented processing. Sunesen, Trihaas, and Stahnke (2004) used an agar sausage models to analyse the effect of starter, sodium ascorbate, sodium nitrate, and temperature on volatiles produced during incubation. They found that nitrate have not a significant impact on any of the variables studied including the 79 volatile compounds analysed.

On the other hand, the effect of glucose on the headspace concentration of the volatile compounds is shown in Fig. 3. Glucose decreased significantly the headspace concentrations of octanal and hexanal exclusively. In this sense Hansson, Andersson, and Leufven (2001), studied the effect of three types of sugar (sucrose, invert sugar, and glucose syrup) and pectin, added at different concentrations to a drink-related model system consisting of water and six flavour compounds (ethyl hexanoate, menthone, hexenylacetate, linalool, isopenthyl acetate, and limonene). They showed that the addition of sucrose (20–60%), invert sugar (20–60%), and glucose syrup (60%) significantly increased the release of the flavour compounds, probably due to a "salting-out" effect. They found that the addition of these ingredients contributes to change the water activity

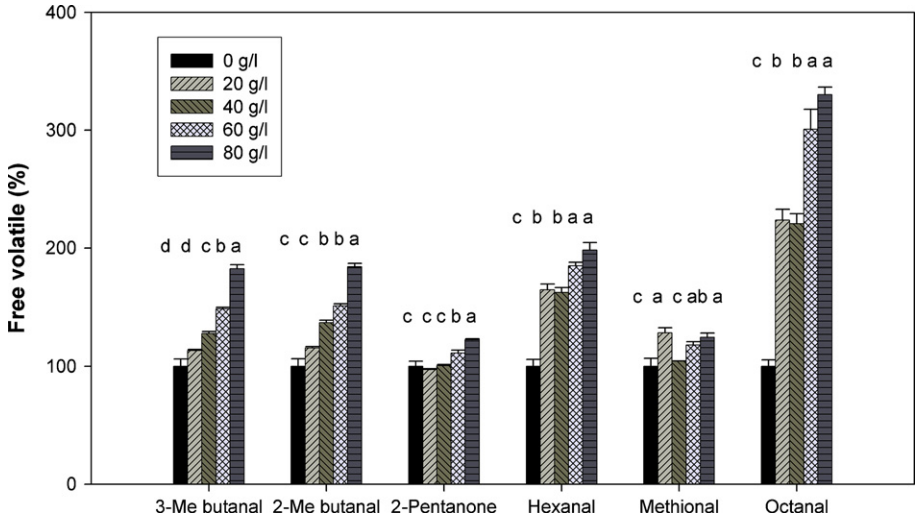

Fig. 1. Effect of sodium chloride on the headspace concentration of the volatile compounds. Results are expressed as a percentage of the free volatile compound (ratio of peak areas) found without any curing agent in the solution.

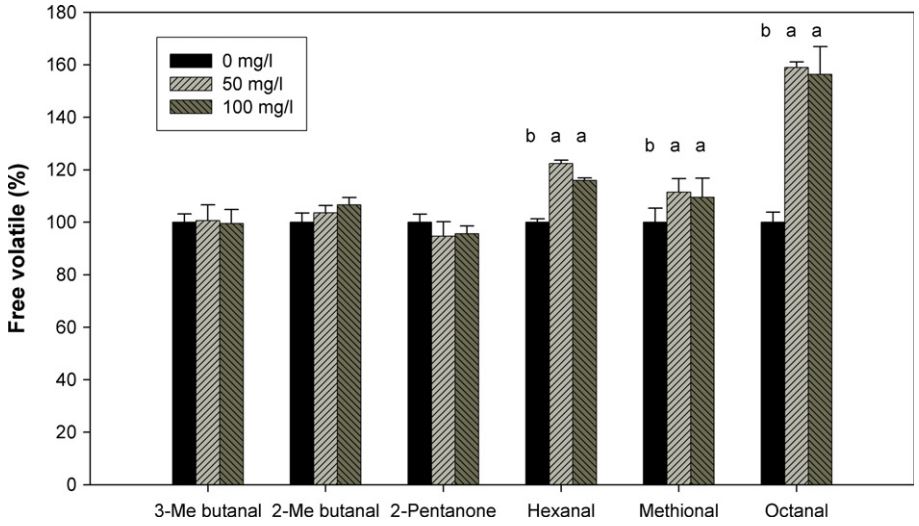

Fig. 2. Effect of sodium nitrite on the headspace concentration of the volatile compounds. Results are expressed as a percentage of the free volatile compound (ratio of peak areas) found without any curing agent in the solution.

as these sugars interact with water, increasing the concentration of flavour compounds in the remaining "free" water. This means that the flavour compound is more concentrated in the available free water and therefore, it is more easily released to the gas phase. In our study, the low glucose concentration used (0–0.1%) was not enough to reduce the water activity of the solution and to produce a release of the volatile compound to the headspace. However, Friel, Linforth, and Taylor (2000) developed an empirical model to describe and predict the change in gas–liquid partition behavior of a wide range of volatile compounds in aqueous sucrose solutions. The static equilibrium headspace concentrations of 40 volatiles (from different chemical classes e.g., pyrazines, alcohols, esters, and ketones) and with different physical properties (e.g., volatility and solubility), were measured above aqueous sucrose solutions (0–65%). These authors found that as sugar concentration increased, the headspace concentration of several compounds increased, whilst others stayed the same or decreased.

Finally, the effect of ascorbic acid on the headspace concentration of the volatile compounds is shown in Fig. 4. Ascorbic acid produced a small significant release to the headspace of 3-methyl-butanal and 2-pentanone. There are few reports where the use of ascorbic acid is discussed. As we mentioned above, Sunesen et al. (2004) studied agar sausage models in order to analyse the effects of starter, sodium ascorbate, sodium nitrate, and temperature on the volatile compounds produced during incubation. These authors found that ascorbate addition showed a clear antioxidative effect because it reduced the amount of more than half of all the volatiles produced by autoxidation or

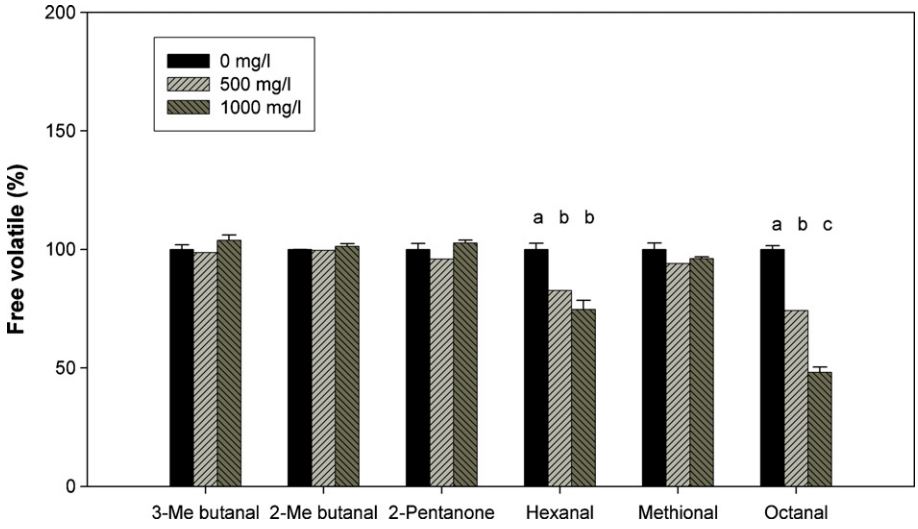

Fig. 3. Effect of glucose on the headspace concentration of the volatile compounds. Results are expressed as a percentage of the free volatile compound (ratio of peak areas) found without any curing agent in the solution.

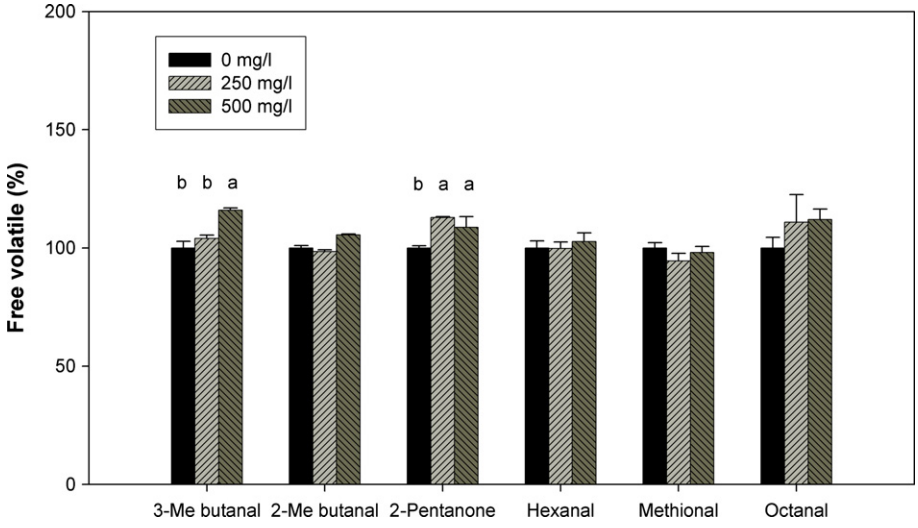

Fig. 4. Effect of ascorbic acid on the headspace concentration of the volatile compounds. Results are expressed as a percentage of the free volatile compound (ratio of peak areas) found without any curing agent in the solution.

microbial oxidation. Furthermore, they found that ascorbate produce an increase of 2-methyl-propanal and 3-methyl-butanal by reducing the oxidation of their corresponding acids and decreasing the *micrococcaceae* responsible of the volatile compound generation.

4. Conclusion

The proportion of dry-cured volatile compounds on the headspace depends on the nature of the volatile compound and on the curing agent assayed. Salt, ascorbic acid, nitrate, and nitrite produce the release of the volatile compounds assayed although in different levels while glucose decreased the headspace concentration of octanal and hexanal, exclusively. The main salting-out effect was produced by sodium chloride in all volatile compounds, being the highest for octanal. The changes in the relative proportion of the volatile compounds due to the effect of curing agents, specially sodium chloride, nitrite, and glucose, should be taken into account as they may change the flavour perception of dry-cured meat products, although further studies considering the effect of other meat components (proteins, lipids, etc.) are required for the comprehension of the flavour release and perception in dry-cured meat products.

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