JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Interaction of Soluble Peptides and Proteins from Skeletal Muscle with Volatile Compounds in Model Systems As Affected by Curing Agents

M. Pía Gianelli, Mónica Flores, and Fidel Toldrá*

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), P.O. Box 73,
46100 Burjassot (Valencia), Spain

The effect of curing agents (salt, glucose, nitrate, nitrite, and ascorbic acid) on the binding of skeletal peptides (carnosine and anserine) and a sarcoplasmic protein (myoglobin) with key flavor compounds (hexanal, octanal, 2-pentanone, 2-methylbutanal, and 3-methylbutanal) has been studied by solid-phase microextraction (SPME). Curing agents had an effect on the interaction process between carnosine and volatile compounds, which was higher than the interactions observed with anserine and myoglobin. Sodium chloride decreased the interaction of volatiles with carnosine except for octanal, which was increased, and 2-pentanone, which was unaltered. Ascorbic acid exerted the highest effect by decreasing the interaction of carnosine with all of the volatile compounds except for octanal and 2-pentanone. The interaction with anserine was affected by sodium chloride, nitrate, and nitrite, producing a decrease in the interaction with hexanal, octanal, and methional. Finally, sodium chloride, glucose, and nitrite increased the interaction of myoglobin with hexanal, octanal, and methional. The effect of simulated stages of the curing process on the binding was also studied. A combined effect of the curing agents resulted in a change in the relative proportions of volatile compounds that can lead to different flavor perceptions of dry-cured meat products.

KEYWORDS: Carnosine; anserine; myoglobin; binding; interaction; curing; sodium chloride; nitrate; nitrite; glucose; ascorbic acid

INTRODUCTION

The aroma perception in meat products depends not only on the concentration and odor thresholds of volatile compounds but also on their interactions with other food components that will affect their gas-phase concentration. Proteins can affect flavor release by binding flavor components, although many factors are known to affect this interaction phenomenon (*1*, *2*). The main factors that affect the interaction between proteins and volatile components are protein nature, volatile compound nature, temperature, pH, ionic strength, and concentration of other food components (*1*, *2*).

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 (*3−6*) including studies of the odor activity of the volatile compounds (*7*, *8*). On the other hand, there are few studies on the interaction process between volatile compounds and peptides and proteins in dry-cured meat products (*9*).

An intense proteolysis has been observed in dry-cured meat products such as dry-cured ham and sausages (*10*, *11*). However, carnosine, the most abundant dipeptide in porcine skeletal muscle, slightly decreases after 100 days of processing, whereas

anserine is not degraded during the processing of dry-cured ham (*12*). Both dipeptides, carnosine and anserine, are important in skeletal muscle due to their buffering and antioxidant activities (*13*). Myoglobin is in low proportion in porcine muscle, whereas myofibrillar proteins are the most abundant proteins. It is important in porcine muscle as it is responsible for the cured meat color due to its reaction with nitric oxide generated from the added nitrite (*14*, *15*). Therefore, the presence of these dipeptides (carnosine and anserine) and a sarcoplasmic protein (myoglobin) during the long processing time of dry-cured meat products is recommended for the investigation of possible interactions.

The study of the interaction between proteins and flavor compounds is important for the flavor modulation and for the improvement of the sensory properties of meat products such as dry-cured ham and sausages. For that reason, the presence of interactions between soluble peptides (carnosine and anserine) and a protein such as myoglobin with volatile compounds responsible for meat flavor was demonstrated in a previous study (*9*). The effect of pH on the binding process was also assayed. In cured meat products, the curing agents (salt, nitrate, nitrite, glucose, and ascorbic acid) are added at the beginning of the dry-curing process, and their respective concentrations depend on the country in which the meat is produced and the kind of product (*16*). The effect of these curing agents on the binding

* Corresponding author (telephone 34 96 3900022; fax 34 96 3636301; e-mail ftoldra@iata.csic.es).

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Table 1. Combination of Curing Agents and pH Corresponding to Three Different Stages in the Processing of Dry-Cured Ham

stage	pH	NO_3^- (mg kg^{-1})	NaCl (%)	ascorbic acid (mg kg^{-1})	glucose (mg kg^{-1})
I	5.5	400	8	500	1000
II	6.0	250	6	300	500
III	6.5	100	6	100	200

of these peptides and proteins (carnosine, anserine, and myoglobin) with volatile compounds remains unknown. The objective of the work was to determine the influence of curing agents on the binding between peptides/proteins and flavor compounds to clarify their role in flavor release and perception in cured meat products.

MATERIALS AND METHODS

Materials. The peptides L-carnosine (*N-β*-alanyl-L-histidine) and anserine (*N-β*-alanyl-3-methyl-L-histidine) and the protein myoglobin from horse skeletal muscle were purchased from Sigma Chemical Co. (St. Louis, MO). The aroma compounds 2-methylbutanal, 3-methylbutanal, hexanal, octanal, 3-(methylthio)propanal (methional), and 2-pentanone were obtained from Fluka Chemika (Buchs, Switzerland).

Preparation of Protein Solution. The protein solution was made by dissolving carnosine (4 mg mL^{-1}), anserine (1 mg mL^{-1}), and myoglobin (4 mg mL^{-1}) in 50 mM phosphate buffer, pH 6.

Preparation of Volatile Compound Solution. A stock solution containing 50000 mg kg^{-1} 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^{-1} for 2-methylbutanal and 3-methylbutanal, 1 mg kg^{-1} for hexanal, octanal, and 2-pentanone, and 5 mg kg^{-1} for methional. All of the volatile compounds were simultaneously present in the solution used for the experiments.

Effect of Curing Agents. The curing agents were solubilized in the peptide/protein solution with a final concentrations of sodium chloride of 0, 20, 40, 60, and 80 g L^{-1}; glucose, 0, 0.25, 0.5, and 1 g L^{-1}; potassium nitrate, 0, 100, 200, and 400 mg of NO_3^- L^{-1}; sodium nitrite, 0, 50, and 100 mg of NO_2^- L^{-1}; and ascorbic acid 0, 250, and 500 mg L^{-1}.

Effect of Combined Dry-Curing Process Parameters. Mixtures of curing agents at different pH values were prepared as a simulation of the three characteristic stages in the processing of dry-cured ham (see composition in **Table 1**). Stage I corresponds to the external *Semimembranosus* muscle of the ham after the salting step. Stages II and III correspond to the *Biceps femoris* muscle in the middle and end, respectively, of the dry-curing process.

Study of Volatile−Protein Bindings. The flavor compounds were added in appropriate concentrations, as mentioned above, to the peptide/protein solution containing the respective concentration of curing agent or simulated stage of processing. The same solution was prepared without peptide/protein and used as control. The solutions were stored at 30 °C for 15 h in the absence of light to allow equilibration. The quantity of aroma compound was extracted by using solid-phase microextraction (SPME) (*9*) and determined by gas chromatography (GC) analysis.

Five milliliters of solution containing the mixture of volatile compounds, peptide or protein, and curing agent was placed in a 10 mL headspace vial and sealed with a PTFE-faced silicone septum (Supelco). The 75 *μ*m carboxen/poly(dimethylsiloxane) (CAR/PDMS) fiber (Supelco, Bellefonte, PA) was then exposed to the headspace for sampling of the aroma compounds. 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 carryover between the samples. The linearity of detection for each aroma compound under these conditions was confirmed within the range of 1−10 mg kg^{-1}.

Gas Chromatography Analysis. An 8000 CE Instruments gas chromatograph (Rodano, Milan, Italy) equipped with a flame ionization detector (FID) was used. The aroma compounds were separated on a DB-624 capillary column (J&W Scientific, 60 m, 0.32 mm i.d., film thickness = 1.8 *μ*m). Helium was used as carrier gas with a linear velocity of 20.4 cm s^{-1}. 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^{-1}, then raised to 220 °C at the rate of 15 °C min^{-1}, 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 found without any peptide or protein in the solution (control solution) and containing the respective curing agent or the simulated stage of processing. All experiments were carried out in triplicate, and the values are represented as the means ± coefficient of variance.

Myoglobin Analysis. The concentrations of oxymyoglobin and metmyoglobin in the solution were determined according to the method of Krzywicki (*17*). The absorbance of the myoglobin solution was measured at 572, 565, 545, and 525 nm in a UV 2101 spectrophotometer (Shimadzu Inc., Columbia, MD) using the absorbance at 730 nm as a blank. The relative fractions of myoglobin, oxymyoglobin, and metmyoglobin were obtained using the equations indicated by Krzywicki (*17*).

Statistical Analysis. The effect of each individual curing agent and the effect of the simulated stages of processing were studied by analysis of variance (ANOVA) using Statgraphics plus v 2.0. The means were compared using Fisher's least significance difference (LSD) procedure ($p < 0.05$).

RESULTS AND DISCUSSION

Salt, nitrate, nitrite, glucose, and ascorbic acid are common substances usually employed as curing agents (*18*). The range of concentrations is variable and depends on the country in which the meat is produced and the kind of product. We studied the effect of the individual agents on the interaction between the volatile compounds and peptides as well as the protein and also the influence of the combined mixture of agents simulating different stages of the processing of dry-cured ham. The selection of six flavor compounds was based on their presence and contribution to the flavor of typical Spanish dry-cured meat products. Hexanal, 2-methylbutanal, 3-methylbutanal, and 2-pentanone were selected because of their high proportion in the headspace of dry-cured ham (*3, 5*), whereas octanal and methional were selected due to their odor activity in the aroma of dry-cured ham (*8*). Moreover, the SPME conditions used in this study have been previously optimized (*9*) to ensure that the concentrations are within a linear range and there is no competition phenomenon among the volatile compounds (**Figure 1**).

Effect of Each Curing Agent on the Interaction between Volatile Compounds and Carnosine, Anserine, and Myoglobin. Sodium chloride is a basic ingredient in the curing mixture. This component is responsible not only for the characteristic salty taste of meat products but also for a slight reduction in water activity, producing a partial inhibition of the microorganisms and an increase in protein solubility. Sodium chloride showed a slight effect on the interaction of carnosine with 2-methylbutanal (**Figure 2A**), 3-methylbutanal (**Figure 2B**), and hexanal (**Figure 2C**). The free percentages of these volatile compounds increased slightly with an increase in sodium chloride concentration. Consequently, the interaction of these compounds with carnosine decreased slightly in the presence of sodium chloride. On the other hand, the interaction between octanal and carnosine was increased ($p < 0.05$) by sodium chloride concentration (**Figure 2D**), whereas the percentage of free methional in the headspace increased ($p < 0.05$) with

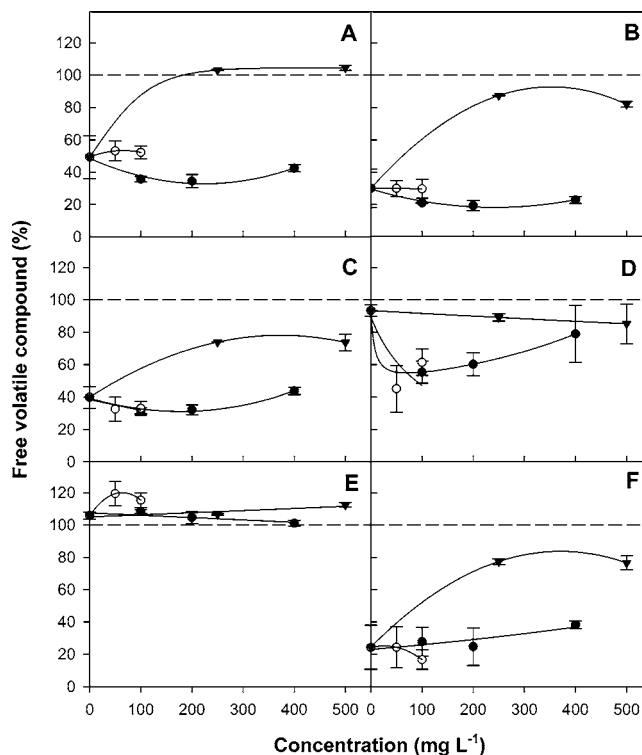**1672** *J. Agric. Food Chem.,* Vol. 53, No. 5, 2005

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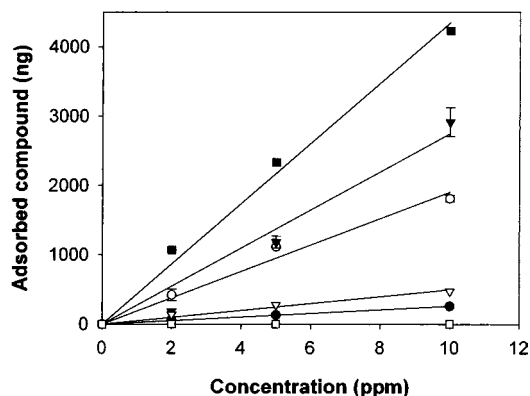
Figure 1. Linearity of detection of each aroma compound using the 75 μm CAR/PDMS fiber exposed for 30 min to the headspace: 2-methylbutanal (●); 3-methylbutanal (○); hexanal (▼); octanal (▽); 2-pentanone (■); methional (□).
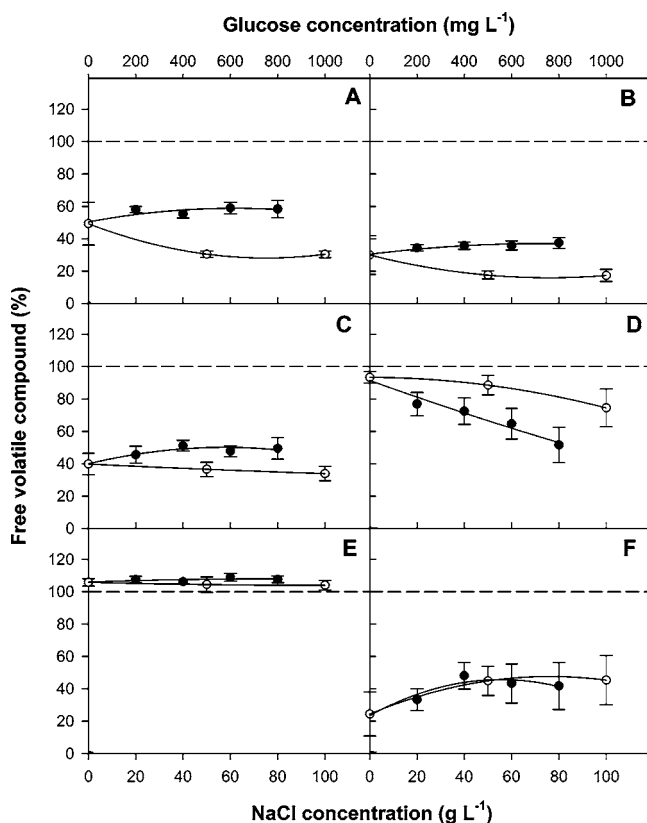
Figure 2. Effect of sodium chloride (●) or glucose (○) concentrations on volatile–carnosine binding: (**A**) 2-methylbutanal; (**B**) 3-methylbutanal; (**C**) hexanal; (**D**) octanal; (**E**) 2-pentanone; (**F**) methional. Results are expressed as a percentage of the free volatile compound found without carnosine in the solution and containing the respective sodium chloride or glucose concentration.

Figure 3. Effect of nitrate (●), nitrite (○), or ascorbic acid (▼) concentrations on volatile–carnosine binding: (**A**) 2-methylbutanal; (**B**) 3-methylbutanal; (**C**) hexanal; (**D**) octanal; (**E**) 2-pentanone; (**F**) methional. Results are expressed as a percentage of the free volatile compound found without carnosine in the solution and containing the respective nitrate, nitrite, and ascorbic acid concentration.

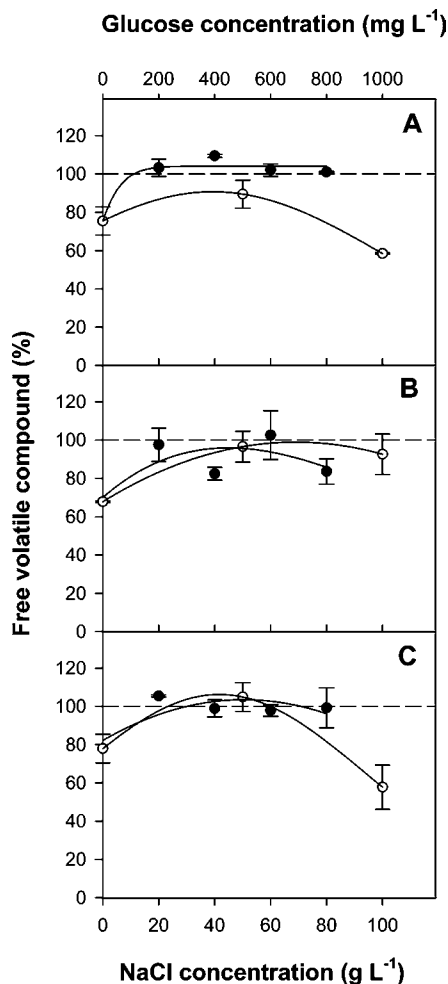increasing sodium chloride concentration up to a maximum at 40 g/L of NaCl (**Figure 2F**).

Glucose is another additive used in meat products to help the formation of a desirable color with improved stability and also serves as substrate for the fermentation process in sausages. Glucose produced a decrease ($p < 0.05$) of the free percentages of 2-methylbutanal (**Figure 2A**), 3-methylbutanal (**Figure 2B**), and hexanal (**Figure 2C**) in the presence of carnosine, thus showing an increase in the interaction process. Octanal showed the greatest interaction in the presence of glucose (**Figure 2D**). Furthermore, an increase in the percentage of free methional

was detected ($p < 0.05$) (**Figure 2F**), indicating a decrease of the interaction between methional and carnosine in the presence of glucose. It was remarkable that both curing agents, glucose and sodium chloride, did not affect the percentage of free 2-pentanone in the presence of carnosine (**Figure 2E**).

Nitrite and nitrate are basic components in the curing mixture, nitrite being responsible for different roles such as color stabilization, contribution to cured flavor, retardation of rancidity, and inhibition of poisoning and spoilage bacteria. Nitrite is very unstable, especially at pH values lower than 7.0, producing nitrous acid and nitric oxide; therefore, the effect of nitrite could be due to its degradation products. On the other hand, nitrate is a curing agent used in dry-cured ham processing and long-processed fermented sausages, during which the slow pH decrease allows its progressive conversion to nitrite. Low concentrations of nitrate (100−200 ppm) decreased the free percentages of 2-methylbutanal (**Figure 3A**), 3-methylbutanal (**Figure 3B**), hexanal (**Figure 3C**), and octanal (**Figure 3D**) in the presence of carnosine. At nitrate concentrations >400 ppm, the free percentages of these compounds increased. In the case of hexanal, the percentage of volatile free compound was slightly higher than in the absence of nitrate. The effect of nitrite concentration depended on the type of volatile compound. In the case of octanal, the nitrite concentration decreased ($p < 0.05$) the free percentage of the compound in the presence of carnosine, showing an increase of the interaction process (**Figure 3D**). The interaction between methional and carnosine was increased at the highest concentration of nitrite ($p < 0.05$) (**Figure 3F**). However, the nitrite concentration produced a release of 2-pentanone ($p < 0.05$) to the headspace in the presence of carnosine higher than 100% (**Figure 3**).
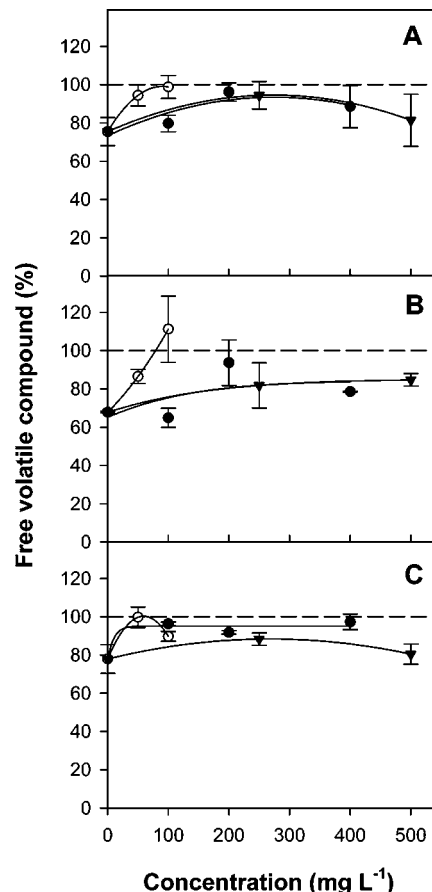
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Figure 4. Effect of sodium chloride (●) or glucose (○) concentrations on volatile–anserine binding: (**A**) hexanal; (**B**) octanal; (**C**) methional. Results are expressed as a percentage of the free volatile compound found without anserine in the solution and containing the respective sodium chloride or glucose concentration.

Figure 5. Effect of nitrate (●), nitrite (○), or ascorbic acid (▼) concentrations on volatile–anserine binding: (**A**) hexanal; (**B**) octanal; (**C**) methional. Results are expressed as a percentage of the free volatile compound found without anserine in the solution and containing the respective nitrate, nitrite, and ascorbic acid concentration.

Ascorbic acid is used to produce reducing conditions, which facilitate nitrite reduction to nitric oxide and color stabilization. Ascorbic acid increased substantially the percentage of free volatile compounds for all of the studied compounds ($p < 0.05$) (**Figure 3**) in the presence of carnosine, except for octanal and 2-pentanone. A slight decrease, although not significant, of the percentage of free octanal in the presence of ascorbic acid was detected, whereas the percentage of free 2-pentanone was not changed (**Figure 3D,E**, respectively).

In a previous study, Gianelli et al. (*9*) showed different interaction percentages for several of the studied compounds. They reported an interaction of 80% between carnosine and octanal, whereas in the present study the interaction in the absence of curing agents but in the presence of other volatile compounds was ∼10% (**Figures 2** and **3**). The reduction in the interaction was due to a competition phenomenon for the binding sites of carnosine by 3-methylbutanal and hexanal that are also present in the same mixture as octanal. Due to the larger affinity constants (*K*) of 3-methylbutanal ($K = 7680$ M^{-1}) and hexanal ($K = 8928$ M^{-1}) compared to octanal ($K = 7215$ M^{-1}) (*9*), these compounds compete for the binding sites of carnosine if they are present in the same solution.

The effect of curing agents on the interaction between volatile compounds and anserine was also studied. The concentration of sodium chloride produced an increase in the free percentages

of hexanal, octanal, and methional ($p < 0.05$) (**Figure 4**) in the presence of anserine. The reduction of the interaction was detected at the lowest concentration of sodium chloride (20 g/L). An addition of glucose in the presence of anserine slightly decreased (∼10% reduction) the free percentages of the branched aldehydes, 2-methyl- and 3-methylbutanal (data not shown). Furthermore, the free percentages of hexanal, octanal, and methional (**Figure 4A−C**) depended on the glucose concentration. For hexanal and methional, low glucose concentrations reduced the interaction, whereas high concentrations increased it ($p < 0.05$). A similar profile was observed in the interaction of octanal and anserine, although at high glucose concentration the interaction was not increased.

In the study of the effect of nitrate in the presence of anserine, an increase of the free percentages of hexanal, octanal (**Figure 5A,B**), and methional (**Figure 5C**) was observed, although it was not significant. The effect of nitrite concentration on the interaction with anserine led to an increase ($p < 0.05$) of the free percentages of the linear aldehydes, hexanal (**Figure 5A**) and octanal (**Figure 5B**), and showed a slight effect on the interaction with branched aldehydes (data not shown). Moreover, other curing agents such as nitrate and ascorbic acid did not affect the interaction between anserine and 2-methylbutanal and 3-methylbutanal (data not shown). On the other hand, the increase of ascorbic acid concentration produced a slight increase in the free percentage of octanal (**Figure 5D**). Finally, no effect on the interaction was detected between anserine and 2-pen-
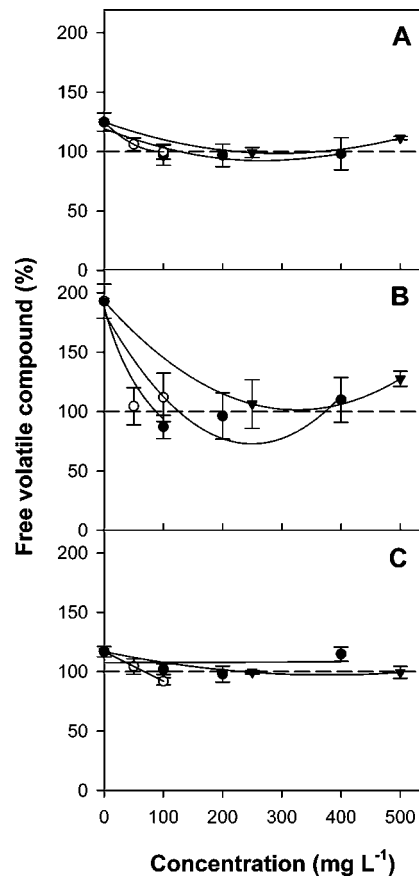
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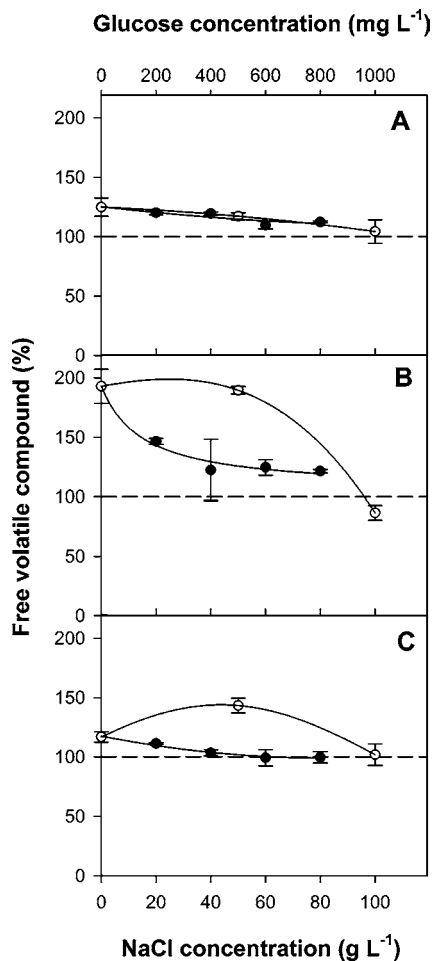
Figure 6. Effect of sodium chloride (●) or glucose (○) concentrations on volatile–myoglobin binding: (**A**) hexanal; (**B**) octanal; (**C**) methional. Results are expressed as a percentage of the free volatile compound found without myoglobin in the solution and containing the respective sodium chloride or glucose concentration.
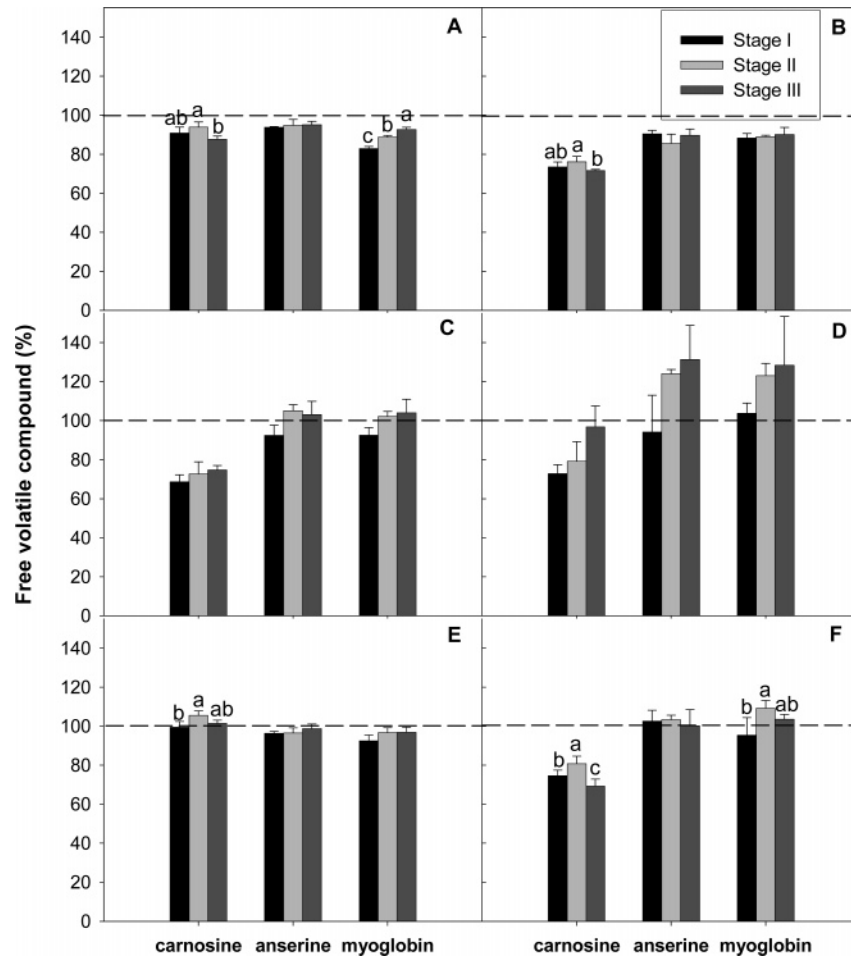

Figure 7. Effect of nitrate (●), nitrite (○), or ascorbic acid (▼) concentrations on volatile–myoglobin binding: (**A**) hexanal; (**B**) octanal; (**C**) methional. Results are expressed as a percentage of the free volatile compound found without myoglobin in the solution and containing the respective nitrate, nitrite, and ascorbic acid concentration.

tanone (data not shown) in the presence of all the examined curing agents.

The interaction percentages of carnosine with 2-methylbutanal, 3-methylbutanal, and methional and of anserine with octanal and methional are higher in this work (**Figures 2**–**5**) than those obtained for each compound assayed individually (*9*). The increment in the percentage of interaction could be due to the lower concentration of each volatile compound used in the mixture of volatile compounds in the present study than in the experiments done by Gianelli et al. (*9*). Therefore, there is a higher ratio peptide per mole of volatile compound for the interaction process resulting in an increase of the interaction percentage by reducing volatile compound concentration. These results were also reported by Ng et al. (*19*) and Landy et al. (*20*), who indicated that when the ratio of protein to volatile compound increased, the percentage of interaction also increased.

Finally, the effect of curing agents on the interaction between volatile compounds and myoglobin was determined (**Figures 6** and **7**). It was remarkable that sodium chloride, glucose, nitrate, nitrite, and ascorbic acid did not affect the interaction between myoglobin and 2-methylbutanal, 3-methylbutanal, or 2-pentanone (data not shown). The presence of sodium chloride decreased ($p < 0.05$) the free percentages of hexanal (**Figure 6A**), octanal (**Figure 6B**), and methional (**Figure 6C**) in the presence of myoglobin. This effect was also observed in the

presence of glucose, but compared to octanal and methional the interaction with myoglobin demonstrated a higher decrease.

The effect of nitrate concentration on the interaction between volatile compounds and myoglobin is shown in **Figure 7**. The most remarkable effect was on the interaction with octanal (**Figure 7B**) by reducing the free percentage ($p < 0.05$). On the other hand, at 400 mg/L of nitrate, hexanal and methional were found as 100% of free percentage in the presence of myoglobin (**Figure 7A**,**C**, respectively). Nitrite concentration decreased the free percentage of octanal in the presence of myoglobin ($p < 0.05$) (**Figure 7B**), producing an increase in the interaction process between octanal and myoglobin. The same effect, although in lower proportion, was observed for the interaction of methional (**Figure 7C**) and hexanal (**Figure 7A**) with myoglobin in the presence of nitrite. Ascorbic acid did not produce any significant effect except a slight decrease of the free percentage of hexanal (**Figure 7A**) and a higher decrease of octanal (**Figure 7B**) in the presence of myoglobin, although a slight increase in the free percentage was detected at the highest concentration of ascorbic acid.

It is important to determine which oxidation state of myoglobin is responsible for the interactions observed with the volatile compounds. The myoglobin used in this study was mainly found in the metmyoglobin form (93−94%) and small percentages in the form of oxymyoglobin (6−7%). The presence of sodium chloride, glucose, and nitrite did not affect the oxidation states of myoglobin. However, ascorbic acid produced an effect on the oxidation states of myoglobin. At 250 mg/L ascorbic acid, metmyoglobin was reduced to 55% and oxymyo-

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Figure 8. Effect of curing processing stages on volatile–peptide/protein binding: (**A**) 2-methylbutanal; (**B**) 3-methylbutanal; (**C**) hexanal; (**D**) octanal; (**E**) 2-pentanone; (**F**) methional. Results are expressed as a percentage of the free volatile compound found without peptide or protein in the solution and containing the curing agents and pH of the respective stage. Bars with different letters are significantly different ($p < 0.05$) among processing stages for a given peptide or protein.

globin and myoglobin were increased to 42 and 2%, respectively. At 500 mg/L ascorbic acid, the proportions of metmyoglobin, oxymyoglobin, and myoglobin were 35, 47, and 18%, respectively. This effect is due to the reduction activity of ascorbic acid. This effect was also observed, although in low proportion, at the highest concentration of nitrate used (400 mg/L), at which metmyoglobin decreased to 85% and oxymyoglobin and myoglobin increased to 14 and 1%, respectively. Although ascorbic acid is the main curing agent responsible for changes in the myoglobin oxidation states, its effect on the interaction process was not remarkable because it affected only the binding of myoglobin to octanal. Therefore, the detected interactions between myoglobin and volatile compounds are probably due to the protein structure (globin) but are not caused by the porphyrin ring of myoglobin, which is responsible for the changes in the oxidation states.

The effect of sodium chloride on the interaction between volatile compounds and carnosine (**Figure 2**) and anserine (**Figure 4**) affected several of the compounds studied (hexanal, octanal, and methional), but the interaction in the presence of sodium chloride was increased between only carnosine and octanal. The effect of sodium chloride on the interaction process was also studied by Damodaran and Kinsella (*21*) using soybean protein; they reported an increase of the affinity constant with an increasing concentration of sodium chloride as also reported by Kinsella (*22*) for the interaction between bovine serum albumin and 2-nonanone. These effects were also observed on

the interaction between myoglobin (**Figure 6**) and octanal and methional, where the sodium chloride concentration decreased the free percentages of these compounds, producing an increase in the interaction process. The effect of the other curing agents (nitrate, nitrite, glucose, and ascorbic acid) on the interaction between volatile compounds and peptides and proteins cannot be discussed in this context because of missing data. The mechanism of interaction was previously studied (*9*), being a reversible interaction between volatile compounds and dipeptides or myoglobin due to van der Waal forces or hydrogen bonds. Probably, the presence of curing agents in the model systems may affect the imidazole group of the dipeptides or the myoglobin structure, producing an effect on the reversible interaction.

Effect of Simulated Stages of Dry-Cured Ham Processing on the Interaction between Volatile Compounds and Skeletal Peptides and Myoglobin. An in vitro simulation of three different stages in the processing of dry-cured ham were prepared by combining different pH values and curing agent concentrations. Stage I represented the conditions in the muscle just after salting, and stages II and III reflected the conditions at the middle and the end of the dry-curing process, respectively. As indicated previously, the oxidation states of myoglobin can vary depending on the concentration of curing agents. In this case, the myoglobin oxidation states in the different processing stages are shown in **Table 2**. As expected, myoglobin was mainly found in the form of metmyoglobin (72–79%), followed

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Table 2. Effect of the Simulation of Dry-Cured Ham Processing Stages on Myoglobin Oxidation States

stage	myoglobin (%)	oxymyoglobin (%)	metmyoglobin (%)
I	8.9	18.7	72.3
II	0.8	19.3	79.8
III		20.1	79.9

by oxymyoglobin (18−20%) and myoglobin that was also present in the first two stages (**Table 2**). The main effect is probably produced by the high concentration of ascorbic acid in stage I, as deduced from the higher proportion of myoglobin and oxymyoglobin.

The effect of the processing stages on the interaction between volatile compounds and skeletal peptides and myoglobin is shown in **Figure 8**. In the in vitro simulation of the processing stages, the free volatile percentages of 2-methylbutanal (**Figure 8A**) and 2-pentanone (**Figure 8E**) in the presence of carnosine were ∼100% or slightly lower. The interactions between carnosine and 3-methylbutanal (**Figure 8B**) and hexanal (**Figure 8C**) were higher than those observed for 2-methylbutanal and 2-pentanone. The free percentage of octanal in the presence of carnosine (**Figure 8D**) in the simulated processing stages was ∼70%. The interaction decreased during the curing process as observed by an increase in its free percentage, although these changes were not significant. The opposite behavior was detected in the interaction between carnosine and methional ($p < 0.05$) (**Figure 8F**) in the processing stages.

The effect of in vitro simulated processing stages on the interaction of anserine and volatile compounds was only remarkable for the lineal aldehydes, although not significant ($p > 0.05$). The free percentages of hexanal and octanal increased during the curing process (**Figure 8C,D**, respectively). Similar results were obtained for the interaction of volatile compounds and myoglobin during the curing process as observed by an increase of hexanal and octanal (**Figure 8C,D**, respectively) ($p > 0.05$) during the processing stages. Moreover, the free percentages of 2-methylbutanal and methional were increased ($p < 0.05$) during the processing stages in the presence of myoglobin.

The higher interaction with volatile compounds detected for carnosine in comparison to anserine and myoglobin is in accordance with the previous results obtained in the study of the effect of each curing agent. During the curing process, the concentrations of curing agents decrease. Therefore, stage I would be comparable with the studies using the highest curing agent concentrations. In the study of the effect of curing process stages on the binding of volatile compounds, ascorbic acid was responsible for the decrease of the binding between carnosine and 2-methylbutanal, 3-methylbutanal, and methional ($p < 0.05$). These compounds were released when assayed individually in the presence of carnosine. On the other hand, there was a reduction in the interaction between carnosine and octanal during the curing process but, in this case, nitrate, nitrite, glucose, and sodium chloride would be responsible for it, as they produced a higher binding to carnosine at the highest concentrations such as those found at the beginning of the processing stages.

In summary, the typical aroma of dry-cured meat products, such as dry-cured ham, cannot be attributed to a single compound but to a mixture of volatile compounds in appropriate amounts (*3*, *23*). The volatile compounds used in this study have low threshold values (*24*, *25*) and are responsible for dry-cured

ham flavor (*7*, *8*), although other odor-active compounds have been found in Parma-type dry-cured ham (*26*). However, changes in their relative proportions due to interaction or binding phenomenon with peptide and proteins, as demonstrated in the present work, must be taken into account because they can change the flavor perception of dry-cured ham.

ABBREVIATIONS USED

SPME, solid-phase microextraction; GC, gas chromatograph; PDMS, poly(dimethylsiloxane); CAR, carboxen.

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