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Model Studies on the Efficacy of Protein Homogenates from Raw Pork Muscle and Dry-Cured Ham in Binding Selected Flavor Compounds

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The binding of sarcoplasmic and myofibrillar proteins extracted from postrigor pork muscle and from 7 and 12 months dry-cured hams with volatile compounds such as 3-methyl-butanal, 2-methyl-butanal, 2-pentanone, hexanal, methional, and octanal was studied using solid phase microextraction and gas chromatography analysis. The binding ability of sarcoplasmic proteins from pork muscle was higher than the ability shown by 7 and 12 months dry-cured ham sarcoplasmic homogenates and also higher than the binding ability of myofibrillar homogenates. The effect of the ionic strength on the binding was also studied. This effect was more important on myofibrillar proteins due to its ability to produce changes on the protein conformation that affect their binding ability. However, the sarcoplasmic protein binding ability was more related to the small compounds present in this homogenate than with the aqueous phase ionic strength.

KEYWORDS: Solid phase micro extraction; SPME; myofibrillar; sarcoplasmic; binding; interaction; dry-cured ham; pork meat

INTRODUCTION

The concentration of free volatile compounds in the gas phase depends on several factors such as their physicochemical properties and their interactions with other food constituents (*1*). Furthermore, the knowledge of the binding behavior of flavor components on different phases has a great practical importance (*2*) and there is an increasing interest on a better comprehension of the interactions between flavor compounds and nonvolatile food ingredients. In this sense, the interaction of proteins with volatile compounds significantly changes their headspace (HS) concentration and this interaction has been studied for vegetable proteins such as soy proteins (*3*−*5*) and pea proteins (*6*) as well as for animal proteins such as β-lactoglobulin (*7*−*9*), bovine serum albumin (*10*), and fish actomyosin (*11*). Furthermore, small components from muscle tissues such as dipeptides carnosine and anserine have been used in interaction studies (*12*, *13*).

On the other hand, dry-cured ham is one of the most typical meat products from the Mediterranean area and its flavor has been extensively studied (*14*−*17*). Its main components (lipids and proteins) experience intense proteolysis and lipolysis during the ripening and drying, which have been extensively studied (*17*−*20*). Sarcoplasmic and myofibrillar proteins constitute the main protein groups present in the skeletal muscle representing about 30−40% of the living animal weight (*21*). The sarcoplasmic proteins, which are soluble in water or in dilute salt solutions, form a mixture of proteins, which are similar to those present in other cell types and mainly include glycolytic enzymes, myoglobin, myoalbumin, and others (*22*). Moreover, using low ionic strength buffers, dipeptides such as carnosine, which is the most abundant dipeptide in pork skeletal muscle, and anserine are also extracted, both of which are important due to their buffering and antioxidant activities (*23*). Moreover, the major constituents of myofibrillar proteins, myosin and actin, are not only important in muscle due to their role on contraction (*21*) but also for their importance on the functional properties in meat products such as water holding, emulsifying capacity, binding ability, and gelation (*24*). In general, the myofibrillar protein fraction undergoes a more intense proteolysis than the sarcoplasmic protein fraction during the ripening process of dry-cured ham, giving rise to a larger number of low molecular weight peptides (*17*). The last step of proteolysis consists of the generation of free amino acids (*17*) that contribute to taste and also to the generation of flavor volatile compounds as a result of Strecker degradations including sulfide compounds, methyl branched aldehydes, alcohols, and pyrazines. These compounds, in combination with the volatile compounds formed by lipid oxidation and nonvolatile compounds generated by proteolysis, have a large impact on the final flavor of the dry-cured ham product (*17*). However, there are few studies about the interaction of soluble molecules with aroma compounds; these studies were mainly done in cheese (*25*). Recent studies in meat products (*26*, *27*) showed the interaction between volatile compounds and peptides and sarcoplasmic proteins from skeletal muscle. However, the effect of the meat structure on the binding of volatile compounds has not yet been elucidated. Therefore, the aim of the present work was to study the role of

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sarcoplasmic and myofibrillar proteins from pork muscle and dry-cured ham in the binding of selected key aromatic compounds to determine the relative impact of these muscle proteins on the release of aroma compounds.

MATERIAL AND METHODS

Materials. 2-Methyl-butanal, 3-methyl-butanal, 2-pentanone, hexanal, methional, and octanal were obtained from Fluka Chemie (Buchs, Switzerland). Postrigor pork muscle (*Longissimus dorsi*) was acquired from a local supermarket. Fat and connective tissues were removed, and the meat was cut in 100 g portions, packaged in vacuum bags, and stored at −20 °C.

Two dry-cured hams were selected from a local industry and processed according to a traditional methodology and ripened for 7 (short process) and 12 (long process) months. The processing consisted of salting (12 days at 3 °C), postsalting (50 days at 4−6 °C), and ripening−drying (1st phase, 60 days at 12 °C; 2nd phase, 60 days at 18 °C; 3rd phase, 30 days at 30 °C, which is the end of the 7 months of processing; and 4th phase, 150 days at 15 °C, the end of 12 months) stages. The final section of the ham, containing mainly *Biceps femoris* and *Gluteous accesorious*, *medious*, and *profundus* muscles, was used. Four portions of this section (approximately 100 g each) without the subcutaneous fat were vacuum packaged and frozen stored at −20 °C until analysis.

Preparation of Sarcoplasmic and Myofibrillar Proteins Homogenates. Sarcoplasmic and myofibrillar proteins were obtained according to the procedure described by Molina and Toldrá (*28*). Pork muscle and dry-cured ham processed for 7 and 12 months were homogenized (dilution 1:10, w/v) with 30 mM sodium phosphate buffer at pH 7.4 with 0.02% NaN_3 during 4 min in a stomacher Lab blender 400 (Seward Medical, London, United Kingdom). The mixture was centrifuged at 10000*g* for 20 min at 4 °C in a Sorvall RC-5B (Dupont Instruments, France). The supernatant contained the sarcoplasmic proteins. The procedure was repeated twice, and the pellet was finally suspended in 9 vol of 100 mM sodium phosphate buffer at pH 7.4 containing 0.7 M potassium iodide and 0.02% NaN_3. The mixture was homogenized in a stomacher for 8 min and then centrifuged at 10000*g* for 20 min at 4 °C in a Sorvall RC-5B. The supernatant contained the myofibrillar proteins. The protein concentration of all supernatants was calculated by the method of Smith et al. (*29*), using the bicinchoninic acid as a reagent and the bovine serum albumin as a standard.

Dialysis of Protein Homogenates. To study the effect of aqueous ionic strength on the binding, the sarcoplasmic and myofibrillar protein homogenates from pork muscle and 7 and 12 months dry-cured ham were dialyzed using dialysis tubing size 6 with 12000−14000 Da molecular mass cutoff (Medicell international Ltd., London). The dialysis of sarcoplasmic and myofibrillar protein homogenates was performed for 24 h. The sarcoplasmic homogenates were dialyzed against two different buffers: 30 mM sodium phosphate buffer at pH 7.4 with 0.02% sodium azide and containing 0.3 M NaCl and the same buffer without NaCl. The dialysis of myofibrillar proteins was performed against two different buffers: 100 mM sodium phosphate buffer at pH 7.4 with 0.02% sodium azide and 0.3 M NaCl and the same buffer without addition of NaCl.

Preparation of Volatile Compound Solution. A stock solution containing 50000 mg kg^{-1} of each volatile compound was prepared in ethanol. The aroma compounds were added in triplicate to the protein homogenates and to control solutions resulting in a final concentration of 2 mg kg^{-1} for 2-methyl-butanal and 3-methyl-butanal; 1 mg kg^{-1} for 2-pentanone, hexanal, and octanal; and 5 mg kg^{-1} for methional. All of the volatile compounds were simultaneously present in the solution used for the experiments.

The selection of the six flavor compounds was based on their presence in the HS and the contribution to the flavor 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 HS of dry-cured ham (*30*, *31*), whereas octanal and methional were selected due to their odor activity in the aroma of dry-cured ham (*14*).

Volatile−Protein Binding. The flavor compounds were added in appropriate concentrations, as mentioned above, to the protein solution. The sarcoplasmic and myofibrillar homogenates from raw pork meat and 7 and 12 months dry-cured hams were used as protein solutions. Also, the dialyzed protein homogenates were used as protein solutions for the study of the effect of ionic strength on the binding. Protein and control vials were prepared for the binding studies. The protein vials contained 5 mL of the homogenate placed in a 10 mL HS vial and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA). The control vial contained the same buffer as the homogenate (including the same NaCl concentration of the protein vial) but without protein. Control and protein vials were stored for 16 h at 30 °C to allow the equilibration.

The quantity of aroma compound present on the HS of the protein and control vials was determined using solid phase microextraction (SPME) and gas chromatography (GC) analysis using optimized conditions (*27*). A 75 μm carboxen/poly(dimethylsiloxane) (CAR/PDMS) fiber (Supelco) was exposed to the HS for sampling 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 for 5 min on the injection port at 220 °C to avoid an analyte carry-over between the samples. The linearity of detection for each aroma compound under these conditions was confirmed within the range of 0.02−5 mg kg^{-1}.

GC Analysis. An 8000 CE instruments 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 the 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 in the HS without protein in the solution. All of the experiments were performed in triplicate, and the values were represented as the mean and coefficient of variance.

Calculation of Volatile Bound per Weight of Protein Homogenate. The flavor compounds were added into the protein and control vials at the same concentration as described above and also diluted 1/100 and 1/500. The quantity of aroma compound was determined by a previous GC after extraction using SPME under the optimized conditions. All of the experiments were done in triplicate.

The concentration in the HS ([HS]) was calculated using eq 1

$$[\mathrm{HS}] = \frac{A_F}{m \times V_F \times K} \quad (1)$$

where A_F is the area (CG-FID) of the volatile compounds adsorbed by the SPME fiber, m is the slope of a standard curve obtained by injecting known quantities of each aroma compound, V_F is the fiber volume (5.3×10^{-7} L), and K is the fiber−air partition coefficient of each volatile compound for CAR/PDMS 75 μm fiber obtained from Gianelli et al. (*26*).

The free volatile in aqueous phase (μM) and bound volatile/protein (μmol/g) were calculated using the concentration in the HS of vials with and without protein (eqs 2 and 3, respectively).

$$\text{free volatile in aqueous phase} = \frac{[\mathrm{HS}]_P}{[\mathrm{HS}]_C} \times O \quad (2)$$

$$\text{bound volatile/protein} = \frac{\dfrac{([\mathrm{HS}]_C - [\mathrm{HS}]_P)}{[\mathrm{HS}]_C} \times O}{C_p} \quad (3)$$

where $[HS]_C$ (mol/L) is the volatile compound HS concentration on the control vials, $[HS]_P$ (mol/L) is the volatile compound HS concentration on the protein vials, O (mol/L) is the initial concentration added

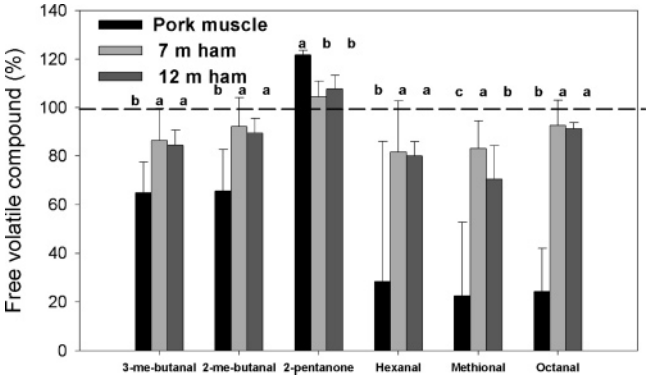

Figure 1. Effect of the sarcoplasmic homogenate type used (pork muscle and 7 and 12 months dry-cured ham) on the binding of volatile compounds. Results are expressed as the percentage of the free volatile compound found in the HS without protein in the solution.

to the solutions, and C_p (g/L) is the protein concentration present on the protein vials.

Statistical Analysis. The effects of the protein in solution, the homogenate type used (pork muscle or 7 and 12 months dry-cured ham), and the effect of ionic strength on the binding of volatile compounds were studied by analysis of variance using Statgraphics plus version 5.1. The means were compared using Fisher's least significance difference procedure ($p < 0.05$).

RESULTS AND DISCUSSION

Binding of Volatile Compounds with Sarcoplasmic Proteins Homogenates. All of the changes occurring in meat proteins during processing are very important because they will affect not only the texture but also the interactions between the generated flavor compounds and the proteins and peptides; thus, these interactions will be responsible for different sensory perceptions (*17*). The free percentage of each volatile compound on the HS in the presence of a sarcoplasmic protein homogenate from pork muscle and 7 and 12 months dry-cured ham is shown in **Figure 1**. The presence of sarcoplasmic proteins produced a decrease ($p < 0.05$) in the free percentages of the volatile compounds in the HS in the case of 3-methyl-butanal, 2-methyl-butanal, hexanal, methional, and octanal. This decrease means that the sarcoplasmic protein homogenates bind these volatile compounds. However, the opposite effect was observed for 2-pentanone where an increase ($p < 0.05$) in the free percentage was detected in the presence of these protein homogenates. On the other hand, the effect was not always the same and it was different depending on the type of sarcoplasmic protein homogenate (pork or dry-cured ham). The presence of pork muscle sarcoplasmic proteins in solution produced a reduction of the free percentages of 3-methyl-butanal, 2-methyl-butanal, hexanal, methional, and octanal and an increase of 2-pentanone that was significantly different from the effect produced by 7 and 12 months dry-cured ham homogenates.

The homogenates obtained in this study contain a complex mixture of sarcoplasmic proteins including glycolitic enzymes, myoglobin, and also small peptides, amino acids, and salts as previously reported by Pérez-Juan et al. (*32*). In this sense, Gianelli et al. (*26*) studied the interaction of soluble peptides and proteins from skeletal muscle on the release of volatile compounds. They showed that carnosine was able to bind the same volatile compounds except 2-pentanone while anserine and myoglobin significantly bound hexanal and 2-methyl-butanal. Myoglobin, as a major component of sarcoplasmic fraction, is present in the sarcoplasmic homogenates of pork muscle and 7 and 12 months dry-cured ham. The dipeptides, carnosine and anserine, that are not degraded during the curing process are also present (*18*). Therefore, they can be responsible for the binding observed in **Figure 1** together with other components present in the homogenate. In this sense, the different binding observed in the homogenates from 7 and 12 months dry-cured ham in comparison to pork muscle may be due to the degradation of sarcoplasmic proteins or also to the higher presence of mineral salts. The presence of mineral salts in cheese extracts produced a release effect of aroma compounds (*25*).

The moles of 3-methyl-butanal, 2-methyl-butanal, hexanal, and methional bound by the sarcoplasmic homogenates from pork muscle and 7 and 12 months dry-cured ham were calculated and shown in **Figure 2**. The increase in volatile compound concentration added to the protein solution produced and increment in the volatile compound bound (**Figure 2**). The pork muscle sarcoplasmic homogenates bound higher quantities of 3-methyl-butanal (**Figure 2A**), 2-methyl-butanal (**Figure 2B**), hexanal (**Figure 2C**), and methional (**Figure 2D**) than 7 and 12 months dry-cured ham homogenates. This fact is in accordance with the results shown in **Figure 1**.

Binding of Volatile Compounds with Myofibrillar Proteins Homogenates. The myofibrillar proteins fraction undergoes a more intense proteolysis than the sarcoplasmic proteins fraction, giving rise to a large number of low molecular mass peptides that contribute to the final flavor of dry-cured ham (*17*). With reference to the interaction, the free percentage of volatile compounds in the HS in the presence of myofibrillar proteins extracted from pork muscle and 7 and 12 months dry-cured ham is shown in **Figure 3**. The presence of myofibrillar protein homogenates produces a decrease ($p < 0.05$) of the free percentage of 3-methyl-butanal, hexanal, and octanal. This means that the myofibrillar proteins bind these volatile compounds. However, the binding of octanal was the only one that depends on the type of myofibrillar homogenate. The presence of the pork muscle myofibrillar protein homogenate produced a significant decrease of the free percentage of octanal while a significant release of octanal was observed in the presence of 7 and 12 months dry-cured ham myofibrillar homogenates. However, the type of myofibrillar homogenate (pork muscle and 7 or 12 months dry-cured ham) did not produce a significant difference on the free percentage in the HS of 3-methyl-butanal, 2-methyl-butanal, 2-pentanone, hexanal, and methional. Although there are few reports about the interaction of myofibrillar proteins, Goodridge et al. (*33*) found that freeze-dried chicken myofibrils act as a significant reservoir for hexanal and they observed a difference of about 20% between the peak area of hexanal alone as compared to the peak area of hexanal with chicken myofibrils at low concentrations of hexanal (0.01 mg/L). This indicated that more hexanal was being retained by the myofibrils in comparison to the standard alone. In our study, we observed a decrease of around 20% of hexanal in the presence of pork myofibrillar proteins; however, the decrease was smaller in the presence of myofibrillar homogenates from 7 and 12 months.

To study the interaction between 3-methyl-butanal, 2-methyl-butanal, hexanal, and octanal, the moles bound by the myofibrillar homogenates from pork muscle and 7 and 12 months dry-cured ham were calculated and are shown in **Figure 4**. The different quantities of volatile compounds added to the myofibrillar homogenates increase the free volatile compounds in the aqueous phase, but only in the case of pork muscle myofibrillar homogenate, a small increase was observed in the bound moles of 3-methyl-butanal (**Figure 4A**), 2-methyl-butanal (**Figure 4B**), and hexanal (**Figure 4C**). However, the addition of different quantities of volatile compounds to the myofibrillar homogenates from 7 and 12 months dry-cured ham did not

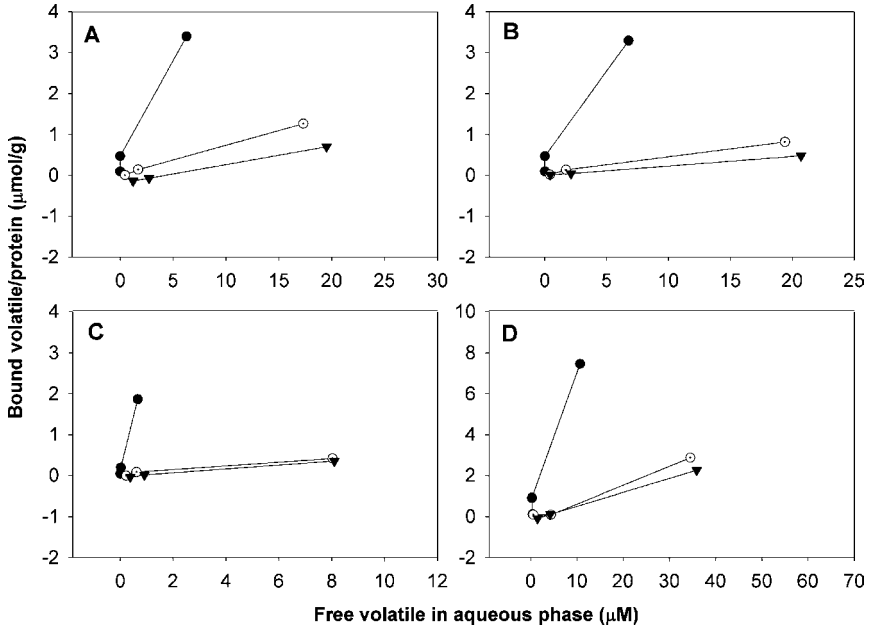

Figure 2. Moles bound by the sarcoplasmic homogenates from pork muscle (●), 7 months dry-cured ham (○), and 12 months dry-cured ham (▼) vs the concentration of free volatile compound in the aqueous phase. Binding of (**A**) 3-methyl-butanal, (**B**) 2-methyl-butanal, (**C**) hexanal, and (**D**) methional.

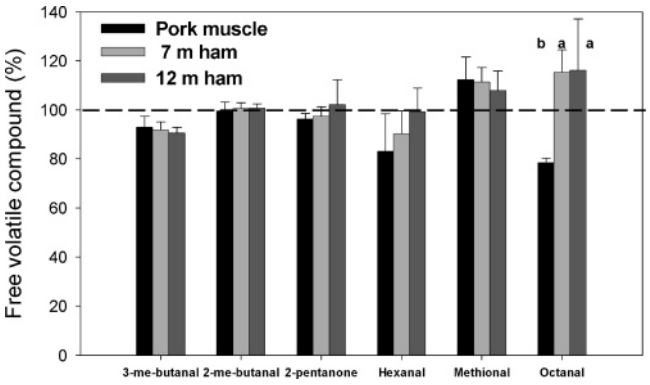

Figure 3. Effect of the myofibrillar homogenate type used (pork muscle and 7 and 12 months dry-cured ham) on the binding of volatile compounds. Results are expressed as a percentage of the free volatile compound found in the HS without protein in the solution.

increase the bound moles of the volatile compounds except in hexanal (**Figure 4 C**). In comparison to the bound moles by the sarcoplasmic proteins (**Figure 2**), the myofibrillar homogenates bound less moles of 3-methyl-butanal, 2-methyl-butanal, and hexanal (**Figure 4**). However, Goodridge et al. (*33*) found that the percentage of hexanal removed from the system in the presence of chicken myofibrils was lower at higher hexanal concentrations. This may be due to the saturation of the limited number of hexanal binding sites. In our study, the saturation of binding sites was not reached due to the low concentration of volatile compound used.

Effect of Aqueous Phase Ionic Strength on the Binding Ability of Sarcoplasmic and Myofibrillar Proteins Homogenates. Salt is one of the most important ingredients used during the dry-curing process; thus, it is present in the sarcoplasmic homogenates of 7 and 12 months dry-cured ham and it can affect its interaction with volatile compounds. The effect of salt on the volatility of flavor compounds has been studied in solution (*34*) and in model systems (*8*) due to its "salting out" effect (*2*). For that reason, the sarcoplasmic proteins extracted from pork muscle and 7 and 12 months dry-cured ham were dialyzed against different buffers, with and without NaCl. The free percentage of each volatile compound in the presence of the sarcoplasmic protein homogenates and after the dialysis process is shown in **Figure 5**.

In the presence of the sarcoplasmic proteins extracted from pork muscle, a significant increase on the free percentages of 3-methyl-butanal (**Figure 5A**), 2-methyl-butanal (**Figure 5B**), hexanal (**Figure 5D**), methional (**Figure 5E**), and octanal (**Figure 5F**) and a significant decrease in the free percentage of 2-pentanone (**Figure 5C**) were observed when the proteins were dialyzed in both cases, against salt and without salt. This effect could probably be due to the loss of small peptides and free amino acids during the dialysis process, which could bind the assayed volatile compounds (*12*, *26*). Furthermore, a significant increase was observed for octanal (**Figure 5F**) between proteins dialyzed against buffer without salt and dialyzed in the presence of salt probably due to the same reason as described above. On the other hand, the sarcoplasmic proteins homogenates extracted from 7 months dry-cured ham only showed a significant increase on the free percentages of methional (**Figure 5E**) and octanal (**Figure 5F**) when they were dialyzed without NaCl. However, a significant decrease of the free percentage of 2-pentanone (**Figure 5C**) was shown when the dialysis was made against 0.3 M NaCl. Finally, a significant increase of the free percentages of 3-methyl-butanal (**Figure 5A**), 2-methyl-butanal (**Figure 5B**), hexanal (**Figure 5D**), methional (**Figure 5E**), and octanal (**Figure 5F**) was observed in 12 months dry-cured ham sarcoplasmic protein homogenates after the dialysis. However, a significant decrease of the free percentage of 2-pentanone (**Figure 5C**) was observed after dialysis against buffer with salt. In summary, the dialysis of sarcoplasmic proteins from pork and dry-cured ham produced a loss of the compounds responsible for the binding of the studied volatile compounds (*12*, *26*), as observed by the increase in the free percentages of all volatile compounds except 2-pentanone after dialysis. Although there are no studies about the effect of ionic strength on sarcoplasmic homogenates binding ability, Guichard et al. (*8*) studied the effect of the solution composition on protein conformation and its ability to bind flavor compounds. They found that the decrease in the retention

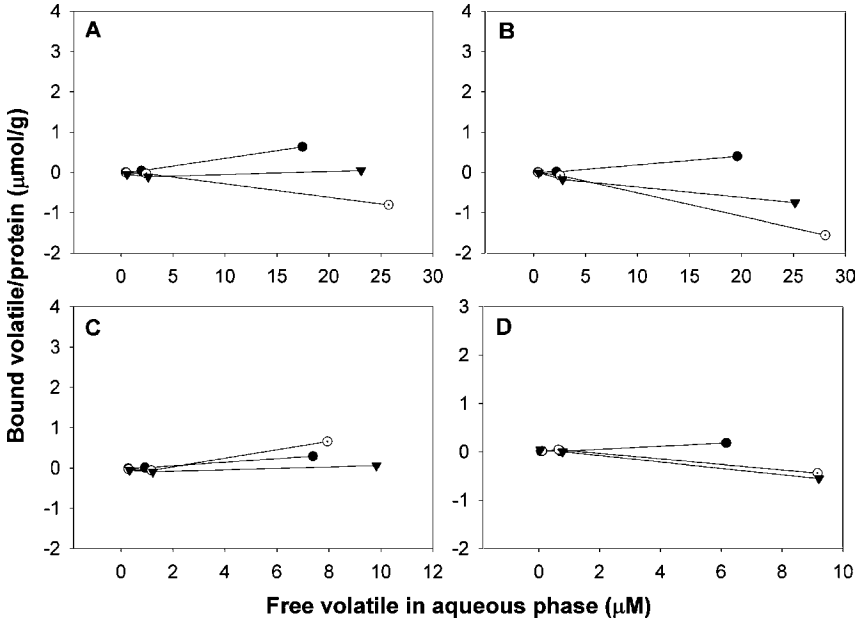

Figure 4. Moles bound by the myofibrillar homogenates from pork muscle (●), 7 months dry-cured ham (○), and 12 months dry-cured ham (▼) vs the concentration of the free volatile compound in the aqueous phase. Binding of (**A**) 3-methyl-butanal, (**B**) 2-methyl-butanal, (**C**) hexanal, and (**D**) octanal.

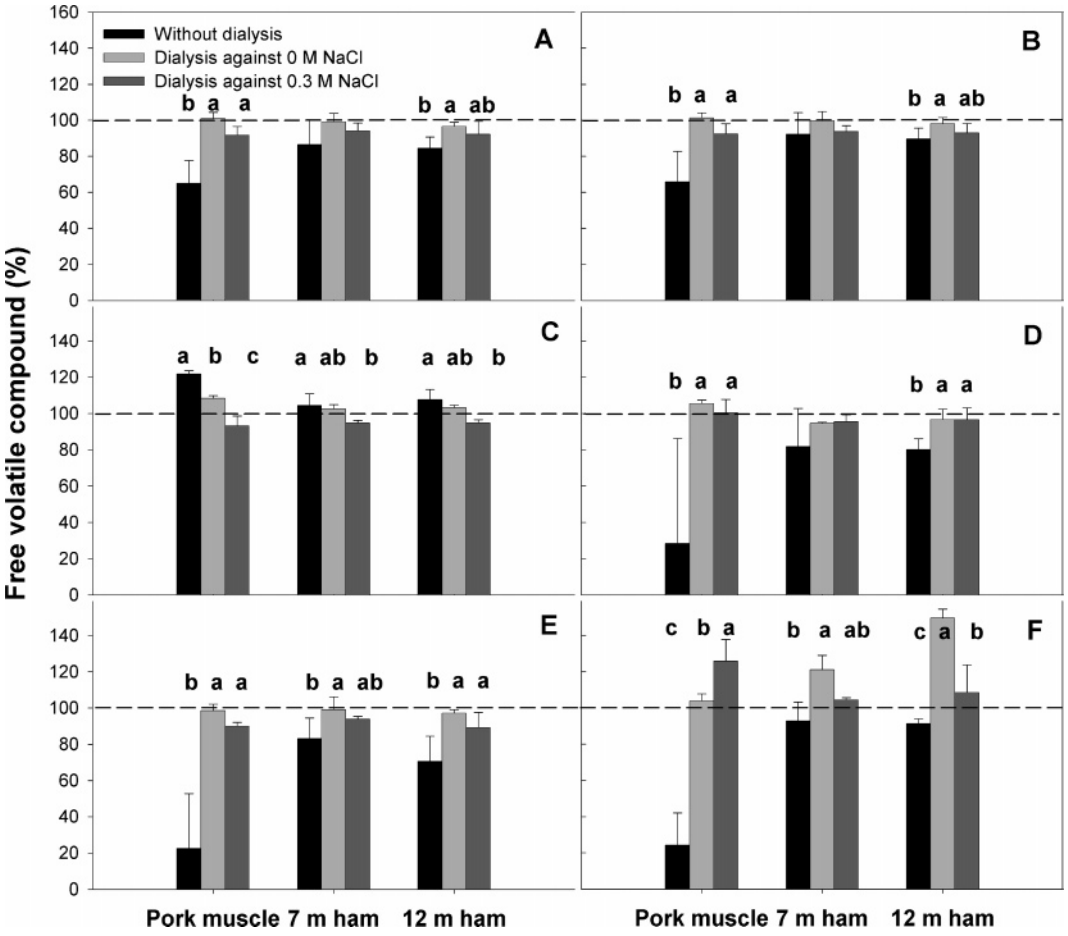

Figure 5. Effect of ionic strength on the binding of sarcoplasmic protein homogenates with volatile compounds. Results are expressed as a percentage of the free volatile compound found in the HS without protein in each buffer solution. (**A**) 3-methyl-butanal, (**B**) 2-methyl-butanal, (**C**) 2-pentanone, (**D**) hexanal, (**E**) methional, and (**F**) octanal.

of benzaldehyde by β-lactoglobulin in the presence of NaCl was due to a salting out effect.

In the case of myofibrillar proteins, it should be taken into account that they were extracted in the presence of 0.7 M potassium iodide. At this high ionic strength, the proteins are solubilized and their conformation is affected (*21*). For that reason, the myofibrillar proteins extracted from pork muscle and 7 and 12 months dry-cured ham were dialyzed against

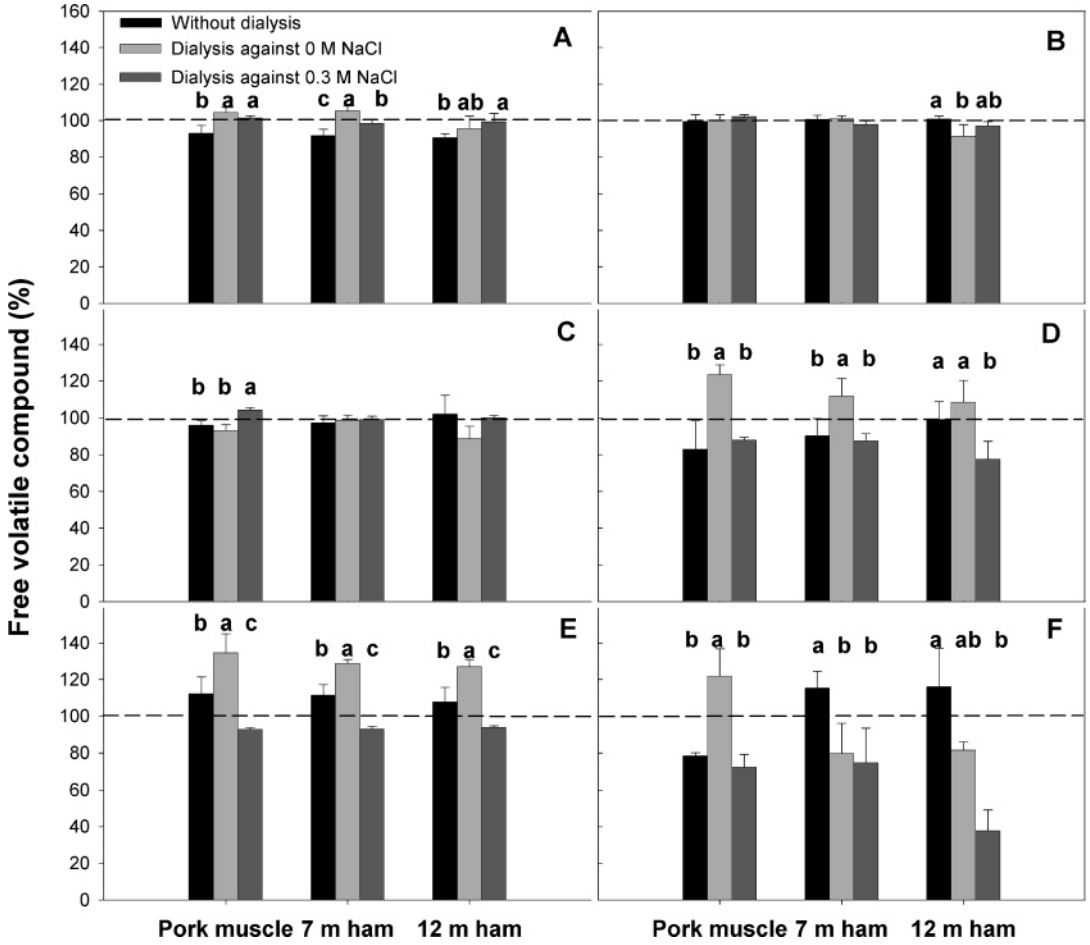

Figure 6. Effect of ionic strength on the binding of myofibrillar protein homogenates with volatile compounds. Results are expressed as a percentage of the free volatile compound found in the HS without protein in each buffer solution. (**A**) 3-Methyl-butanal, (**B**) 2-methyl-butanal, (**C**) 2-pentanone, (**D**) hexanal, (**E**) methional, and (**F**) octanal.

different buffers, with and without NaCl, to study how the change in protein conformation affects the protein−volatile binding ability. The free percentage of each volatile compound in the presence of myofibrillar protein homogenates and after dialysis process is shown in **Figure 6**.

In the presence of the myofibrillar proteins extracted from pork muscle, significant increases in the free percentages of 3-methyl-butanal (**Figure 6A**), hexanal (**Figure 6D**), methional (**Figure 6E**), and octanal (**Figure 6F**) were observed when the dialysis buffer did not contain NaCl. However, when the dialysis buffer contained 0.3 M NaCl, only the free percentage of 2-pentanone (**Figure 6C**) was significantly increased and the free percentage of methional (**Figure 6E**) was significantly decreased. These differences could be explained by the ionic strength change that affects the conformation of myofibrillar proteins (*21*) and also their binding ability. This fact was observed in the case of hexanal, methional, and octanal where the binding ability of myofibrillar proteins was lost in the absence of salt while it was kept in the presence of 0.3 M NaCl. The same effect was observed in the case of the myofibrillar 7 months dry-cured ham homogenates for hexanal (**Figure 6D**) and methional (**Figure 6E**) and in the case of the myofibrillar 12 months dry-cured ham homogenates only for methional (**Figure 6E**). However, a significant decrease of the free percentage of octanal (**Figure 6F**) was observed when 7 and 12 months dry-cured ham were dialyzed with and without salt. The observed differences in octanal between the homogenates from dry-cured ham and pork muscle could be due to the proteolysis occurring during the ripening process that changed the protein composition of these homogenates (*32*) and affected the binding properties. The effect of ionic strength on the binding ability of actomyosin to several carbonyl compounds was studied by Damodaran and Kinsella (*11*). They found that binding affinity and binding capacity were greater in the presence of 0.15 M NaCl, approaching the conditions found in muscle, than at 0.6 M NaCl, where actomyosin is fully solubilized. Although the studied volatile compounds were different, this result confirms that the conformation of myofibrillar proteins is important for their binding ability although it depends on the type of the volatile compound. O'Keefe et al. (*35*) studied the effect of NaCl concentration on the soy proteins (glycinin and β-conglycinin) binding ability. These authors showed that the presence of 0.5 M NaCl increased the presence of hexanal in the HS and reduced the affinity of hexanal for β-conglycinin while it had no effect for glycinin. As happens with myofibrillar proteins, the ionic strength of the medium affects the conformation of these two soy proteins.

Finally, it is difficult to compare our results with those obtained by other authors because of the different experimental conditions used. However, the effect of meat structure on the binding of volatile compounds has not yet been elucidated, so more research is still required to study the effect of isolated skeletal proteins on the binding of flavor compounds in order to determine the relative impact of muscle components on flavor release.

In summary, the binding ability of the sarcoplasmic fraction was higher than the myofibrillar fraction for the studied volatile compounds. All of the studied volatile compounds were retained

by the sarcoplasmic and myofibrillar protein homogenates except 2-pentanone, which was released. This was confirmed for 3-methyl-butanal, 2-methyl-butanal, hexanal, and methional, which were more bound by the sarcoplasmic proteins than by the myofibrillar ones. Furthermore, the effect of the aqueous phase ionic strength on the protein ability to bind flavor compounds was discussed. The effect of aqueous ionic strength was more important on myofibrillar proteins due to its ability to produce changes on the protein conformation that affect the binding ability of these proteins. However, the sarcoplasmic protein binding ability seems to be more related to the small compounds present in this homogenate than with the aqueous phase ionic strength.

ABBREVIATIONS USED

SPME, solid phase microextraction; GC, gas chromatography; FID, flame ionization detector; CAR/PDMS, carboxen/poly-(dimethylsiloxane); HS, headspace.

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