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Interactions of Soluble Peptides and Proteins from Skeletal Muscle on the Release of Volatile Compounds

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The ability of skeletal dipeptides (carnosine and anserine) and a sarcoplasmic protein (myoglobin) to interact with key flavor compounds (hexanal, octanal, methional, 2-pentanone, 2-methylbutanal, and 3-methylbutanal) has been studied using the solid phase microextraction (SPME) technique. Conditions for SPME analysis (fiber coating, sampling time, and linearity of detection) were optimized. The effect of pH on the binding was also investigated. Thermodynamic models were applied to evaluate the binding parameters *n* (number of binding sites), *K* (affinity constant), and ΔG (Gibb's free energy) to all of the flavor compounds studied, and they showed an absence of cooperative effect. Carnosine was the peptide with the highest affinity for all of the volatile compounds except 2-pentanone. Its interaction with hexanal and methional was significantly affected by pH. Anserine showed a lower level of interactions with hexanal, methional, 2-methylbutanal, and 3-methylbutanal, whereas myoglobin interacted with only hexanal and 2-methylbutanal. Differences in aroma retention can thus result in different sensory perceptions of muscle foods.

KEYWORDS: Solid phase microextraction; SPME; carnosine; anserine; myoglobin; binding; interaction

INTRODUCTION

The compounds responsible for flavor perception must be released from the food matrix and transported to the flavor receptors in the mouth and nose. The volatile compounds generated in meat will contribute to the aroma depending on their concentrations, odor thresholds, and interactions with other food components that will affect its gas phase concentration and, therefore, the perceived aroma. The main influence of proteins on flavor release and perception is caused by the interaction of the flavor component with the protein. Knowledge of the factors affecting this interaction is important for flavor perception (*1*). The main factors are protein nature, volatile compound nature, ionic strength of the medium, concentration of other food components, temperature, and pH (*2*). However, many studies on food protein interactions are based on milk proteins such as casein (*3*, *4*) and β-lactoglobulin (*5*), due to their importance for the dairy industry, and soy protein (*6*, *7*), because it is a functional ingredient in food products. Few papers deal with protein interactions from animal origin such as bovine serum albumin (*8*), fish actomyosin (*9*), and ovalbumin (*10*). On the other hand, the studied aroma compounds are mainly ketones and esters due to their fruity and sweet aroma. However, there is an absence of studies of the interaction between muscle proteins and volatile compounds responsible for meat flavor (*11*).

Carnosine is the most abundant dipeptide in skeletal muscle, and like other histidine-containing dipeptides such as anserine, has buffering capacity and antioxidant activity (*12*). The ability of carnosine and other histidine-containing dipeptides to quench unsaturated aldehydic products has been reported by Zhou and Decker (*13*, *14*), indicating the important contribution of these compounds to decrease lipid oxidation products and minimize rancidity in muscle foods. On the other hand, myoglobin is the main pigment in beef, pork, and dark muscles, representing 70−90% of the total concentration in heme proteins (*15*). The postmortem process and especially the pH fall stimulate the acid-catalyzed autoxidation of Fe^{2+} to Fe^{3+} of myoglobin in meats. The typical color of cured meat products is due to the reaction of myoglobin with nitric oxide generated from the added nitrite (*16*).

The study of the interaction between proteins and flavor compounds can be useful to solve problems such as off-flavor originating from the presence of compounds interacting with the protein and, therefore, it is very important not only for flavor modulation but also to improve the sensory properties of meat products. A relatively new technique, solid phase microextraction (SPME), has been successfully applied to the study of interactions between proteins and volatile compounds (*10*, *17*, *18*).

The SPME is an equilibrium process in which the concentration of the analyte in the fiber coating is directly related to its concentration in the headspace (*19*). The concentration of the analyte in the SPME fiber is based on the equilibrium among three phases: the polymeric coating, the headspace, and the matrix. The different concentrations of the analyte among the three phases depend on the driving forces that move the analyte from the matrix to the fiber coating. For aqueous samples, the

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headspace/water partition coefficients are determined by their volatility and hydrophobicity (*20*).

Due to the lack of previous studies on interactions of meat proteins with flavor compounds, the objective of this investigation was to optimize the appropriate conditions for SPME analysis and its application to the study of the binding behavior of flavor compounds to skeletal dipeptides (carnosine and anserine) and a sarcoplasmic protein (myoglobin).

MATERIALS AND METHODS

Materials. The dipeptides L-carnosine (β-Ala-His) and L-anserine (β-Ala-1-methyl-His) 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 Chemie (Buchs, Switzerland).

Optimization of the Extraction Method. *Sample Preparation.* A stock solution containing 10000 mg kg^{-1} of each aroma compound was made up in water except for octanal and methional, which were 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 50 mg kg^{-1}. The solutions were stored during 15 h at 30 °C in the absence of light to allow equilibration.

SPME. Different parameters (affinity for the fiber, trapping time, and the combination of water/air and air/fiber partition coefficients) were tested to optimize the conditions for flavor compounds.

Appropriate fiber coating was determined for each aroma compound by a combination of its affinity for a particular fiber and its partitioning in the sample vial. The appropriate fiber coating was selected from among the following fiber coatings: 100 μm polydimethylsiloxane (100 μm PDMS), 7 μm PDMS, 85 μm polyacrylate (85 μm PA), 75 μm carboxen/PDMS (75 μm CAR/PDMS), and 50/30 μm divinylbenzene/carboxen/PDMS (50/30 DVB/CAR/PDMS), all purchased from Supelco (Bellefonte, PA). The trapping time was chosen as a time lying within the linear range of the adsorption by time plots. The linearity of detection for each aroma compound with the selected fiber and trapping time was confirmed in the range of 20−200 mg kg^{-1}.

Five milliliters of solution containing the volatile compound was placed in a 10 mL headspace vial and sealed with a PTFE-faced silicone septum (Supelco). A SPME fiber was then exposed to the headspace for sampling the aroma compound. The SPME needle was pushed through the septum and the fiber exposed by depression on the plunger. After an appropriate adsorption time, the fiber was retracted into the needle and the holder was withdrawn. The needle was then inserted into the gas chromatograph, the fiber extended by depression of plunger, and chromatographic analysis started (*21*). The aroma compounds were desorbed by inserting the fiber into the GC injection port set at 220 °C for 5 min in splitless mode. The split valve was opened 1 min after injection. After desorption, the fiber was retracted and the needle was removed from the injection port. All analyses were carried out in triplicate. The fiber was baked at 220 °C for an additional 10 min to eliminate possible analyte carry-over between samples.

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 carrier gas with a linear velocity of 20.4 cm s^{-1}. The GC oven temperature program began when the fiber was injected at 70 °C and maintained for 5 min; then, the temperature was increased to 220 °C at a rate of 10 °C min^{-1} and held for 5 min, giving a total time of 25 min. The detector temperature was set at 240 °C.

Standard curves for each aroma compound were made at the proper range by injecting known quantities of aroma compound and by headspace analysis using a gastight syringe in order to calculate the linear response of the GC and the quantity present in the headspace, respectively.

Results are expressed in nanograms of compound adsorbed by the fiber. All of the experiments were done in triplicate.

Determination of Partition Coefficients. For determination of air/water partition coefficients of each compound, air phase concentrations (w/v) were divided by the concentration in the water phase (w/v). The determinations were made using 50 mM phosphate buffer, pH 6.0, as water phase and at 30 °C.

The partition coefficients of the fiber coatings were determined according to the method of Zhang and Pawliszyn (*22*): $K = (A_f V_g)/(A_g V_f)$ where A_f and A_g were the peak areas from the GC corresponding to fiber coating and direct gas injections, respectively. V_f was the volume of the coating and V_g the volume of gas sample injected. The time of exposure of the fiber coating was 30 min at 30 °C.

Volatile—Protein Binding. *Preparation of Protein Solutions.* The protein solutions were made by dissolving carnosine (4 mg mL^{-1}), anserine (1 mg mL^{-1}), and myoglobin (4 mg mL^{-1}) in 50 mM phosphate buffered at different pH 5.0, 5.5, 6.0, and 6.5.

Effect of pH on Volatile−Protein Binding. The flavor compounds were added at a concentration of 50 mg kg^{-1} into the peptide/protein solutions described above. The quantity of aroma compound was determined by a previous gas chromatography extraction using SPME under the optimized conditions for each compound. Results are expressed as a percentage of the free volatile compound found without any peptide or protein in the solution at the respective pH. All experiments were done in triplicate.

Calculation of Binding Parameters. The flavor compounds were added into the peptide/protein solutions in the linear range of each compound. The pH of the solution for each compound was selected from the higher binding determined above.

The quantity of aroma compound was determined by a previous gas chromatography extraction using SPME under the optimized conditions. Results are expressed in nanograms of compound adsorbed by the fiber. All experiments were done in triplicate.

The non-covalent and reversible binding of volatile compounds with proteins can be represented by the Scatchard equation (*23*)

$$\nu/[\mathrm{L}] = nK - \nu K$$

where ν is the number of ligand moles (volatile compound) bound per mole of protein, [L] is the molar concentration of free ligand, n is the total number of binding sites in the protein, and K is the intrinsic binding constant.

The concentration of free ligand [L] is calculated using the equation of O'Keefe et al. (*24*, *25*)

$$[\mathrm{L}] = (P/C) \times T$$

where P (mol/L) is the measured headspace concentration for the protein buffer solution, C (mol/L) is the measured headspace concentration in the control buffer, and T is the initial concentration added in the solution.

ν, the number of ligand moles (volatile compound) bound per mole of protein, is calculated using the equation of O'Keefe et al. (*25*)

$$\nu = \frac{\left(\frac{C-P}{C}\right) \times T}{[\text{protein}]}$$

The Scatchard equation can also be expressed in the form of a double-reciprocal equation (Klotz plot):

$$\frac{1}{\nu} = \frac{1}{n} + \frac{1}{nK[\mathrm{L}]}$$

A plot of $1/\nu$ versus $1/[\mathrm{L}]$ should give a straight line with a slope of $1/nK$ and an intercept of $1/n$ from where the binding parameters (n and K) are calculated.

The experimental determination of the equilibrium binding constant K as a function of temperature facilitates the determination of the thermodynamic parameter Gibb's free energy of binding (ΔG):

$$\Delta G = -RT \ln K$$

Statistical Analysis. The effect of pH on the interaction between peptides, protein, and volatile compounds was studied by analysis of

Table 1. Partition Coefficients for Each Volatile Compound

volatile compd	$K_{air-water}$ (30 °C)	$K_{fiber-air}$ (30 °C, 30 min)				
		CAR/PDMS	DVD/CAR/PDMS	PDMS 100 μm	PA	PDMS 7 μm
2-methylbutanal	1.31×10^{-2}	12 008	6122	2796	2560	2618
3-methylbutanal	1.08×10^{-2}	17 392	2734	458	317	105
hexanal	6.96×10^{-3}	17 233	12 322	1378	1285	282
octanal	2.67×10^{-3}	57 118	47019	28 383	21 632	5950
2-pentanone	4.73×10^{-3}	58 327	10173	511	523	
methional	1.95×10^{-5}	414 321	81 948	2821	8497	6343

Table 2. Selected Operating Conditions for Each Volatile Compound

volatile compd	SPME fiber coating	time (min)
2-methylbutanal	PDMS 100 μm	10.00
3-methylbutanal	CAR/PDMS 75 μm	0.33
hexanal	PDMS 100 μm	30.00
octanal	PDMS 7 μm	20.00
2-pentanone	CAR/PDMS 75 μm	1.00
methional	CAR/PDMS 75 μm	30.00

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

The selection of six flavor compounds to study the interactions with skeletal peptides and myoglobin was based on their presence and contribution to the flavor of a typical Spanish cured meat product such as dry-cured ham. The six compounds selected have been detected by SPME in the headspace of Spanish dry-cured ham (*26*). Hexanal, 3-methylbutanal, 2-methylbutanal, and 2-pentanone were selected because of their high proportion in the headspace of dry-cured ham (*26*, *27*), whereas octanal and methional were selected due to their odor activity in the aroma of dry-cured ham (*28*).

The rate-limiting step in SPME analysis is considered to be the diffusion of the analyte from the aqueous phase to the headspace (*31*). Therefore, the appropriate fiber coating was selected for each flavor compound (**Table 1**), and the partition coefficients were determined (**Table 2**). The values for $K_{air-water}$ were all much lower than those obtained for the $K_{fiber-air}$ for all of the compounds, including all of the fiber coatings studied. These partition coefficients can explain why methional was adsorbed by the five fiber coatings in very low amounts (**Figure 1**). Although it has a high $K_{fiber-air}$, it was the compound with the lowest $K_{air-water}$.

On the other hand, 2-methyl- and 3-methylbutanal are compounds with high $K_{air-water}$ and moderate $K_{fiber-air}$ (**Table 1**). These compounds are present at high concentrations in the headspace and, to avoid a saturation of the fiber, the conditions chosen for the analysis consisted of the use of CAR/PDMS fiber with a short sampling time for 3-methylbutanal. In the case of 2-methylbutanal, the PDMS 100 μm fiber was selected because of its low $K_{fiber-air}$ (see **Table 1**).

There are few studies dealing with partition coefficients between fiber coatings and headspace. The value of $K_{fiber-air}$ obtained for 3-methylbutanal with PDMS 100 μm fiber coating was similar to the one obtained by Roberts et al. (*32*). However, the value of $K_{fiber-air}$ obtained for hexanal with PDMS 100 μm fiber coating was lower than the value reported by Song et al. (*33*).

Optimization of SPME for the Study of Volatile—Protein Binding. The optimization of appropriate conditions for SPME sampling depends on the compounds to be analyzed. In this case, it is necessary to select an appropriate fiber coating and optimize the necessary time for trapping the volatile compound and control the response of the fiber to different concentrations (linearity of detection) (*29*). The optimization process was done on the six selected flavor compounds. The first step in the selection of the appropriate fiber consisted of the study of the different affinities of the coatings to the six flavor compounds as shown in **Figure 1**. The CAR/PDMS coating was the fiber that presented the highest affinity for all of the selected compounds followed by the DVB/CAR/PDMS coating. The PDMS 100 μm, PA 85 μm, and PDMS 7 μm coatings resulted in lower affinities for the studied compounds. The higher affinities of these bipolar coatings have been previously reported (*30*).

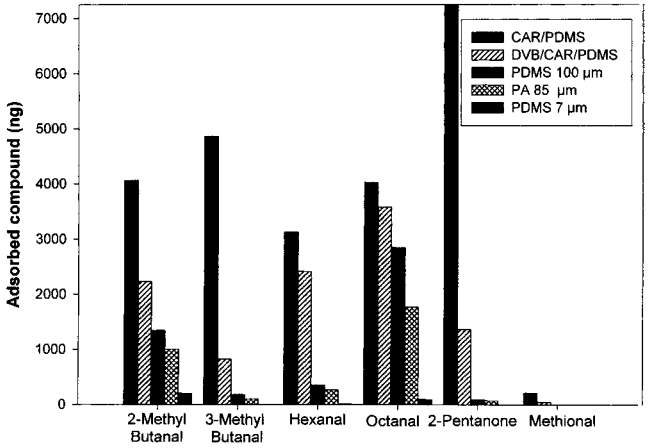

Figure 1. Affinity of the different fiber coatings. Results are expressed as nanograms of compound adsorbed by the fiber coating after 30 min of exposure in the headspace of a solution containing 50 ppm of volatile compound in 50 mM phosphate buffer, pH 6.0.

The selected operation conditions (fiber coating and sampling time) for each volatile compound are described in **Table 2**. The sampling time selected for each compound and its fiber coating was determined by analyzing the adsorbed compound during sampling time (**Figure 2**). The amount of analyte removed by the fiber is proportional to the concentration of the compound in the sample when the fiber and the sample reach equilibrium or before equilibrium but as long as the sampling parameters are carefully controlled (*31*). In this case, the selected sampling time was before reaching equilibrium in all cases except for hexanal, for which the selected time was at equilibrium. Therefore, these selected conditions have to be carefully controlled to allow correct quantification of the compounds. Furthermore, the temperature effect on equilibrium is not reported because all of the experiments were done at a fixed temperature of 30 °C in order to avoid variations in the concentration.

The linearity of detection of each aroma compound using the selected operation conditions reported in **Table 2** can be

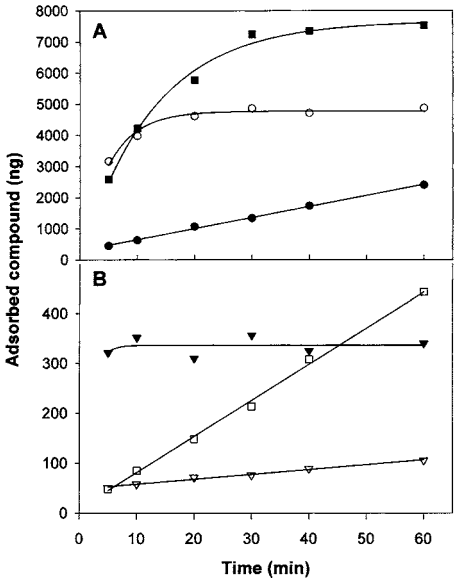

Figure 2. Effect of sampling time on compound uptake by the selected SPME fiber coating. Results are expressed as nanograms of compound adsorbed by the fiber coating after exposure for different times in the headspace of a solution containing 50 ppm of volatile compound in 50 mM phosphate buffer, pH 6.0: (A) 2-methylbutanal to PDMS 100 μm (●), 3-methylbutanal to CAR/PDMS 75 μm (○), 2-pentanone to CAR/PDMS 75 μm (■); (B) hexanal to PDMS 100 μm (▼); octanal to PDMS 7 μm (▽); methional to CAR/PDMS 75 μm (□).

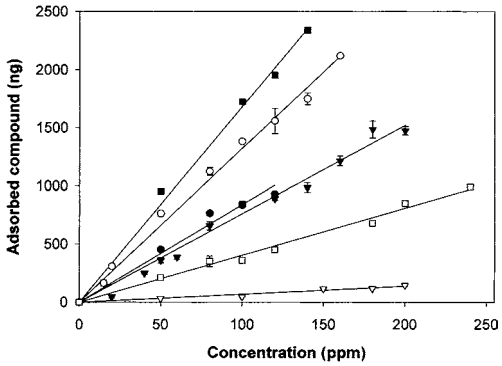

Figure 3. Linearity of detection of each aroma compound: 2-methylbutanal (●); 3-methylbutanal (○); 2-pentanone (■); hexanal (▼); octanal (▽); methional (□). Results expressed as nanograms of compound adsorbed by the fiber coating under the previously optimized selected conditions (see **Table 1**). Verticals bars represent means of three replicates ± standard error of means.

observed in **Figure 3**. The SPME technique can be applied, under the selected conditions, to quantify changes in volatile concentrations ranging from 20 to 150 mg kg^{-1} for the six studied volatile compounds.

Interaction of Volatile Compounds with Skeletal Peptides and Myoglobin. In the case of meat proteins, all of the changes that occur 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 and, finally, these interactions will be responsible for different sensory perceptions.

Due to the absence of previous studies on interactions between muscle peptides and proteins with flavor compounds (*11*), two skeletal muscle dipeptides, carnosine and anserine, which are not degraded during the processing of meat products such as dry-cured ham and dry-cured loin (*34*, *35*), were selected. In addition, a sarcoplasmic protein, myoglobin, was selected due

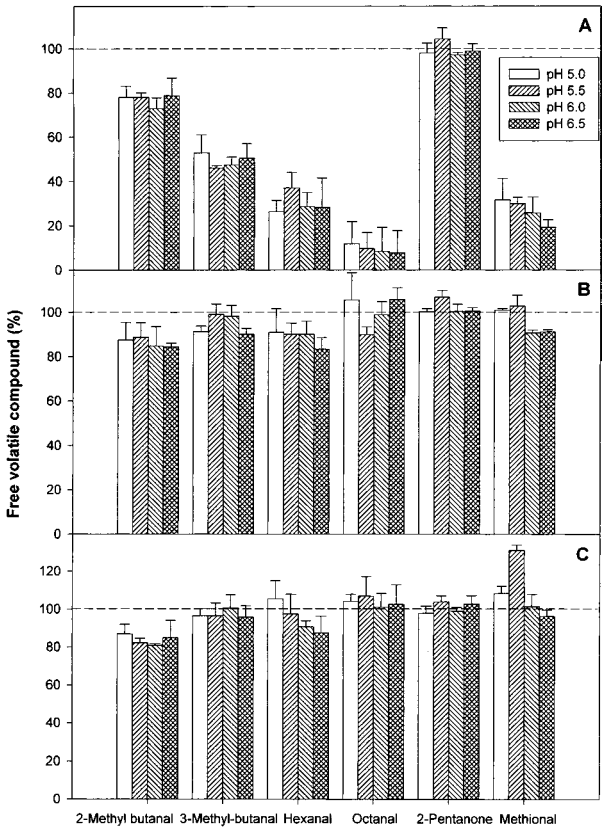

Figure 4. Effect of pH on volatile–peptide/protein binding: (A) carnosine; (B) anserine; (C) myoglobin. Results are expressed as a percentage of the free volatile compound found without any peptide or protein in the solution at the respective pH.

to its importance in color formation. We studied the effect of pH as a factor that can modify the protein conformation and thus exert a considerable influence on the binding of volatile compounds.

The interaction of carnosine with the studied volatile compounds was remarkable except for 2-pentanone, which did not present interaction (reduction of the percentage of free volatile compounds) at any of the studied pH values (**Figure 4A**). The other flavor compounds reacted with carnosine, particularly octanal. The free hexanal in the presence of carnosine was significantly ($p < 0.05$) lower at pH 5.0, 6.0, and 6.5 than at pH 5.5. In the case of methional, the free volatile compound was significantly ($p < 0.05$) lower at pH 6.0 and 6.5 than at pH 5.0 and 5.5. A low free volatile compound in solution means that at this pH exists a higher interaction with carnosine. Therefore, in the cases of hexanal and methional the interaction with carnosine was increased at higher pH values.

On the other hand, anserine showed interactions with 2-methylbutanal, 3-methylbutanal, hexanal, and methional (**Figure 4B**), although these interactions were low because a reduction of only ~10−20% of the free volatile compound present in the headspace was detected. The free methional in the presence of anserine was significantly lower ($p < 0.05$) at pH 6.0 and 6.5 than at pH 5.0 and 5.5. In the case of 3-methylbutanal, the free volatile compound was lower at pH 5.0 and 6.5 than at pH 5.5 and 6.0.

The structure of amino acids and peptides is important in their binding potential for volatile compounds. Zhou and Decker (*13*, *14*) have reported the interaction of a variety of amino acids and peptides present in the skeletal muscle with aldehydic lipid oxidation products. They suggested that the imidazole nitrogen

group, and not the β-amino group, is the major reaction site in histidine-containing dipeptides. However, our results indicate a different interaction behavior between carnosine and anserine. These differences might be attributed to the presence of a methyl group in the ring of anserine.

The interaction of myoglobin with the volatile compounds and the effect of the pH on the binding is shown in **Figure 4C**. The hexanal−myoglobin binding showed a significantly lower ($p < 0.05$) free hexanal percentage at pH 6.0 and 6.5 than at pH 5.0 and 5.5. On the other hand, the interaction of 2-methylbutanal and myoglobin was not affected by pH, although it was higher than the interaction observed with hexanal.

There are no papers in the literature about myoglobin binding. However, there are studies indicating that pH may produce alterations on the binding sites of proteins induced by conformational changes such as in the case of the interaction of BSA with 2-nonanone that varied with the pH. The intrinsic binding constant increased at pH 3, 5, and 6 and decreased at pH 9 (*36*). In the case of β-lactoglobulin the retention increased from pH 3 to 9, whereas it decreased at pH 11 (*37*). Adams et al. (*10*) reported a stronger interaction between ovalbumin and disulfide compounds (diethyl, diallyl, dipropyl, dibutyryl, and 2-furfuryl methyl sulfides) with the increase in pH. They indicated that when the pH increased from 6.7 to 8.0, the amount of disulfides in the headspace of native and heat-denatured ovalbumin was decreased.

For binding studies, we studied only those cases in which the interaction caused a reduction of >20% of the free volatile compound found without peptide or protein in the solution and the pH selected was that of the highest interaction (**Figure 4**). The binding curves obtained at 30 °C for carnosine, anserine, and myoglobin with the selected volatile compounds are presented in **Figure 5**.

Klotz (*38*) indicated that in order to be able to calculate the total number of receptor sites, it is necessary that a graph showing the moles bound plotted against the concentration of free ligand on a logarithmic scale have the following characteristics: (1) an inflection point appears at half-maximum binding; (2) the S-shaped curve is symmetric about the inflection point; and (3) a plateau is reached as the concentration of free ligand approaches very large values. In our experiments, all of the binding curves (**Figure 5**) showed the behavior proposed by Klotz (*38*) and the binding parameters, binding sites (n) and affinity constants (K), were obtained from the double-reciprocal plot of these data and presented in **Table 3**. Also, O'Keefe et al. (*25*) indicated that the binding curves (on a linear scale) have to show a hyperbolic pattern because a sigmoidal pattern would indicate the presence of cooperation in the binding sites. We represented the data in linear scale (data not shown), and all of the curves were hyperbolic and also the double-recripocal plots were linear, indicating the absence of cooperativity.

The numbers of binding sites n obtained (**Table 3**) were very low for carnosine and anserine, probably due to the dipeptide structure, meaning that there is only one binding site for each dipeptide. Myoglobin, however, showed three binding sites for 2-methylbutanal.

The obtained affinity constants K (**Table 3**) indicated that carnosine affinity was higher for the two linear aldehydes, hexanal and octanal, and for 3-methylbutanal. However, carnosine presented a very low affinity for 2-methylbutanal. These results indicate that the structure of 2-methylbutanal produces a steric effect resulting in less affinity for carnosine binding. Anserine showed a higher affinity constant for 2-methylbutanal

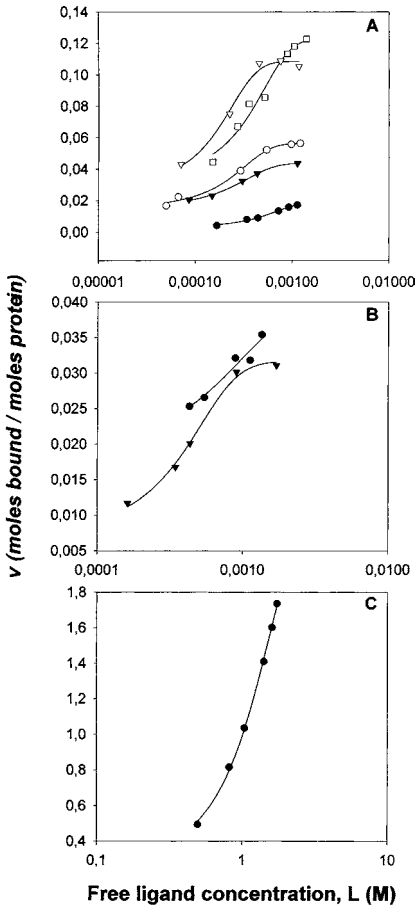

Figure 5. Number of ligand moles bound per mole of protein (ν) against the molar concentration of free ligand [L]. Binding isotherms of (A) carnosine to 2-methylbutanal (●), 3-methylbutanal (○), hexanal (▼), octanal (▽), and methional (□); (B) anserine to 2-methyl-butanal (●) and hexanal (▼); and (C) myoglobin to 2-methylbutanal (●).

Table 3. Binding Parameters of Reversible Interactions

			binding parameters		
protein	volatile compd	pH	sites (n)	K (M^{-1})	ΔG (kJ/mol)
carnosine	2-methylbutanal	6.5	0.036	831	–16.9
	3-methylbutanal	6.5	0.062	7680	–22.5
	hexanal	6.5	0.045	8928	–22.9
	octanal	6.5	0.126	7215	–22.4
	methional	6.5	0.159	2620	–19.8
anserine	2-methylbutanal	6.0	0.041	3535	–20.6
	hexanal	6.5	0.038	2658	–19.9
myoglobin	2-methylbutanal	6.0	3	1083	–17.6

than for hexanal, whereas myoglobin showed a medium affinity constant for 2-methylbutanal.

The free energy of binding (ΔG) values obtained in these experiments were similar for all of the compounds studied (**Table 3**). The functional groups are important in binding, and several authors have used the thermodynamic data to postulate types of bonds (*6*). These authors indicated that the increase in free energy (ΔG) of binding with chain length was consistent with hydrophobic interactions. In our experiments, this fact was not observed in the binding of hexanal and octanal.

Zhou and Decker (*14*) have reported that the interaction between hexanal and various peptides showed that the increase in size of alkyl groups increases hexanal binding (e.g., Leu-His > Val-His > Ala-His > Gly-His). The peptide Ile-His had a lower binding activity than Leu-His, indicating that the

branched methyl group at the first carbon of the side chain has less impact on hexanal binding than the branched methyl group at the second carbon of the side chain (*14*). In our experiments, when the interactions of hexanal with carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-L-1-methylhistidine) were compared, a higher affinity by carnosine than for anserine was observed (**Table 3**), indicating that the methyl group in anserine makes the amino group unreactive. However, the opposite behavior was observed for the interaction of 2-methylbutanal with carnosine and anserine as a higher binding affinity (*K*) was obtained in the case of anserine with 2-methylbutanal binding.

In summary, the retention of flavor compounds by skeletal peptides, carnosine and anserine, and the sarcoplasmic protein, myoglobin, was determined by their volatility measured using SPME analysis. The results showed that carnosine was the peptide with the higher affinity for volatile compounds. However, the myoglobin showed a higher number of binding sites than the dipeptides. These results indicate different aroma retentions by skeletal dipeptides and myoglobin that can result in different sensory perceptions of muscle foods such as dry-cured products.

ABBREVIATIONS USED

SPME, solid phase microextraction; GC, gas chromatograph; PDMS, polydimethylsiloxane; CAR, carboxen; PA, polyacrylate; DVB, divinylbenzene.

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