Effect of pork meat proteins on the binding of volatile compounds

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Abstract

The binding ability of pork meat proteins (sarcoplasmic, myofibrillar and isolated actin and actomyosin) was determined by measuring the relative headspace concentration of the volatile compounds in the presence of each protein (expressed as percentages of the free volatiles relative to a standard solution without protein) using solid-phase microextraction (SPME) and gas chromatography analysis. The sarcoplasmic homogenates bound higher quantities of the volatile compounds assayed (3-methylbutanal, 2-methylbutanal, 2-pentanone, hexanal, methional and octanal) than myofibrillar homogenates. The addition of salts also affected the binding ability of sarcoplasmic and myofibrillar proteins. Actomyosin was able to bind all the assayed volatile compounds although the binding depended on protein concentration and conformation, and it was highly affected by frozen storage. On the other hand, G-actin was unable to bind any of the assayed volatile compounds although the polymerized form (F-actin) bound higher quantities of the volatile compounds.
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Keywords: Meat proteins; Flavour; Aroma; Sarcoplasmic proteins; Myofibrillar proteins; Actin; Actomyosin; Salts; Binding; Interaction

1. Introduction

There is an increasing interest on a better comprehension of the interactions between flavour compounds and non-volatile food components. Furthermore, the concentration of free volatile compounds in the gas phase depends on factors such as their physicochemical properties and their interactions with other food constituents (Landy, Courthaudon, Dubois, & Voilley, 1996).

Sarcoplasmic and myofibrillar proteins constitute the main proteins present in skeletal muscle (Pearson & Young, 1989). The sarcoplasmic proteins form a mixture of proteins that are soluble in water or in dilute salt solutions whereas the myofibrillar ones are soluble in high salt concentration solutions (Syrovy, 1984). Moreover, myosin and actin constitute the two major myofibrillar proteins in meat. Myosin (43% of the total myofibrillar meat proteins) is the major component of thick filaments and actin (20% of the total) is the second most abundant and it exists in two forms: G-actin (globular) and F-actin (fibrous). These myofibrillar proteins are not only important in muscle due to their role on contraction (Pearson & Young, 1989) but also for their importance on the functional properties in meat products such as water-holding, emulsifying capacity, binding ability and gelation (Asghar, Samejima, & Yasui, 1985).

The main influence of proteins on flavour release and perception is caused by the interaction of the flavour components with proteins that changes the headspace concentration. This interaction is affected by several factors such as protein nature (Adams, Mottram, Parker, & Brown, 2001; Damodaran & Kinsella, 1981a; Jouenne & Crouzet, 2000), volatile compound nature (Damodaran & Kinsella, 1981b, 1983; Zhou & Decker, 1999), ionic strength of the medium (Damodaran & Kinsella, 1983; Guichard, 2002), protein concentration (Pérez-Juan, Flores, & Toldrá, 2007a), temperature (Damodaran & Kinsella, 1981b; Fischer & Widder, 1997; Leland, 1997) and pH (Adams et al., 2001; Damodaran & Kinsella, 1981a; Jouenne & Crouzet, 2000; Lubbers, Landy, & Voilley, 1998; Oneill & Kinsella, 1987).

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The comprehension of all the factors that affect the binding between proteins and aroma compounds is important for flavour modulation and to improve the sensory properties of meat products. In this sense, sodium chloride is an essential ingredient in the manufacture of cured meat products where it has three major functions: preservation, flavour contribution and dietary sodium source (Ruusunen & Puolanne, 2005). However, in order to obtain healthier products there is a trend to reduce salt content in the traditional cured meat products by means of the substitution with other salt types (Flores, Nieto, Ferrer, & Flores, 2005; Gimeno, Astiasaran, & Bello, 1998, 1999, 2001; Ibañez, Quintanilla, Cid, Astiasaran, & Bello, 1996; Ibañez et al., 1995).

Recent studies in meat products showed the interaction between volatile compounds and peptides and protein homogenates from skeletal muscle (Gianelli et al., 2003, 2005; Pérez-Juan, Flores, & Toldrá, 2006a, 2007a, 2007b; Zhou and Decker, 1999). However, there are few studies about the effect of muscle structure on the interaction with aroma compounds. Therefore, this work reports the effect of the main meat proteins on the binding of selected key aroma compounds to determine the relative impact of the muscle components on flavour release. Furthermore, the effect of different factors such as, protein concentration, conformation and different salts on the binding are reported.

2. Materials and methods

2.1. Materials

The aroma compounds, 2-methylbutanal, 3-methylbutanal, 2-pentanone, hexanal, methional and octanal, were obtained from Fluka Chemie (Buchs, Switzerland).

Post-rigor porcine muscle (*Longissimus dorsi*) was acquired from a local supermarket. Fat and connective tissue were removed and the meat was cut in 100 g portions, packaged in vacuum bags and stored at −20 °C.

2.1.1. Preparation of sarcoplasmic and myofibrillar homogenates

Sarcoplasmic and myofibrillar proteins were obtained according to the procedure described by Molina and Toldrá (1992). The sarcoplasmic proteins were obtained as a supernatant in 30 mM sodium phosphate buffer at pH 7.4 with 0.02% NaN_3 whereas the myofibrillar proteins were suspended in 100 mM sodium phosphate buffer at pH 7.4 containing 0.7 M potassium iodide and 0.02% NaN_3.

The protein concentration of sarcoplasmic and myofibrillar homogenates were determined according to the Bicinchoninic acid method (Smith et al., 1985) using bovine serum albumin as standard.

2.2. Effect of salt ionic strength

The effect of the ionic strength of different salts on the binding of volatile compounds was studied by the solubili-zation of each individual salt (1 M NaCl, 0.6 M KCl, 0.2 M $MgCl_2$ and 0.1 M $CaCl_2$) in the sarcoplasmic or myofibrillar homogenate that contained a protein concentration of 6.0 mg/ml (Pérez-Juan et al., 2007b).

2.3. Preparation of actomyosin and actin

The major myofibrillar proteins, actomyosin and actin, were isolated using the simultaneous process described by Pérez-Juan, Flores, and Toldrá (2007c). Actomyosin was isolated from porcine post-rigor muscle and suspended in 20 mM potassium phosphate buffer at pH 7.0 and 0.5 M NaCl, and used for the freshly actomyosin binding studies. For the frozen studies, the actomyosin pellet obtained as indicated (Pérez-Juan et al., 2007c) was stored at −20 °C in the presence of 25% glycerol. Previous to the binding study, the glycerol was removed by centrifugation at 11,700 g for 30 min (Doerscher, Briggs, & Lonergan, 2004) and the pellet was diluted in the same buffer as that used for the fresh actomyosin.

G-actin was obtained in buffer A containing 2 mM Tris–HCl at pH 8.0 and 0.2 mM $ATPNa_2$, 0.5 mM β-mercaptoethanol, 0.2 mM $CaCl_2$ and 0.005% NaN_3.

The protein concentration of isolated actomyosin and actin was determined according to the Bicinchoninic acid method (Smith et al., 1985) using bovine serum albumin as standard.

2.4. Effect of protein concentration

Frozen actomyosin, processed to eliminate glycerol as described previously, was diluted using 20 mM potassium phosphate buffer at pH 7.0 with 0.5 M NaCl to a final protein concentration of 0.8, 1.6, 3.3, 4.9 and 8.2 mg/ml. G-actin, obtained as previously described, was diluted using the buffer A without $ATPNa_2$ to a final protein concentration of 0.01, 0.1, 0.3, 0.5 and 0.8 mg/ml.

2.5. Effect of protein conformation

The effect of actomyosin conformation on the binding was studied by comparing freshly prepared actomyosin to the actomyosin samples stored at −20 °C during one week in the presence of 25% glycerol. The actomyosin concentration used was 8 mg/ml.

The effect of actin conformation on the binding was assayed using G- and F-actin at a concentration of 0.8 mg/ml. F-actin was prepared by adding to the isolated G-actin a solution containing 50 mM KCl, 2 mM $MgCl_2$ and 1 mM $ATPNa_2$ (Pardee & Spudich, 1982).

2.6. Preparation of volatile compound solution

A stock solution containing 50,000 mg/kg of each volatile compound was prepared in ethanol. The aroma compounds were added to the protein homogenates and control solutions resulting in a final concentration of

2 mg/kg for 2-methylbutanal and 3-methylbutanal; 1 mg/kg for 2-pentanone, hexanal and octanal and 5 mg/kg for methional. All the volatile compounds were simultaneously present in the solution used for the experiments. All the assays were done per triplicate.

The selection of the six flavour compounds was based on their presence in the headspace and the contribution to the flavour of typical Spanish dry-cured meat products (Carrapiso, Jurado, Timon, & Garcia, 2002; Flores, Grimm, Toldrá, & Spanier, 1997; Gianelli, Flores, & Toldrá, 2002). The concentrations used were those present in the headspace of dry-cured ham. They were approximately calculated using the partition coefficients (air–water and fiber–air) calculated for each volatile compound (Table 1) (Gianelli et al., 2003) and through the areas obtained under the same conditions (fiber, etc.) in the headspace of dry-cured ham (Gianelli et al., 2002), although for methional it was increased due to its low presence in the headspace that made unable its analysis by FID detector.

2.7. Volatile-protein binding

The flavour compounds were added in appropriate concentrations, as mentioned above, to the protein solutions. The sarcoplasmic and myofibrillar homogenates and isolated actomyosin and actin were used as protein solutions. Protein and control vials were prepared for the binding studies. The protein vials contained 5 ml of the homogenate or isolated protein placed in a 10 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA, USA). The control vial contained the same buffer as the homogenate or isolated protein but without protein. Control and protein vials were stored during 16 h at 30 °C to allow the equilibration.

The quantity (expressed as percentages of the free volatiles relative to a standard solution without protein) of aroma compound present on the headspace of protein and control vials was determined using solid-phase microextraction (SPME) and gas chromatography analysis using optimized conditions (Gianelli et al., 2005). A 75 μm carboxen/poly(dimethylsiloxane) (CAR/PDMS) fiber (Supelco) was exposed to the headspace for sampling

the aroma compounds. After 30 min of adsorption, the aroma compounds were desorbed by inserting the fibre 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 fibre was heated 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.

2.7.1. Gas chromatography analysis

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

The results are expressed as a percentage of the free volatile compound relative to a standard solution without protein. All the experiments were performed in triplicate and the values represented as the means and coefficients of variance.

2.7.2. Statistical analysis

The effect of the homogenate type used (sarcoplasmic or myofibrillar), the addition of different salts and the protein concentration and conformation on the binding of volatile compounds was studied by analysis of variance (ANOVA) using Statgraphics plus v. 5.1. The means were compared using Fisher's least significance difference (LSD) procedure ($p < 0.05$).

3. Results and discussion

All the changes occurring on meat proteins during processing are very important because they will affect not only the texture but also the interactions between the generated flavour compounds and the proteins and peptides and, finally, these interactions will be responsible for different sensory perceptions (Toldrá & Flores, 1998).

3.1. Binding of volatile compounds by sarcoplasmic and myofibrillar homogenates

During the processing of dry-cured meat products, the myofibrillar fraction undergoes a more intense proteolysis than the sarcoplasmic fraction, giving rise to a large number of low molecular weight peptides that contribute to the final flavour of dry-cured ham (Toldrá & Flores, 1998).

With reference to the interaction, the free percentage of volatile compounds in the headspace in the presence of myofibrillar and sarcoplasmic homogenates are shown in

Table 1
Chemical and physicochemical properties of the analysed volatile compounds

Compound	MW	Boiling point (°C)[a]	$K_{(CAR/PDMS\ 75\,\mu m)/air}$ (30 °C, 30 min)[b]	$K_{air/water}$ (30 °C)[b]
2-Methylbutanal	86.13	93	12,008	1.31×10^{-2}
3-Methylbutanal	86.13	93	17,392	1.08×10^{-2}
Hexanal	100.16	131	17,233	6.96×10^{-3}
Octanal	128.22	171	57,118	2.67×10^{-3}
2-Pentanone	86.13	102	58,327	4.73×10^{-3}
Methional	104.17	165–166	414,321	1.95×10^{-5}

[a] Obtained from Burdock, G.A. (2002). *Fenaroli's handbook of flavour ingredients* (4th ed.). Boca Raton, FL: CRC Press, Inc.
[b] Obtained from Gianelli et al. (2003).

Fig. 1. The presence of sarcoplasmic proteins produced a significant ($p < 0.05$) decrease in the free percentage of 3-methylbutanal, 2-methylbutanal, hexanal, methional and octanal. This decrease means that the sarcoplasmic protein homogenate bind these volatile compounds. However, the opposite effect was observed for 2-pentanone where a significant increase ($p < 0.05$) in the free percentage was detected. On the other hand, the presence of the myofibrillar homogenate only produce a significant decrease ($p < 0.05$) in the case of octanal.

Many different proteins can be responsible of the binding because the sarcoplasmic homogenate includes glycolitic enzymes, myoglobin and also small peptides, amino acids and salts as previously reported (Pérez-Juan, Flores, & Toldrá, 2006b). The binding capacity of peptides has been reported by Gianelli et al. (2003) who showed the ability of carnosine to bind the same volatile compounds except 2-pentanone while other peptides such as anserine and the protein myoglobin were able to bind hexanal and 2-methylbutanal. Therefore, these compounds can be responsible of the binding observed in Fig. 1 together with other components present in the homogenate.

Few studies have shown the ability of myofibrillar proteins to bind volatile compounds. Goodridge, Beaudry, Pestka, and Smith (2003) found that freeze-dried chicken myofibrils act as a significant reservoir for hexanal. However, in Fig. 1 only a small binding ability of the myofibrillar proteins was observed for octanal. Probably, other factors such as ionic strength or protein conformation affects this binding.

In this sense, the effect of different salts NaCl, KCl, $MgCl_2$, $CaCl_2$, and their concentration on the binding with sarcoplasmic proteins was studied by Pérez-Juan et al. (2006a). It is not only important to determine the effect of these salts on the headspace concentration of the volatile compounds but also to determine their effect in the presence of proteins. In the presence of myofibrillar and sarco-plasmic homogenates the addition of different salts produced different results on the binding of volatile compounds that not only depended on the volatile compound but also on the type of protein and salt (Fig. 2).

Sarcoplasmic proteins are able to bind volatile compounds as previously observed (Pérez-Juan et al., 2006a). The addition of NaCl to a solution of sarcoplasmic proteins produced a significant increase of the free percentage of all the volatiles assayed (Fig. 2a). However, the addition of KCl only increases the percentage of 3-methylbutanal (Fig. 2a). That means that the addition of these salts in a sarcoplasmic solution decreases the binding ability of the sarcoplasmic homogenate although the effect was higher in the presence of NaCl. On the other hand, the addition of $CaCl_2$ and $MgCl_2$ did not affect the binding of this volatile compounds.

The addition of different salts to a myofibrillar homogenate produced different results (Fig. 2b). The addition of NaCl and KCl produced only few effects on the binding. NaCl produced the release of octanal while KCl produce a retention of hexanal. However, the biggest changes were observed in the presence of $CaCl_2$ and $MgCl_2$ that produced the release of 3-methylbutanal, 2-methylbutanal, hexanal and methional from the myofibrillar homogenate.

The salting out observed was expected because salts are often added to aqueous samples to increase the concentration of the aroma compounds in the vapour phase (Guichard, 2002). Similar results were reported by Guichard and Langourieux (2000) who observed a decrease of retention of benzaldehyde by β-lactoglobulin in the presence of NaCl that was attributed to the salting out effect or to the modification of the protein surface polarity by the presence of salt which affected its binding ability.

In our study, the salting out effect was observed in all the volatile compounds assayed except for 2-pentanone although the effect depends on the type of salt and type of homogenate present in the solution. The different solubility
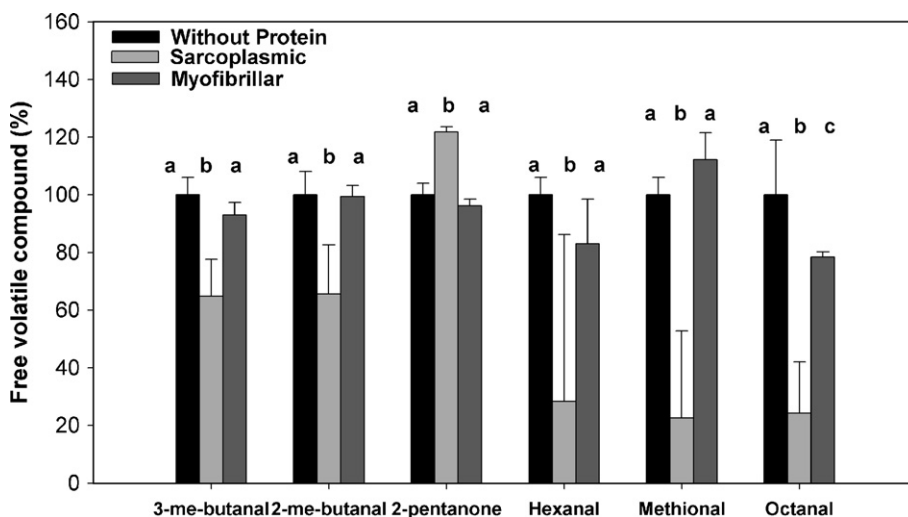

Fig. 1. Effect of sarcoplasmic and myofibrillar homogenates on the binding of volatile compounds. Results are expressed as the percentage of the free volatile compound found without protein in the solution.
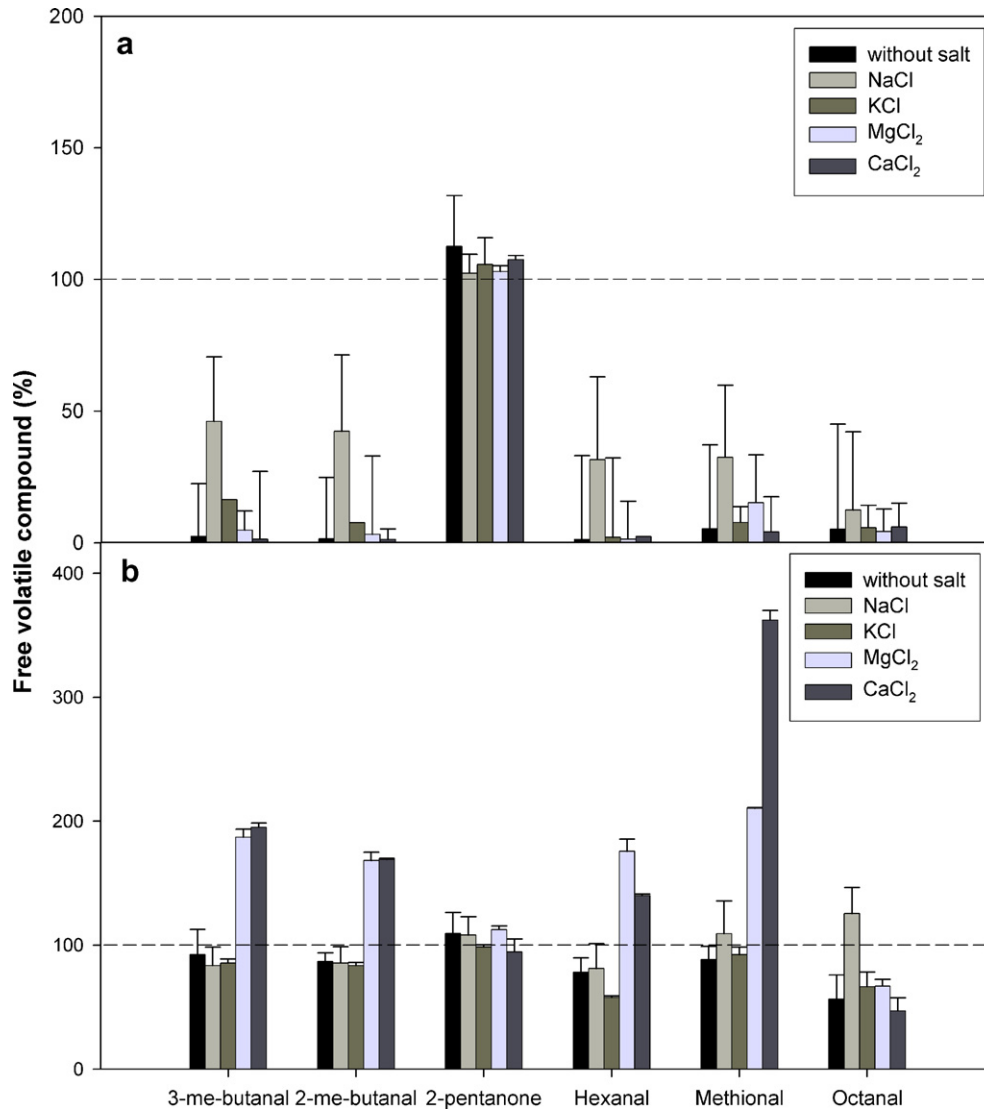
Fig. 2. Effect of different salts on the binding of the volatile compounds: (a) sarcoplasmic homogenates and (b) myofibrillar homogenates. Results are expressed as a percentage of the free volatile compound found without protein in solution.

of sarcoplasmic and myofibrillar proteins in salt solutions could explain their different binding ability but the presence of dichloride salts affect their cuaternary structure. The reason of the release to the headspace of volatile compounds in the presence of myofibrillar proteins and dichloride salts could be due to a myofibrillar denaturation.

3.2. Binding of volatile compounds by isolated actomyosin and actin proteins

The main myofibrillar proteins (myosin and actin) were purified from post-rigor porcine skeletal muscle obtaining a purification grade of 80% for actomyosin and 90% for G-actin, using silver stained SDS–PAGE (Pérez-Juan et al., 2007c). These isolated proteins were used to study their binding ability and the factors affecting it.

The effect of protein concentration on the interaction with volatile compounds was studied and shown in

Fig. 3. The free percentage of each volatile compound in the headspace in the presence of different concentrations of actomyosin (0.8–8.2 mg/ml) and G-actin (0.01–0.8 mg/ml) was shown (Fig. 3). Actomyosin retained hexanal and octanal as seen by the significant ($p < 0.05$) reduction of the free percentages. On the other hand, actomyosin was not able to retain the other compounds. Furthermore, the increase in protein concentration produced a significant increase in the free percentages of octanal and 2-pentanone while a slight decrease ($p < 0.05$) was observed for methional at the highest concentration (Fig. 3a).

In the presence of G-actin (Fig. 3b), all the volatile compounds assayed were not bound. However, at high G-actin concentrations (0.5 and 0.8 mg/ml) a significant increase in the free percentages of 3-methylbutanal, hexanal, methional and octanal was observed indicating that these volatile compounds were released to the headspace (Fig. 3b).
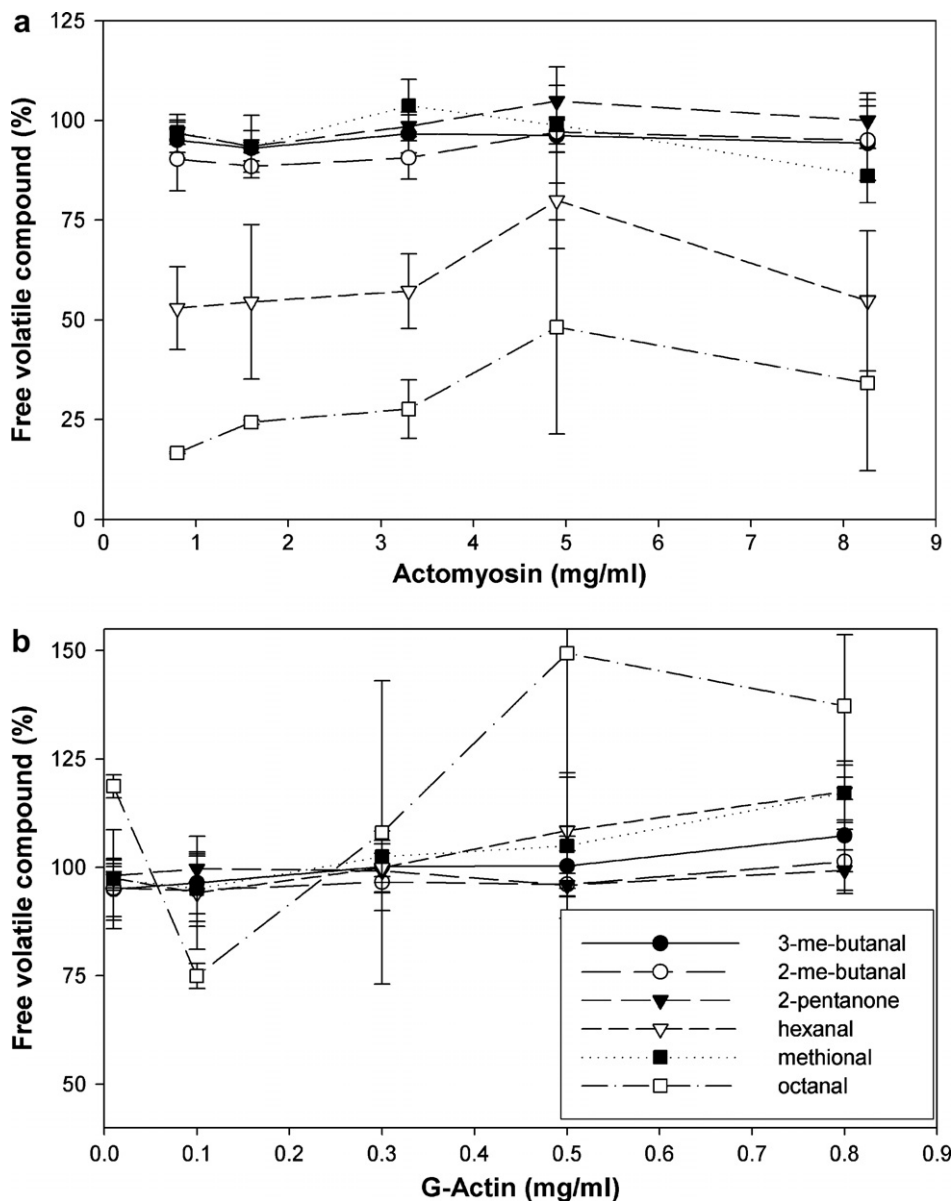
Fig. 3. Effect of protein concentration on the binding of volatile compounds: (a) actomyosin (0.8–8.2 mg/ml) and (b) G-actin (0.01–0.8 mg/ml). Results are expressed as percentage of the free volatile compound found in the headspace without protein in solution.

The release to the headspace of volatile compounds in the presence of proteins could be due to protein–protein interaction that results in an apparent decrease in the protein–ligand interaction as suggested (Damodaran & Kinsella, 1980). This fact could explain the G-actin behaviour. However, other authors have seen that the volatile retention increased with protein concentration although it depended on the volatile compound (Landy, Druaux, & Voilley, 1995).

All these studies have been done in model systems because it is very difficult to reproduce the muscle structure. However, the conformation of proteins should not be ignored. It has been reported that a change in the protein conformation affects the interaction between volatile compounds and proteins due to the modification of the available protein binding sites (Adams et al., 2001; Dam-

odaran & Kinsella, 1981a, 1981b; Jouenne & Crouzet, 2000).

In the case of pork muscle, the proteins are responsible for the maintenance of the myofibrillar structure. Therefore, different myofibrillar conformations were studied in the binding of volatile compounds (Pérez-Juan et al., 2007a). Freshly prepared actomyosin, frozen actomyosin, G-actin and F-actin were studied to see its binding ability (Fig. 4). The presence of fresh actomyosin produced a significant reduction ($p < 0.05$) of the free percentages of hexanal, methional and octanal meaning that fresh actomyosin was able to bind these volatile compounds. Moreover, 3-methylbutanal and 2-methylbutanal were also bound by fresh actomyosin although in lower quantities. However, when frozen actomyosin was used less quantities of hexanal and methional were bound while no differences were
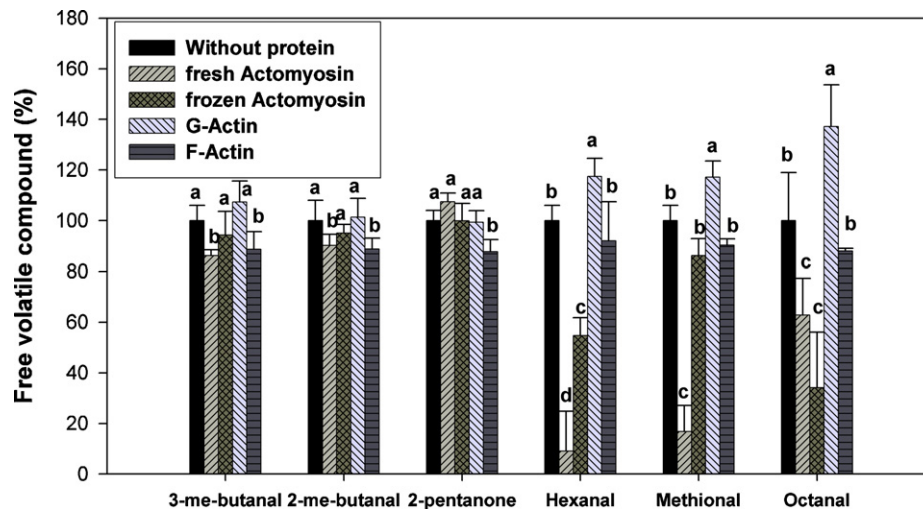
Fig. 4. Effect of protein conformation (fresh actomyosin, frozen actomyosin, G-actin and F-actin) on the binding of volatile compounds. The concentration of actomyosin and actin used was 8 mg/ml and 0.8 mg/ml, respectively. Results are expressed as a percentage of the free volatile compound found in the headspace without protein in solution.

observed in the octanal bound. The changes observed during the frozen storage of actomyosin could be due to an increase in hydrophobicity as indicated (Careche, Cofrades, Carballo, & Colmenero, 1998) during the frozen storage of pork myofibrillar extracts.

In the case of actin, the fibrous form (F-actin) produced a significant decrease of the free percentages of all the studied volatile compounds while no decrease was observed in the presence of G-actin. The change between the G-actin to F-actin involves a conformational modification before completing the polymerisation process (Pearson & Young, 1989) probably related to their sulfhydryl groups (Jiang, Wang, & Chen, 1989). This conformational change affects its binding ability even though it depends on the volatile compound studied.

In conclusion, the binding ability of the muscle proteins has been studied including the main factors that affect the binding of volatile compounds. Although it seems that sarcoplasmic proteins are the main responsible for the binding of volatile compounds also myofibrillar proteins bind the volatile compounds assayed. However, the binding ability of myofibrillar proteins is highly dependent on protein concentration and conformation. Finally, the presence of salts also affects the binding ability of sarcoplasmic and myofibrillar proteins and it is different depending on the type of salt, volatile compound and muscle homogenate. Therefore, the results presented can be very interesting in the processing of meat products because the substitution of sodium chloride during processing will affect the release of flavour compounds from the meat matrix and finally, the flavour perception.

References

Adams, R. L., Mottram, D. S., Parker, J. K., & Brown, H. M. (2001). Flavour–protein binding: disulfide interchange reactions between ovalbumin and volatile disulfides. *Journal of Agricultural and Food Chemistry, 49*, 4333–4336.

Asghar, A., Samejima, K., & Yasui, T. (1985). Functionality of muscle proteins in gelation mechanisms of structured meat products. *Critical Reviews in Food Science and Nutrition, 22*, 27–101.

Careche, M., Cofrades, S., Carballo, J., & Colmenero, F. J. (1998). Emulsifying and gelation properties during freezing and frozen storage of hake, pork, and chicken actomyosins as affected by addition of formaldehyde. *Journal of Agricultural and Food Chemistry, 46*, 813–819.

Carrapiso, A. I., Jurado, A., Timon, M. L., & Garcia, C. (2002). Odor-active compounds of Iberian hams with different aroma characteristics. *Journal of Agricultural and Food Chemistry, 50*, 6453–6458.

Damodaran, S., & Kinsella, J. E. (1980). Flavor protein interactions – binding of carbonyls to bovine serum-albumin – thermodynamic and conformational effects. *Journal of Agricultural and Food Chemistry, 28*, 567–571.

Damodaran, S., & Kinsella, J. E. (1981a). Interaction of carbonyls with soy protein – conformational effects. *Journal of Agricultural and Food Chemistry, 29*, 1253–1257.

Damodaran, S., & Kinsella, J. E. (1981b). Interaction of carbonyls with soy protein – thermodynamic effects. *Journal of Agricultural and Food Chemistry, 29*, 1249–1253.

Damodaran, S., & Kinsella, J. E. (1983). Binding of carbonyls to fish actomyosin. *Journal of Agricultural and Food Chemistry, 31*, 856–859.

Doerscher, D. R., Briggs, J. L., & Lonergan, S. M. (2004). Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. *Meat Science, 66*, 181–188.

Fischer, N., & Widder, S. (1997). How proteins influence food flavor. *Food Technology, 51*, 68–70.

Flores, M., Grimm, C. C., Toldrá, F., & Spanier, A. M. (1997). Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *Journal of Agricultural and Food Chemistry, 45*, 2178–2186.

Flores, M., Nieto, P., Ferrer, J. M., & Flores, J. (2005). Effect of calcium chloride on the volatile pattern and sensory acceptance of dry-

fermented sausages. *European Food Research and Technology, 22*, 624–630.

Gianelli, M. P., Flores, M., & Toldrá, F. (2002). Optimisation of solid phase microextraction (SPME) for the analysis of volatile compounds in dry-cured ham. *Journal of the Science of Food and Agriculture, 82*, 1703–1709.

Gianelli, M. P., Flores, M., & Toldrá, F. (2003). Interactions of soluble peptides and proteins from skeletal muscle on the release of volatile compounds. *Journal of Agricultural and Food Chemistry, 51*, 6828–6834.

Gianelli, M. P., Flores, M., & Toldrá, F. (2005). Interaction of soluble peptides and proteins from skeletal muscle with volatile compounds in model systems as affected by curing agents. *Journal of Agricultural and Food Chemistry, 53*, 1670–1677.

Gimeno, O., Astiasaran, I., & Bello, J. (1998). A mixture of potassium, magnesium, and calcium chlorides as a partial replacement of sodium chloride in dry fermented sausages. *Journal of Agricultural and Food Chemistry, 46*, 4372–4375.

Gimeno, O., Astiasaran, I., & Bello, J. (1999). Influence of partial replacement of NaCl with KCl and CaCl$_2$ oil texture and color of dry fermented sausages. *Journal of Agricultural and Food Chemistry, 47*, 873–877.

Gimeno, O., Astiasaran, I., & Bello, J. (2001). Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausages: effect on colour, texture and hygienic quality at different concentrations. *Meat Science, 57*, 23–29.

Goodridge, C. F., Beaudry, R. M., Pestka, J. J., & Smith, D. M. (2003). Solid phase microextraction–gas chromatography for quantifying headspace hexanal above freeze-dried chicken myofibrils. *Journal of Agricultural and Food Chemistry, 51*, 4185–4190.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*, 49–70.

Guichard, E., & Langourieux, S. (2000). Interactions between β-lactoglobulin and flavour compounds. *Food Chemistry, 71*, 301–308.

Ibañez, C., Quintanilla, L., Cid, C., Astiasaran, I., & Bello, J. (1996). Dry fermented sausages elaborated with *Lactobacillus plantarum-Staphylococcus carnosus*. 1. Effect of partial replacement of NaCl with KCl on the stability and the nitrosation process. *Meat Science, 44*, 227–234.

Ibañez, C., Quintanilla, L., Irigoyen, A., Garciajalon, I., Cid, C., Astiasaran, I., et al. (1995). Partial replacement of sodium-chloride with potassium-chloride in dry fermented sausages – influence on carbohydrate fermentation and the nitrosation process. *Meat Science, 40*, 45–53.

Jiang, S. T., Wang, F. J., & Chen, C. S. (1989). Properties of actin and stability of the actomyosin reconstituted from milkfish (Chanos-Chanos) actin and myosin. *Journal of Agricultural and Food Chemistry, 37*, 1232–1235.

Jouenne, E., & Crouzet, J. (2000). Effect of pH on retention of aroma compounds by beta-lactoglobulin. *Journal of Agricultural and Food Chemistry, 48*, 1273–1277.

Landy, P., Courthaudon, J. L., Dubois, C., & Voilley, A. (1996). Effect of interface in model food emulsions on the volatility of aroma compounds. *Journal of Agricultural and Food Chemistry, 44*, 526–530.

Landy, P., Druaux, C., & Voilley, A. (1995). Retention of aroma compounds by proteins in aqueous-solution. *Food Chemistry, 54*, 387–392.

Leland, J. V. (1997). Flavor interactions: the greater whole. *Food Technology, 51*, 75–80.

Lubbers, S., Landy, P., & Voilley, A. (1998). Retention and release of aroma compounds in foods containing proteins. *Food Technology, 52*, 68–74, 208–214.

Molina, I., & Toldrá, F. (1992). Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *Journal of Food Science, 57*, 1308–1310.

Oneill, T. E., & Kinsella, J. E. (1987). Binding of alkanone flavours to beta-lactoglobulin – effects of conformational and chemical modification. *Journal of Agricultural and Food Chemistry, 35*, 770–774.

Pardee, J. D., & Spudich, A. (1982). Purification of muscle actin. *Methods in Enzymology, 85*, 164–179.

Pearson, A. M., & Young, R. B. (1989). Muscle and meat biochemistry. In B. S. Schweigert & S. L. Taylor (Eds.), *Food science and technology. A series of monographs* (pp. 1–29). London: Academic Press, 66–94 and 98–127.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2006a). Model studies on the efficacy of protein homogenates from raw pork muscle and dry-cured ham in binding selected flavor compounds. *Journal of Agricultural and Food Chemistry, 54*, 4802–4808.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2006b). Generation of volatile flavour compounds as affected by the chemical composition of different dry-cured ham sections. *European Food Research and Technology, 222*, 658–666.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2007a). Binding of aroma compounds by isolated myofibrillar proteins: effect of protein concentration and conformation. *Food Chemistry, 105*(3), 932–939.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2007b). Effect of ionic strength of different salts on the binding of volatile compounds to sarcoplasmic proteins homogenates in model systems. *Food Research International*. doi:10.1016/j.foodres.2006.11.013.

Pérez-Juan, M., Flores, M., & Toldrá, F. (2007c). Simultaneous process to isolate actomyosin and actin from post-rigor porcine skeletal muscle. *Food Chemistry, 101*(3), 1005–1011.

Ruusunen, M., & Puolanne, E. (2005). Reducing sodium intake from meat products. *Meat Science, 70*, 531–541.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry, 150*, 76–85.

Syrovy, I. (1984). Separation of muscle proteins. *Journal of Chromatography, 300*, 225–247.

Toldrá, F., & Flores, M. (1998). The role of muscle proteases and lipases in flavour development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition, 38*, 331–352.

Zhou, S. Y., & Decker, E. A. (1999). Ability of carnosine and other skeletal muscle components to quench unsaturated aldehydic lipid oxidation products. *Journal of Agricultural and Food Chemistry, 47*, 51–55.