Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 932–939
Food Chemistry
www.elsevier.com/locate/foodchem

Binding of aroma compounds by isolated myofibrillar proteins: Effect of protein concentration and conformation

María Pérez-Juan, Mónica Flores *, Fidel Toldrá

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apartado de correos 73, 46100 Burjassot (Valencia), Spain

Received 11 October 2006; received in revised form 12 January 2007; accepted 19 April 2007

Abstract

Actomyosin and G-actin were isolated from post-rigor porcine muscle and used to study their binding ability with selected volatile aroma compounds (3-methyl-butanal, 2-methyl-butanal, 2-pentanone, hexanal, methional and octanal). The binding ability was determined by measuring the headspace concentration of the volatile compounds in the presence of each protein using solid-phase microextraction (SPME) and gas chromatography analysis. Actomyosin was able to bind to all the assayed volatile compounds, although the binding was dependent on protein concentration and conformation, and highly affected by frozen storage. On the other hand, G-actin was unable to bind any of the assayed volatile compounds and furthermore, it caused the release of several of them (3-methyl-butanal, hexanal, methional and octanal) at the highest protein concentration. However, the polymerized form (F-actin) was the isolated myofibrillar protein that was able to bind higher quantities of the assayed volatile compounds.

Keywords: Actomyosin; Actin; Myofibrillar proteins; Volatile compounds; SPME; Interaction

1. Introduction

Myosin and actin constitute the two major myofibrillar proteins in meat. Myosin (43% of the total myofibrillar meat protein) is the major component of thick filaments which contain about 300 myosin molecules per filament. Actin (20% of the total) is the second most abundant myofibrillar protein in meat myofibrils, and it exists in two forms: G-actin (globular) and F-actin (fibrous). The formation of the actomyosin complex takes place during muscle contraction. It can also be produced *in vitro*, increasing the viscosity of solution (Morrissey, Mulvihill, & O'Neill, 1987). The composition and the molecular weight of actomyosin mostly depend on the experimental conditions, such as pH, salt concentration (KCl, $MgCl_2$) or protein concentration.

It has been reported, that proteins can contribute to flavour release (Guichard, 2002) due to their ability to bind volatile compounds. Binding, being defined as a molecular bond existing between an aroma compound and a protein. This ability has been widely studied (Damodaran & Kinsella, 1980; Gianelli, Flores, & Toldrá, 2003; Jouenne & Crouzet, 2001) and several authors have even attempted to investigate it through the study of model solutions (Kinsella, 1990; Landy, Druaux, & Voilley, 1995; Okeefe, Wilson, Resurreccion, & Murphy, 1991).

However, few studies have dealt with the contribution of muscle proteins to flavour perception (Guichard, 2002; Leland, 1997). In this sense, Damodaran and Kinsella (1983) studied the binding of alkanones to fish actomyosin in order to provide relevant data to remove the off-flavours from fish protein concentrates. Recently, Gianelli et al. (2003) studied the ability of skeletal dipeptides (carnosine and anserine) and a sarcoplasmic protein (myoglobin) to interact with key flavour compounds (3-methyl-butanal, 2-methyl-butanal, 2-pentanone, hexanal, methional and octanal). These authors studied the effect of pH on the binding and calculated the thermodynamic binding parameters (n, number of binding sites; K, affinity constant) for these dipeptides and protein. Furthermore, the binding ability of sarcoplasmic and myofibrillar

* Corresponding author. Fax: +34 963636301.
E-mail address: mflores@iata.csic.es (M. Flores).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.04.051

homogenates, obtained from fresh pork muscle and dry-cured ham, was studied by Pérez-Juan, Flores, and Toldrá (2006). In this study, a higher binding ability of sarcoplasmic and myofibrillar proteins was detected when the proteins were obtained from fresh pork muscle than from dry-cured ham. These authors also reported an important effect of ionic strength on the binding ability of myofibrillar homogenates. However, nothing is known about the contribution of the individual myofibrillar proteins to the binding of flavour compounds in order to establish their possible contribution to flavour release in meat products. So, the aim of the present work was to elucidate the binding ability of the major myofibrillar proteins through the study of the binding using isolated proteins from fresh pork muscle at different concentration and conformation stages.

2. Material and methods

2.1. Materials

The aroma compounds, 2-methyl-butanal, 3-methyl-butanal, 2-pentanone, hexanal, methional and octanal, were obtained from Fluka Chemie (Buchs, Switzerland). The selection of the six aroma compounds was based on their presence in the headspace of dry-cured ham and the contribution to the flavour of typical Spanish dry-cured meat products (Pérez-Juan et al., 2006).

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

2.2. Protein isolation

The myofibrillar proteins, actomyosin and actin, were isolated using the simultaneous process described by Pérez-Juan, Flores, and Toldrá (2007). Actomyosin was isolated from porcine post-rigor muscle with 0.1 M Tris–HCl at pH 7.0 containing 20 mM EDTA in order to eliminate the sarcoplasmic proteins. Afterwards, the myofibrillar proteins were extracted with the Hasselbach–Schneider solution (0.1 M KH_2PO_4/K_2HPO_4 at pH 6.4 with 0.6 M KCl, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 1 mM $MgCl_2$ and 20 mM EGTA) and the supernatant obtained after centrifugation at 11,700*g* for 30 min in a Sorvall RC-5B was precipitated by diluting 1/20 with deionised water. This new pellet, containing actomyosin, was diluted (1:1) with 20 mM potassium phosphate buffer at pH 7.0 and 0.5 M NaCl, and used for the fresh actomyosin binding studies. For the frozen studies, the actomyosin pellet was stored at −20 °C in the presence or absence 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.

The initial pellet obtained after the extraction with Hasselbach–Schneider solution, which contained actin, was used to prepare the acetone powder. Afterwards, G-actin was extracted from the acetone powder with 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 with different extraction times, as described Pérez-Juan et al. (2007). The last fraction, enriched in G-actin, was used for actin binding studies.

The protein concentration in each fraction, actomyosin and actin, was determined according to the bicinchoninic acid method (Smith et al., 1985) using bovine serum albumin as standard.

2.3. 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 final protein concentrations of 0.01, 0.1, 0.3, 0.5 and 0.8 mg/ml.

2.4. 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 or absence of 25% glycerol. In the three cases, the protein concentration 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).

2.5. Preparation of volatile compounds solution

A stock solution containing 50,000 mg/kg of each volatile compound was prepared in ethanol. The aroma compounds were added per triplicate to the isolated protein and control solutions resulting in a final concentration of 2 mg/kg for 2-methyl-butanal and 3-methyl-butanal; 1 mg/kg for 2-pentanone, hexanal and octanal and 5 mg/kg for methional. All volatile compounds were simultaneously present in the solution, and used for the experiments.

2.6. Volatile-protein binding

The aroma compounds were added in appropriate concentrations, as mentioned above, to the protein and control solution. The protein vials contained 5 ml of the isolated proteins (actomyosin or actin) placed in a 10 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Bellefonte, PA). The control vial contained the same buffer as the actomyosin or actin vials but without protein.

Control and protein vials were stored 16 h at 30 °C to allow equilibration.

The quantity of aroma compound present in the headspace of protein and control vials, was determined using solid-phase microextraction (SPME) and gas chromatography analysis using optimised conditions (Gianelli, Flores, & Toldrá, 2005). A 75 μm carboxen/poly(dimethylsiloxane) (CAR/PDMS) fibre (Supelco, Bellefonte, PA) 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 for 5 min in the injection port at 220 °C to avoid analyte carry-over between the samples.

Gas chromatography analysis was performed with a 8000 CE instruments gas chromatograph (Rodano, Milan, Italy) equipped with a flame ionisation detector (FID). The aroma compounds were separated on a DB-624 capillary column (J&W Scientific, 60 m, 0.32 mm i.d. film thickness = 1.8 μm), using the conditions reported by Pérez-Juan et al. (2006). The linearity of detection for each aroma compound under these conditions was confirmed within the range of 0.02–5 mg/kg (Gianelli et al., 2003, 2005).

The results are expressed as a percentage of the free volatile compound found in the headspace without protein in solution. All the experiments were carried out in triplicate and the data are reported as means and coefficients of variance.

2.6.1. Volatile compound bound per weight of protein

The aroma compounds were added into the protein and control vials at the same concentrations as those described above and also diluted 100 and 500 times. The (G- and F-) actin and actomyosin concentrations used were 0.8 and 5 mg/ml, respectively. The quantity of each aroma compound was determined by the previously described gas chromatography analysis after extraction using SPME under the optimised conditions. All the experiments were carried out in triplicate.

The concentration in the headspace ([HS]) was calculated as described by Pérez-Juan et al. (2006) using the following equation:

$$[\mathrm{HS}] = \frac{A_\mathrm{F}}{m \times V_\mathrm{F} \times K} \quad (1)$$

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 fibre volume (5.3×10^{-7} l) and K is the fibre–air partition coefficient of each volatile compound for CAR/PDMS 75 μm fibre obtained from Gianelli et al. (2003).

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

$$\text{Free volatile in aqueous phase} = \frac{[\mathrm{HS}]_\mathrm{P}}{[\mathrm{HS}]_\mathrm{C}} \times O \quad (2)$$

$$\text{Bound volatile/protein} = \frac{\frac{([\mathrm{HS}]_\mathrm{C} - [\mathrm{HS}]_\mathrm{P})}{[\mathrm{HS}]_\mathrm{C}} \times O}{C_\mathrm{p}} \quad (3)$$

where $[\mathrm{HS}]_\mathrm{C}$ (mol/l) and $[\mathrm{HS}]_\mathrm{P}$ (mol/l) is the volatile compound headspace concentration in the control and protein vials, respectively, O (mol/l) is the initial concentration added to the solutions and C_p (g/l) is the protein concentration present in the protein vials.

2.7. Statistical analysis

The effect of the protein in solution 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

3.1. General

Actomyosin and G-actin samples were purified from post-rigor porcine skeletal muscle, obtaining a purification grade of 80% and 90%, respectively, as confirmed by silver stained SDS–PAGE (Pérez-Juan et al., 2007).

3.2. Effect of protein concentration

In order to study the effect of protein concentration on the interaction with volatile compounds, 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) are shown in Figs. 1a and b, respectively. One hundred percent of free volatile compound corresponds to the headspace concentration present in the control vial that does not contain protein. As shown in Fig. 1a, the presence of actomyosin produced a significant reduction of the percentages of free hexanal and octanal; this means that actomyosin was able to bind both volatile compounds. Furthermore, the increase in actomyosin concentration did not affect the percentage of free hexanal except at 4.9 mg/ml. On the other hand, the increase in protein concentration produced a significant increase in the percentages of free octanal and 2-pentanone, while a slight decrease ($p < 0.05$) was observed for methional (Fig. 1a). Nevertheless, the range of studied protein concentrations did not affect the percentages of free 3-methyl-butanal and 2-methyl-butanal (Fig. 1a).

Some of the chemical and physicochemical properties of the studied volatile compounds are shown in Table 1. The partition coefficients air/water ($K_{air/water}$) of the volatile compounds indicate the distribution of these compounds in the two phases. In this sense, methional presented the

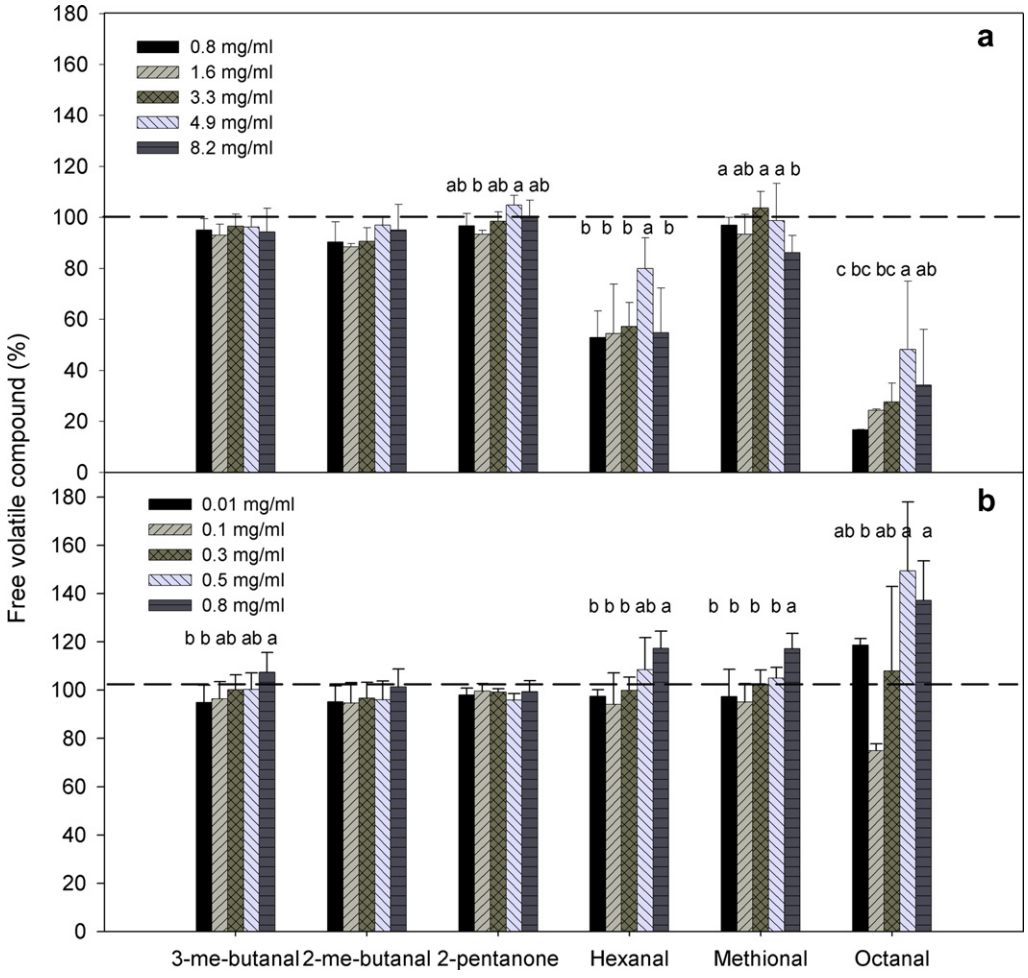

Fig. 1. Effect of protein concentration on the binding of volatile compounds: (a) actomyosin (0. 8–8.2 mg/ml) and (b) G-actin (0.01–0.8 mg/ml). Results are expressed as percentage of the free volatile compounds found in the headspace without protein in solution. Bars with different letters are significantly different ($p < 0.05$) among protein concentrations.

lowest $K_{air/water}$ meaning that is mainly present in the aqueous phase, while 2-methyl and 3-methyl-butanal presented the highest $K_{air/water}$ meaning that are highly concentrated in the vapour phase. The presence of actomyosin in solution affected mainly the compounds with the lowest $K_{air/water}$, hexanal, octanal, methional and 2-pentanone, whose concentration in the solution is higher.

In the case of the presence of G-actin (Fig. 1b), it did not significantly bind any of the assayed volatile compounds. However, at high G-actin concentrations (0.5 and 0.8 mg/ml) a significant increase in the percentages of free 3-methyl-butanal, hexanal, methional and octanal was produced, indicating that these volatile compounds were released to the headspace (Fig. 1b). On the other hand, the studied G-actin concentration did not affect the percentages of free 2-methyl-butanal and 2-pentanone. In the case of octanal, the presence of low G-actin concentration (0.1 mg/ml) produced a significant decrease which was not detected at the other concentrations.

The presence of G-actin in the solution also affected mainly the compounds with the lowest $K_{air/water}$ (see Table 1), hexanal, octanal, and methional, whose concentration in the solution is higher but in this case, the protein produced a release of these compounds to the headspace. However, 3-methyl-butanal that has a high $K_{air/water}$, was also affected by the presence of G-actin although in lower proportion.

The release of several compounds to the headspace (octanal in Fig. 1a and 3-me-butanal, hexanal, methional and octanal in Fig. 1b) at high actomyosin and G-actin concentrations could be due to the presence of protein–protein

Table 1
Chemical and physico-chemical properties of the analysed volatile compounds

Compounds	MW	Boiling point (°C)[a]	$K_{air/water}$ (30 °C)[b]
2-Methyl-butanal	86.13	93	1.31×10^{-2}
3-Methyl-butanal	86.13	93	1.08×10^{-2}
Hexanal	100.16	131	6.96×10^{-3}
Octanal	128.22	171	2.67×10^{-3}
2-Pentanone	86.13	102	4.73×10^{-3}
Methional	104.17	165–166	1.95×10^{-5}

[a] Obtained from Burdock (2002).
[b] Obtained from Gianelli et al. (2003).

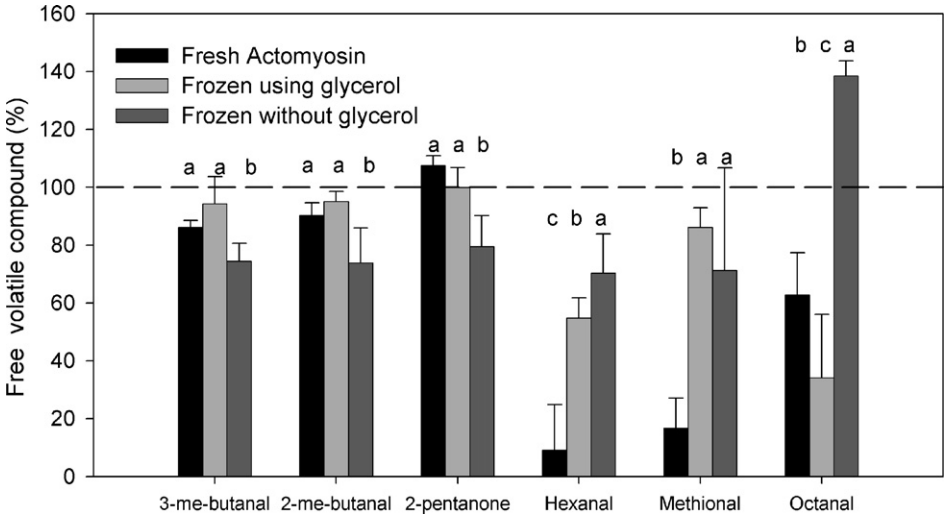

Fig. 2. Actomyosin conformation effect on the binding of volatile compounds. Conformation was studied by comparing actomyosin samples (8 mg/ml) frozen with and without glycerol to freshly prepared actomyosin. Results are expressed as a percentage of the free volatile compound found in the headspace without protein in solution. Bars with different letters are significantly different ($p < 0.05$) among protein samples.

interactions that diminished their binding ability. This fact has been reported by Damodaran and Kinsella (1980) who suggested that, in the case of carbonyls and bovine serum albumin, at high protein concentrations, protein–protein interaction exists, that results in an apparent decrease in the protein–ligand interaction. However, Landy et al. (1995) reported that the volatile retention increased with protein concentration (sodium caseinate), although it varied depending on the volatile compound.

Another possible cause of the release to the headspace could be due to the decrease of the surface tension that is produced at increasing protein concentrations, that could facilitate the partition in the vapour phase of volatiles. In this sense, O'Neill, Morrissey, and Mulvihill (1990) reported the ability of myofibrillar proteins as effective surface tensions depressors, myosin being the most effective depressor, followed by actomyosin, F-actin and G-actin.

3.3. Effect of protein conformation

Several authors have studied the effect of freezing on myofibrillar proteins (Careche, Cofrades, Carballo, & Colmenero, 1998; Cofrades, Careche, Carballo, & Colmenero, 1996) due to the impact that this treatment has on the protein conformation affecting their properties. In the present study, the effect of freezing on the structure of actomyosin was studied in relation to its binding ability. The percentage in the headspace of each volatile compound in the presence of freshly prepared actomyosin and actomyosin samples, stored at −20 °C during one week in the presence

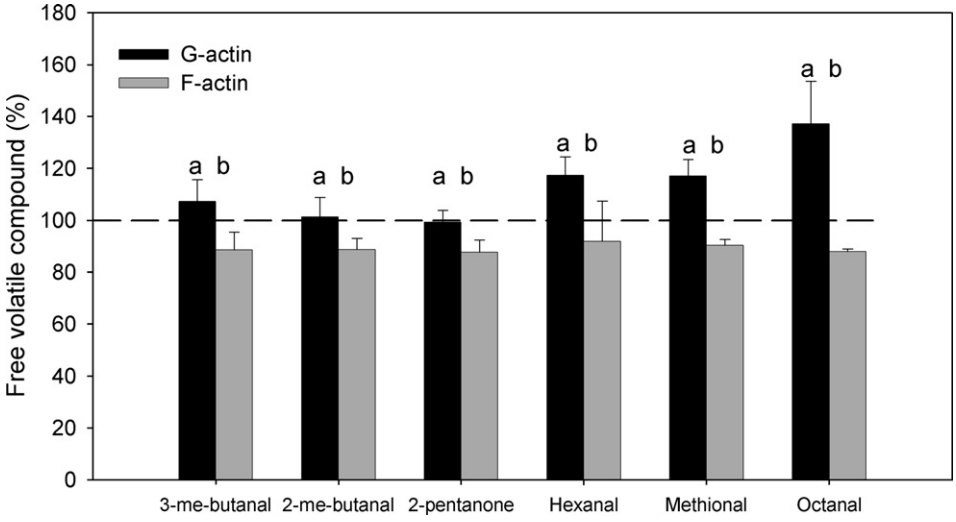

Fig. 3. Effect of actin conformation (G-actin and F-actin, both at 0.8 mg/ml) on the binding of volatile compounds. Results are expressed as a percentage of the free volatile compound found in the headspace without protein in solution. Bars with different letters are significantly different ($p < 0.05$) among protein conformations.

or absence of 25% glycerol, was compared as shown in Fig. 2.

The presence of fresh actomyosin, produced a significant reduction ($p < 0.05$) of the percentages of free hexanal, methional and octanal, meaning that the fresh actomyosin was able to bind these volatile compounds. However, the actomyosin binding ability was affected by the freezing storage in different ways (Fig. 2). When actomyosin was frozen with and without glycerol, the percentages of free hexanal and methional increased significantly. This means that less volatile compound were bound in comparison to the fresh actomyosin. The percentage of free octanal decreased significantly when actomyosin was frozen using glycerol and increased without glycerol (Fig. 2).

On the other hand, the presence of fresh actomyosin did not bind 3-methyl-butanal, 2-methyl-butanal and 2-pentanone and the free percentages of these compounds were not affected by frozen storage when glycerol was used, but they were significantly decreased in the absence of glycerol.

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, Mottram, Parker, & Brown, 2001; Damodaran & Kinsella, 1981; Jouenne & Crouzet,

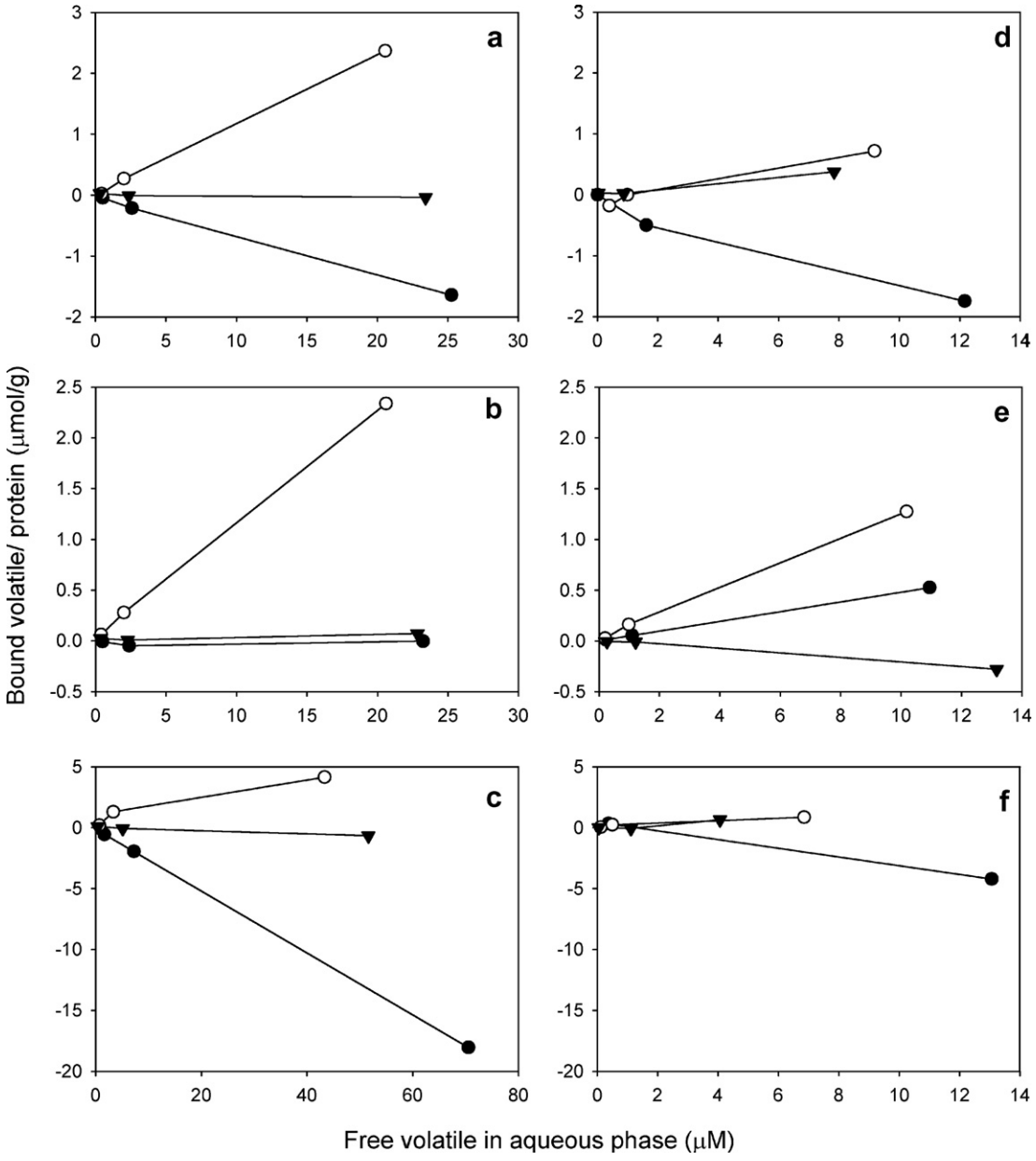

Fig. 4. Moles of volatile compound bound by actomyosin (–▼–), G-actin (–●–) and F-actin (–⊙–) *vs* the concentration of free volatile compound in the aqueous phase. Binding of: (a) 3-methyl-butanal, (b) 2-methyl-butanal, (c) methional, (d) hexanal (e) 2-pentanone and (f) octanal. The actin (G- and F-) and actomyosin concentrations used were 0.8 and 5 mg/ml, respectively.

2000). The changes in protein hydrophobicity take place during processes of induced denaturation and aggregation produced by heating and freezing (Lichan, Nakai, & Wood, 1985). In this sense, Careche et al. (1998) analyzed the effect of freezing and frozen storage on hydrophobicity and properties of myofibrillar extracts from chicken, pork and hake. They found that hydrophobicity, emulsifying activity index and gel strength of the natural actomyosin, were generally influenced by species, freezing and storage process. With reference to pork myofibrillar extract, they found that the hydrophobicity increased during the first week of frozen storage.

The increase of actomyosin hydrophobicity due to the frozen storage (Careche et al., 1998) could explain the decrease of the free percentages of 3-methyl-butanal, 2-methyl-butanal and 2-pentanone when actomyosin was frozen without glycerol, meaning that frozen actomyosin was able to retain these volatile compounds. However, the increase of the free percentages of hexanal, methional and octanal when actomyosin was frozen without glycerol, imply a release of these compounds, suggesting that the actomyosin interaction with these compounds is not hydrophobic and, moreover, glycerol is not able to protect the binding sites for these compounds.

On the other hand, actin exists in two forms: G-actin or globular and F-actin or fibrous (Pearson & Young, 1989). The change between these two forms involves a conformational change before completing the polymerisation process (Pearson & Young, 1989), probably related to their sulfhydryl groups (Jiang, Wang, & Chen, 1989). In order to study the effect of actin conformation (globular or fibrous) on its binding ability, the free percentages of each volatile compound in the presence of F-actin or G-actin are shown in Fig. 3. The fibrous form (F-actin) produced a significant decrease of the free percentages of all the studied volatile compounds. This means that F-actin was able to bind these compounds. The effect of actin conformation on the binding ability has not been previously studied. The conformation effect, produced by actin polymerisation, affects its binding ability even though it depends on the volatile compound studied.

Finally, the moles bound by each isolated myofibrillar protein were calculated (Fig. 4). The addition of increasing concentrations of volatile compounds, shows that the more volatile compound was added to the aqueous phase, the more volatile compound was bound by F-actin (Fig. 4). Furthermore, F-actin bound higher quantities of 3-methyl-butanal (Fig. 4a), 2-methyl-butanal (Fig. 4b), methional (Fig. 4c), hexanal (Fig. 4d) and 2-pentanone (Fig. 4e) than G-actin and actomyosin. On the other hand, the moles bound by G-actin were decreased in the case of 3-methyl-butanal (Fig. 4a), hexanal (Fig. 4d), methional (Fig. 4c) and octanal (Fig. 4f). These volatile compounds were released to the headspace and the results shown in Figs. 2 and 3.

In summary, the binding ability of isolated meat myofibrillar proteins, actomyosin and actin, was affected by their concentration and conformation. Actomyosin was able to bind all the assayed volatile compounds, although the binding was dependent on protein concentration and conformation, as was observed by the effect of frozen storage.

On the other hand, G-actin was not able to bind any of the assayed volatile compounds and even caused a release of several of them (3-methyl-butanal, hexanal, methional and octanal) when the protein concentration was increased. However, the polymerisation of G-actin to F-actin showed that F-actin was the myofibrillar protein which bound higher quantities of the assayed volatile compounds.

Acknowledgements

This work has been supported by Grants AGL2004-05064-CO2-01 (MEC, Spain) and GV 05/067 (Generalitat Valenciana, Spain). The FPI/MEC scholarship to M. Pérez-Juan is also fully acknowledged.

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.

Burdock, G. A. (2002). *Fenaroli's handbook of flavor ingredients* (4th ed.). Boca Raton, FL: CRC Press Inc.

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.

Cofrades, S., Careche, M., Carballo, J., & Colmenero, F. J. (1996). Freezing and frozen storage of actomyosin from different species. *Zeitschrift fur Lebensmittel – Untersuchung Und-Forschung, 203*, 316–319.

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

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

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

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.

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.

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

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.

Jouenne, E., & Crouzet, J. (2001). Aroma compounds–proteins interaction using headspace techniques. In R. L. Rouseff & K. R. Cadwallader (Eds.). *Advances in experimental medicine and biology. Headspace analysis of foods and flavours. Theory and practice* (Vol. 488, pp. 33–41). New York: Kluwer Academic/Plenum Publishers.

Kinsella, J. E. (1990). Flavour perception and Binding. *Inform, 1*(3), 215–226.

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). Flavour interactions: The greater whole. *Food Technology, 51*, 75–76, 78–80.

Lichan, E., Nakai, S., & Wood, D. F. (1985). Relationship between functional (fat binding, emulsifying) and physicochemical properties of muscle proteins – Effects of heating, freezing, pH and species. *Journal of Food Science, 50*, 1034–1040.

Morrissey, P. A., Mulvihill, D. M., & O'Neill, E. M. (1987). Functional properties of muscle proteins. In B. J. F. Hudson (Ed.). *Developments in food proteins* (Vol. 5, pp. 195–256). London and New York: Elsevier Applied Science.

Okeefe, S. F., Wilson, L. A., Resurreccion, A. P., & Murphy, P. A. (1991). Determination of the binding of hexanal to soy glycinin and beta-conglycinin in an aqueous model system using a headspace technique. *Journal of Agricultural and Food Chemistry, 39*, 1022–1028.

O'Neill, E., Morrissey, P. A., & Mulvihill, D. M. (1990). The surface-active properties of muscle proteins. *Food Chemistry, 35*, 1–12.

Pardee, J. D., & Spudich, A. (1982). Purification of muscle actin. *Methods in Enzymolgy, 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. (2006). Model studies on the efficacy of protein homogenates from raw pork muscle and dry-cured ham in binding selected flavour compounds. *Journal of Agricultural and Food Chemistry, 54*, 4805–4808.

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

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.