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.