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The ability of peptide extracts obtained at different dry cured ham ripening stages to bind aroma compounds

Isadora Martínez-Arellano, Mónica Flores *, Fidel Toldrá

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Avda. Agustín Escardino 7, 46980 Paterna, Valencia, Spain

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ABSTRACT

The ability of peptide extracts, obtained at different dry-cured ham ripening-stages, to bind volatile compounds has been examined using solid-phase microextraction and gas-chromatography. The peptide extracts from dry-cured ham were previously defatted and deodorised in order to be able to study peptide–volatile interactions. The binding effect of each peptide extracts to volatile compounds was analysed at different concentrations. In the presence of peptide extracts, a release was observed for ethyl butyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate and 3-methylthiopropanal. On the other hand, retention of about 20% and 30% was observed for 2-methylpropanal, hexanal and ethyl acetate while the highest interaction was observed for trimethylpyrazine. All peptide extracts did not exert any binding effect on 2-methylbutanal. No significant differences in binding-ability were detected for the peptides obtained at different ripening-stages; therefore, the binding-ability of peptide extracts was mainly based on volatile chemical characteristics and not on the type of peptide extract obtained.

1. Introduction

Nearly 39 million dry-cured hams per year are manufactured in Spain, making the country the world's biggest producer (Estévez, Ventanas, Morcuende, & Ventanas, 2015). Currently, there are five Protected Designations of Origin (PDO) in Spain; one of them is named "Teruel dry-cured ham", where hams are produced from heavy white pigs (Latorre, Ripoll, García-Belenguer, & Ariño, 2009). Colour, texture, appearance and flavour determine the dry-cured ham quality (Ruiz, García, Muriel, Andrés, & Ventanas, 2002). A long process of manufacturing, including the stages of salting, post salting and ripening is necessary to obtain a high quality dry-cured ham. Different biochemical reactions like proteolysis, lipolysis, glycolysis and transformations of nucleotides take place during these stages (Toldrá & Flores, 1998). Within these reactions, proteolysis and lipolysis integrate two of the most important enzymatic phenomena. They generate compounds with high influence on taste, aroma and texture (Toldrá, 2006). Proteolysis is responsible for tenderisation, due to an intense degradation of the myofibrillar structure being detected during the drying–curing process. Also, a large number of peptides resulting from muscle proteins like myosin, actin, troponin T, glycolytic enzyme, titin and LIM-domain breakdown have recently been identified in dry-cured ham (Gallego, Mora, Fraser, Aristoy, & Toldrá, 2014; Mora, Sentandreu, & Toldrá, 2010; Mora et al., 2009). These peptides were identified by proteomic strategy and can be useful as industrial biomarkers for quality optimisation. These peptides also show antihypertensive effect and antioxidant activity (Escudero, Mora, & Toldrá, 2014). Regarding flavour, several amino acids, such as glutamic acid, glycine, alanine, valine, proline, histidine and leucine, have been found in savoury fractions of dry-cured ham. Some small peptides were also selected with interesting characteristics, such as ham flavour, salty, bitter and umami tastes in Spanish dry-cured ham (Sentandreu et al., 2003).

Flavour is related to non-volatile compounds, like free amino acids and small peptides, while aroma is due to the formation of volatile compounds (Flores, Sanz, Spanier, Aristoy, & Toldrá, 1998). Several studies have identified and quantified more than 261 volatile compounds in dry-cured ham from different origins, such as French, Italian and Spanish (Toldrá, Aristoy, & Flores, 2009). The identified volatile compounds belonged to the different chemical classes: alkanes, alkenes, aldehydes, ketones, alcohols, aromatic hydrocarbons, carboxylic acids, esters, terpenes, sulphur compounds, furans, pyrazines, amines and chloride compounds (Flores, Grimm, Toldrá, & Spanier, 1997). The final dry-cured ham aroma is based on the volatile compounds concentration, odour threshold and the binding with the food matrix. Polarity, pH and molecular weight among others characteristics of volatile compounds affect the binding; therefore, it is fundamental to consider

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

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them (Seuvre, Espinosa Diaz, & Voilley, 2002). However, there are a few studies dealing with protein–flavour binding, being milk (Andriot, Marin, Ferona, Relkin, & Guichard, 1999; Kühn, Considine, & Singh, 2006) and soy proteins (Solina, Baumgartner, Johnson, & Whitfield, 2005). In addition, several binding studies on animal proteins and peptides, like fish actomyosin (Damodaran & Kinsella, 1983), sarcoplasmic and myofibrillar proteins from dry-cured ham (Pérez-Juan, Flores, & Toldrá, 2006) and myoglobin and carnosine and anserine peptides (Gianelli, Flores, & Toldrá, 2003) were investigated. Likewise, the structure of amino acids and peptides is important in their binding capacity to volatile compounds (Gianelli, Flores, & Toldrá, 2002). In the food matrix, several volatile compounds are bound to proteins while others are solubilised in fat. However, in fat reformulated foods these compounds are lost because fat acts as a reservoir for the majority of volatile compounds (Kühn et al., 2006). Therefore, the information about the physicochemical interactions between proteins and volatile compounds can help in the development of reformulated food products.

Nevertheless, there are no studies about the role of small peptides, produced endogenously during the ripening stages of dry-cured ham, on the binding of volatile compounds. Therefore, the objective of this work was to analyse the ability of peptides obtained, at different ripening stages of dry-cured ham, to bind volatile compounds. In addition, the influence of the chemical characteristics of the volatile compounds and the source of peptide extracts was analysed.

2. Materials and methods

2.1. Samples and reagents

Dry-cured hams (P.D.O. Teruel, Spain, Large White × Duroc; Escudero et al., 2014) at different stages of processing (post-salting (90 days), 5, 10 and 14 months of drying) were used. The muscle *Bicep femoris* was removed and used for the peptide extraction.

The volatile compounds were obtained from Fluka Chemie Gmbh (Buchs, Switzerland): 3-methyl l-butanol (99%), 3-methyl 2-butanol (99%), 1-octen 3-ol (98%), 3-methylbutanal (97%), 2-methylbutanal (95%), ethyl acetate (99%), ethyl butyrate (99%), ethyl 2-methylbutyrate (98%), 2,3-butanedione (97%), 2-heptanone (98%) and Sigma Aldrich (St. Louis, Mo., USA): hexanal (98%), 3-methylthiopropanal (98%), ethyl 3-methylbutyrate (98%), 2,6-dimethylpyrazine (98%), 2,3-dimethylpyrazine (99%), 2,3,5-trimethylpyrazine (99%).

2.1.1. Preparation of peptide extracts

A 50 g sample of *Bicep femoris* was obtained at each ripening stage, this was placed in a stomacher (IUL Instrument, Barcelona, Spain) with 100 ml 0.01 N HCl for 8 min. The homogenate was centrifuged at 10,000 rpm, 4 °C for 20 min. Then, the supernatant was filtered through glass wool and deproteinised with the addition of 3 volumes of ethanol. The sample was stored for 24 h at 4 °C. Afterwards, it was centrifuged under the same conditions. The ethanol in the supernatant was distilled to dryness, and the sample was lyophilised (Escudero et al., 2014). This constituted the peptide extract (PE).

The presence of lipids in the peptide extract (4 g of PE) was determined by extracting the total lipids using dichloromethane: methanol (2:1; Folch, Lees, & Sloane Stanley, 1957). The residual peptides were diluted again in 0.01 N HCl and further lyophilised to obtain the defatted peptide extract (Def-PE), which was further subjected to a deodorisation process to remove the volatile compounds. The Def-PE (4 g) was dissolved in 200 ml HCl 0.01 N, and sonicated during 20 min. Then, it was deodorised with 40 g of Amberlite XAD-2 resin (Sigma Aldrich, St. Louis, Mo., USA), using gentle agitation for 1.5 h. The resin was eliminated by filtration through glass wool. The obtained defatted–deodorised peptide extract (Def–DO-PE) was lyophilised and kept at −20 °C until use.

2.2. Chemical characterisation of dry-cured ham and peptide extract

Sodium chloride in the dry-cured ham and peptide extracts was determined by ion chromatography (Corral, Salvador, & Flores, 2013), through the analysis of the sodium ion. Dry-cured ham moisture was measured using a moisture analyser Mettler Toledo HB43 Halogen (Mettler-Toledo, Greifensee, Switzerland). As described above, total lipids were extracted from 4 g of PE according to the method of Folch et al. (1957). The content of free and total fatty acids in the total lipids was determined by conversion of fatty acids into fatty acids methyl esters (FAME), and analysed by gas chromatography with flame ionisation detector (GC–FID) as described by Corral, Salvador, Belloch, and Flores (2014) using an Agilent HP 7890A gas chromatograph (Hewlett Packard, Palo Alto, CA).

2.3. Volatile compounds of dry-cured ham

Five grams of minced dry-cured ham with 0.75 mg of BHT were placed in 20 ml headspace (HS) vial, sealed with PTFE faced silicone septum. Subsequently, the extraction of volatile compounds was done by using a solid phase microextraction (SPME) device with a CAR/PDMS fibre, as described by Corral et al. (2013).

The analysis of the volatile compounds was performed in a gas chromatograph Agilent 7890 CG with a mass spectrometer 5975 MS system (Hewlett Packard, Palo Alto, CA, USA). The equipment was supply with a Gerstel MPS2 autosampler (Gerstel, Mülheim and der Ruhr, Germany). The headspace vial was kept at 37 °C during 30 min to equilibrate its headspace. Afterwards, the SPME fibre was exposed to the headspace while maintaining the sample during 60 min at 37 °C for volatile extraction. Before each injection the fibre was baked at 250 °C for 15 min. The compounds adsorbed by the fibre were desorbed in the injection port for 5 min at 240 °C with purge valve off (splitless mode). The volatiles were separated on a DB-624 column (30 m, 0.25 mm i.d., film thickness 1.4 μm). The carrier gas was helium with a flow rate of 34.3 cm/s. The oven temperature program began at 38 °C for 13 min, ramped to 100 °C at 3 °C/min and maintained at 100 °C for 5 min, then ramped to 150 °C at 4 °C/min, ramped to 210 °C at 10 °C/min, and lastly held at 210 °C for 5 min, the total run time was 62.2 min. Furthermore, the compounds were identified by comparison with mass spectra from the library database (Nist'05), Kovats retention index (Kovats, 1965) and by comparison with authentic standards. The quantification of the volatile compounds present in the headspace was done in SCAN mode employing either the area of total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale.

2.4. Volatile compounds of peptide extract

The analysis of the volatile compounds was done by using 2 g of PE in 20 ml HS vials. The volatile analysis was done by SPME-GC–MS, using the same conditions as described above for dry-cured ham. The relative quantification of the volatile compounds present in the headspace was done in SCAN mode employing either the area of total or extracted ion chromatogram (TIC or EIC), expressed as a percentage of the total area present initially in the dry-cured ham.