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Analytical Methods

Optimisation of stir-bar sorptive extraction (SBSE), targeting medium and long-chain free fatty acids in cooked ham exudates

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ABSTRACT

The purpose of our research was to optimise the extraction conditions of the stir-bar sorptive extraction (SBSE) targeting the identification of lipid compounds particularly medium and long-chain free fatty acids in cooked cured pork ham exudates. The analytical conditions of extraction (including sample volume, extraction time, stirring speed, pH and dilution of the sample) were checked using the Simplex method approach. As a result of the SBSE optimisation, improved detection limits and linear ranges for hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic and tetradecanoic fatty acids were obtained. When comparing results with those obtained by the commonly used SPME methodology, optimisation of SBSE achieved better results for volatile compounds of low volatility, such as medium and long-chain free fatty acids, whereas compounds with high volatility and polarity were only detected by SPME. SBSE also confirmed its potential as a tool to help identify undesirable contaminants/residues in meat products.

1. Introduction

Cooked cured pork ham (hereinafter cooked ham) is a popular, ready-to-eat meat product with a high worldwide acceptance, partially related to its pleasant sensory characteristics (Bryhni et al., 2002; Myers et al., 2009; O'Mahony, 1991). However, the high fat and sodium content of the final processed product is a cause for concern among consumers, due to potential negative influences on human health such as hypercholesterolaemia (Guerrero, Marín, Mejías, & Barroso, 2007; Karwowska & Dolatowski, 2013) and high blood pressure (Doyle & Glass, 2010; Kotchen, Cowley, & Frohlich, 2013). Regarding fats, the cooking process of hams generates a number of volatile compounds following fatty acid oxidation. These volatiles, in turn, react with metabolites of Maillard reactions and thiamine degradation, thus becoming one of the main determinants of pork ham flavour (Estévez, Morcuende, Ventanas, & Cava, 2003; Wood et al., 2008).

Acids of high molecular weight (decanoic acid, dodecanoic acid, tetradecanoic acid and hexadecanoic acid) have a high odour threshold related to a waxy-fatty nuance but have only a moderate direct contribution to the overall ham aroma (Dirinck, Van Opstaele, & Vandendriessche, 1997). However, these acids are known to be precursors of other volatile compounds of high aromatic relevance (Shahidi, Rubin, D'Souza, Teranishi, & Buttery, 1986). On the other hand, some medium-chain fatty acids have been identified as key aroma compounds in cooked hams (Guillard, Le Quere, & Vendeuvre, 1997; Thomas, Mercier, Tournayre, Martin, & Berdagué, 2013) which may convey unpleasant cheesy and goat-like notes (Carrapiso, Martín, Jurado, & García, 2010). Consequently, a precise description of the fatty acid profile in pork products is fundamental to characterise and understand the development of ham flavour.

Several extraction techniques have been used to analyse free fatty acids, such as distillation, solid-phase extraction (SPE), supercritical-fluid extraction, purge-and-trap techniques, liquid–liquid extraction (LLE) and headspace liquid-phase microextraction (Clark & Bunch, 1997; Collin, McCormick, & Schmitt, 1974; Spaepen, Van Oevelen, & Verachtert, 1978; Tan, Zhao, Liu, Ju, & Li, 2005). The comparison of all these techniques is out of the scope of the current manuscript. However, SPME has been the most popular and optimised technique to analyse free fatty acids in food products (Pan, Adams, & Pawliszyn, 1995; Tomaino, Parker, & Larick, 2001) and more precisely meat volatile compounds from lipid oxidation (Elmore, Mottram, & Hierro, 2001; Fernando, Berg, & Grün, 2003). However, SPME possesses several disadvantages: firstly it is not very effective in extracting highly polar substances; and secondly the volume of the extraction phase is limited, which

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affects the detection and identification of some volatile compounds, such as medium and long-chain free fatty acids (Kataoka, Lord, & Pawliszyn, 2000).

Based on similar principles to SPME, stir-bar sorptive extraction (SBSE), introduced by Baltussen, Sandra, David, and Cramers (1999), allows for an improved extraction of volatile and semi-volatile compounds in aqueous systems (Almeida & Nogueira, 2006). This technique has been applied successfully for trace compound analysis in environmental, biomedical and food applications (Bicchi, Cordero, Liberto, Sgorbini, & Rubiolo, 2008; Guerrero, Marín, Mejías, & Barroso, 2007; León, Álvarez, Cobollo, Muñoz, & Valor, 2003). SBSE has been shown to have low detection limits (i.e. down to ppt levels) for volatile compounds present in an aqueous complex matrix (Prieto et al., 2010). Compared to most meat products cooked ham has a high water content which makes SBSE a potential method to analyse the volatile profile of its post-cooking exudates if/when properly developed.

Given the relevance of medium and long-chain fatty acids in the development of overall pork ham aroma, our research aimed to develop the SBSE of medium and long-chain free fatty acids by optimising sample volume, extraction time, stirring speed, pH and sample dilution of cooked ham exudates. Other extraction parameters had been optimised in a previous work (Ibáñez & Solá, 2006).

2. Material and methods

2.1. Chemicals and reagents

The chemical standards hexanoic and octanoic acids were acquired from Sigma–Aldrich (St. Louis, MO) and heptanoic, nonanoic, decanoic, dodecanoic and tetradecanoic acids were acquired from Ernesto Ventós S.A. (Sant Just Desvern, Spain). The internal standard 1,4-dibromobenzene was supplied by Sigma–Aldrich (St. Louis, MO) and acetone was supplied by Panreac Quimica (Castellar del Vallès, Spain). The volatile standards used to compare SPME and SBSE were from Sigma–Aldrich and Ernesto Ventós S.A.

2.2. Cooked ham samples

For the optimisation of the SBSE parameters by the Simplex method cooked ham samples were prepared following the procedure reported in our previous work (Benet et al., 2015). All hams were from left-side gilt carcasses. *Post-mortem* pH was checked to meet quality standards (pH in the *semimembranosus* muscle at 45 min *post mortem* was above 6.0 and at 24 h (pH_{24}) lower than 6.2. Ten whole-leg green hams were deboned and trimmed of subcutaneous and intermuscular fat, connective tissue and rind. Brine was injected into the meat to increase its weight by 21% reaching 0.3% pentasodium tripolyphosphate, 0.05% sodium ascorbate, 1.8% NaCl and 0.01% sodium nitrite after injection. Hams were then individually placed in a vacuum tumbler at 4 °C at a pressure of 200 mbar. The tumbling schedule was set for the ham to rotate a total of 2000 times at 14 rpm. Then, the hams were packed in bags (CN330; Sealed Air, Elmwood Park, NJ), matured at 2 ± 1 °C for 8 days and moulded in stainless steel moulds and placed in a steam oven and cooked to an internal temperature of 68 °C using an external temperature of 70 °C. The cooked hams were then refrigerated at 2 ± 1 °C for 48 h and vacuum-packaged individually in PA/PE sealed bags and stored at 0 ± 1 °C.

For the validation of the optimised SBSE method and the comparative study between SPME and optimised SBSE, three additional cooked hams were prepared following the same procedure reported above.

2.3. Stir-bar sorptive extraction (SBSE) analysis

2.3.1. Preparation of the exudate samples

Samples of 10 mL each of post-cooked ham exudates were collected, chemically characterised and stored in 50-mL vials immediately after unsealing the cooking bags. The chemical characterisation was based on the determination of the two main components of the exudate (water and NaCl) and the macronutrients fat (lipid derivatives being one of the main targets of our study) and crude protein. Volatile compound extraction of each sample was performed using the optimised conditions: 15 h, 300 rpm, pH 2 and use of 10 mm × 0.5 mm PDMS phase thickness stir bars (Twister®, GERSTEL GmbH & Co. KG, Mülheim an der Ruhr, Germany). Afterwards, the stir bars were rinsed with distilled water (Milli-Q system, Millipore, Bedford, MA,), dried with a clean tissue and transferred to desorption tubes in an MPS2 Gerstel Multipurpose Automatic Sampler System.

2.3.2. Instrumentation and conditions

A gas chromatograph (GC) Agilent 6890 (Agilent Technologies, Santa Clara, CA) was equipped with an MPS2, a thermal desorption unit (TDU) and a cooled injection system (CIS4) by Gerstel. To inject the volatiles absorbed onto the stir bars into the injection port of the GC, the TDU was held at 30 °C for 1 min, raised to 250 °C at a rate of 90 °C/min and then held at this temperature for 10 min. Splitless mode was used for the TDU transfer of the volatiles to the cooled CIS4 injector during thermal desorption. The desorption flow was kept at 50 mL/min and 103 kPa, while the injection port was maintained at −110 °C (cooled using liquid N_2) (Ibáñez & Solá, 2006). After total desorption of the volatiles, the CIS4 temperature was ramped at a rate of 12 °C/s from −110 °C to 250 °C and held at this temperature for 3 min.

2.4. Solid phase microextraction (SPME) analysis

The SPME conditions were set as previously reported (Benet et al., 2015). Two millilitres of post-cooked exudates were placed into 20-mL glass vials and capped with silicone-PTFE septa. The vials were transferred to a CTC SPME Automatic Sampler (CTC Analytics AG, Zwingen, Switzerland). The SPME fibre used was 50/30 μm Carboxen/PDMS/DVB (Supelco, Bellefonte, PA). The fibres were exposed to the headspace of the vial for 30 min at 40 °C and the volatiles were desorbed in the injection port of the chromatograph for 10 min at 250 °C in splitless mode.

2.5. Gas chromatography and mass spectrometry (GC–MS) conditions

Separation of volatiles was performed using a Zebron ZB-Wax Plus capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas. The oven program included an initial temperature of 60 °C and a programmed rate of 4 °C/min up to 230 °C, subsequently holding this temperature for 15 min. The mass spectrometer (MS) transfer line temperature was held at 250 °C. Electronic impact at 70 eV was used to obtain the mass spectra. The MS scanned from m/z 35 to 300, keeping the ion source temperature at 230 °C and the quadrupole temperature at 150 °C. Identification of the volatiles was done using AMDIS deconvolution software (NIST08) in order to separate the chromatographic peaks from interferences in the studied matrices. Volatile compounds were identified by comparing their mass spectra with those of the commercial standards and with references from several commercial database libraries: NIST08 library (NIST 08, version 2.0, Gaithersburg, MD) and Wiley (Wiley-VCH, Weinheim, Germany). Each compound was further confirmed by comparing its linear retention index with those obtained from the standards and/or from literature sources. If standards were unavailable,