Meat Science 92 (2012) 715–720

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

A novel method for determination and quantification of 4-methyloctanoic and 4-methylnonanoic acids in mutton by hollow fiber supported liquid membrane extraction coupled with gas chromatography

Haigui Chen [a], Yunfan Wang [a,c], Houyang Jiang [a], Guohua Zhao [a,b,*]

[a] College of Food Science, Southwest University, Tiansheng Road 2, Chongqing 400715, PR China
[b] Key Laboratory of Food Processing and Technology of Chongqing, Chongqing 400715, PR China
[c] School of Chemistry and Chemical Engineering, Chongqing University of Science & Technology, Chongqing, 401331, PR China

ARTICLE INFO

Article history:
Received 16 October 2011
Received in revised form 12 April 2012
Accepted 14 June 2012

Keywords:
Branched fatty acids
Sweaty odor
Hollow fiber supported liquid membrane extraction
Determination
Gas chromatography

ABSTRACT

4-methyloctanoic acid (MOA) and 4-methylnonanoic acid (MNA) are the main compounds responsible for "sweaty" odor of mutton. A novel method for their determination has been developed and validated. Hollow fiber supported liquid membrane (HF-SLM) was applied to selectively extract MOA and MNA prior to gas chromatography (GC) analysis. For HF-SLM, the donor outside the fiber was the acidified supernatant (pH 4) from aqueous mutton slurry. Liquid membrane was 5% tri-*n*-octylphoshphine oxide in di-*n*-hexyl ether and 0.3 M NaOH aqueous solution filled in the lumen of the fiber was used as the acceptor. The extraction last for 4 h. After acidification with HCl, the acceptor was directly analyzed by GC. Importantly, HF-SLM provided high enrichment factors for MOA (133) and MNA (116). The method developed had low detection limits of 0.0007–0.0015 mg/kg, good linearity ($R^2 > 0.9956$), reasonable recovery (88.54–122.13%), satisfactory intra-assay (7.83–9.73%) and inter-assay (15.68–16.14%) precision.

1. Introduction

Flavor is one of the most important sensory attributes of meat, which significantly affects the acceptance of consumers. Mutton is widely consumed all over the world with an annual consumption up to 900 thousand tons (Madruga, Elmore, Dodson, & Mottram, 2009). As known to all, mutton has been often rejected by a considerable number of consumers because of its so-called "sweaty" odor or "soo" in Chinese (Prescott, Young, & O'Neill, 2001).

Numerous researches have been focusing on the chemistry of meat flavor, which researchers had explored its forming mechanism and key responsible components. Researches showed that 4-methyloctanoic acid (MOA) and 4-methylnonanoic acid (MNA) are the main components responsible for the mutton "sweaty" flavor, or so called "soo" in Chinese (Ha & Lindsay, 1991; Mottram, 1998; Wong, Johnson, & Nixon, 1975; Wong, Nixon, & Johnson, 1975). The presence and concentration of MOA and MNA in mutton are dependent on the feed, animal sex, breeding style and slaughter method (Young, Lane, Priolo, & Fraser, 2003). It was found that mutton from animals grazed on grass pasture is less acceptable than that on legume pasture (Nixon, 1981). Although preslaughter adrenalin injection always resulted in an ultimate high pH of mutton, it was proved to be a positive measure in controlling the "sweaty" odor (Braggins, 1996). Biochemically, propionate plays a vital role in mutton flavor as an important precursor of MOA and MNA (Berthelot, Bas, Schmidely, & Duvaux-Ponter, 2001).

The quantitative determination of MOA and MNA is crucial to the flavor control of mutton. Among the developed methods, the use of gas chromatographic method to detect and quantify acids has been extensively used. In gas chromatographic methods, the isolation of the analytes is necessary prior to determination. Distillation and dynamic headspace were often used in the determination of MOA and MNA (Sutherland & Ames, 1996a; Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997). Because of the very low concentration of MOA and MNA in isolated samples, mass spectrometer (MS) was usually required as a highly sensitive detector of gas chromatography. Obviously, valuable gas chromatography–mass spectrometry is not affordable for numerous small-scale research groups and enterprises (Tanaka, Hine, West-Dull, & Lynn, 1980).

Supported liquid membrane extraction (SLME) is an effective technique for selective extraction and enrichment of analytes from complicate matrices (Lee, Lee, Rasmussen, & Pedersen-Bjergaard, 2008; Zhao & Lee, 2002). SLME has many merits such as high selectivity, low cost and negligible consumption of organic solvent (Jönsson

Abbreviations: HF-SLME, hollow fiber supported liquid membrane extraction; SLME, supported liquid membrane extraction; MOA, 4-methyloctanoic acid; MNA, 4-methylnonanoic acid; TOPO, tri-*n*-octylphoshphine oxide; DHE, di-*n*-hexyl ether; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry.

* Corresponding author at: College of Food Science, Southwest University, Tiansheng Road 2, Chongqing, 400715, PR China. Tel.: +86 23 68 25 03 74; fax: +86 68 25 19 47.
E-mail address: zhaogh@swu.edu.cn (G. Zhao).

0309-1740/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2012.06.027

& Mathiasson, 1999a; Liu, Toränq, Mayer, & Jönsson, 2007). It has been successively applied to pre-enrichment and selective extraction of various organic acids. The successful examples include phenylacetic acid in fermentation slurry (Dai, Yang, Luo, & Sirkar, 2000), carboxylic acids in soil (Shen, Obuseng, Grönberg, & Jönsson, 1996), haloacetic acids in tap water (Kou, Wang, & Mitra, 2004), and short chain-fatty acids in serum (Zhao, Liu, Nyman, & Jönsson, 2007). The high enrichment factor and selective mass transfer of SLME provided high limits of detection and quantification and good performance of chromatographic analysis. To our best knowledge, the feasibility of utilization of SLME to extract MOA and MNA from mutton has never been investigated. The use of SLME makes it possible to quantify MOA and MNA in mutton with GC instead of GC–MS.

With an aim to determine MOA and MNA in mutton with GC, a novel sample pre-treatment for the selective extraction and enrichment of MOA and MNA from mutton, called hollow fiber supported liquid membrane extraction (HF-SLME), was investigated and its operation parameters were optimized. Finally, hollow fiber supported liquid membrane extraction coupled with gas chromatography was validated for the determination of MOA and MNA in mutton which paving ways to detect and quantify the amounts of MOA and MNA.

2. Material and methods

2.1. Apparatus

Chromatographic analysis was performed on a GC-2010 instrument (Shimadzu, Japan) with a flame ionization detector. Separation was achieved on a DB-FFAP fused-silica capillary column with free fatty acid phase (30 m × 0.53 mm i.d., 0.50 μm film thickness, Zhonghuida, China). A 50/280 Accurel PP polypropylene hollow fiber with a thickness of 50 μm (0.1 μm pore size) and an internal diameter of 280 μm from Membrana GmbH (Wuppertal Germany) was used as the support for liquid membrane. BD Micro-Fine Syringes (with a needle of 0.30 mm outer diameter and 8 cm length, 0.5 ml) obtained from BD Consumer Healthcare, were used to inject the acceptor solution into the lumen of the hollow fiber for extraction and to flush out the acceptor from the lumen of the hollow fiber into a 100 μl pulled point conical glass vial (Agilent, USA).

2.2. Chemicals and materials

MOA and MNA with a purity of 97% were obtained from Alfa Aesar (USA). 2-Ethylbutyric acid, used as internal standard, was obtained from Sigma-Aldrich (Chemie GmbH, Steinheim, Germany).

Aqueous stock standard solutions were prepared for MOA and MNA. A working standard solution containing the two target analytes was prepared by mixing appropriate volumes of the individual standard stock solutions and diluting it with water. An internal standard stock solution containing 2-ethylbutyric acid with 12% formic acid was also prepared. All the stock standard and working solutions were stored at −20 °C until used.

Tri-*n*-octylphosphine oxide (TOPO) and dihexyl ether (DHE) were obtained from Sigma (St. Louis, MO, USA). Petroleum ether, n-hexane, n-octanol, n-heptanol and n-dodecane were purchased from Kaifu (Tianjing, China). The organic membrane liquid was prepared by dissolving appropriate amounts of TOPO in a specific organic solvent. Analytical-grade HCl (30%) and formic acid (98–100%) were used. The donor was acidified using 2 M HCl and 12% (v/v) formic acid was used to clean the GC column. The aqueous solution of NaOH (0.1–0.4 M) was used as the basic acceptor.

Fresh mutton was purchased from a local supermarket and stored at −18 °C prior to use. All water obtained by a Milli-Qbiocel system (Millipore, Bedford, MA, USA) was applied throughout the experiment.

2.3. Sample preparation

Frozen mutton samples were defrosted at 4 °C and homogenized with addition of water (sample/water ratio of 1:60 (w/v)) by a homogenizer (XHF-D, Xinzhi, China) at 10 000 r/min for 1 min. The resulting slurry was transferred into a polypropylene tube and centrifuged at 3000 r/min for 15 min in a centrifuge (TGL-16 G, Anting, China), giving a clear supernatant. To prepare donor with varying pH for HF-SLME, the supernatant obtained was acidified by adding an appropriate amount of 2 M HCl. The free exudate of meat slurry was wrapped in 11 cm filter paper (Xinxing, China) with a sample/water ratio of 1:3 (w/v) was directly analyzed by GC as a control after acidified to have a pH of 2.

2.4. Extraction procedure

The extraction was carried out as described by Liu et al. (2007) with some modifications. The fiber was cut into 16 cm length, and then using the BD Micro-Fine Syringe with acceptor solution to fill the lumen of a hollow fiber. Next, the fiber was dipped into the membrane liquid for a few seconds to impregnate the pores of the fiber wall. Then, completely fill the lumen of fiber with the acceptor solution. The two ends of the fiber were then folded and enveloped with a piece of aluminum foil and sealed. Fiber surplus membrane liquid was washed away by soaking in water for a few minutes. This prepared hollow fiber supported liquid membrane extraction device was immersed in the 1.5 ml sample solution in a 2 ml capped vial. The vial was capped and shaken on a shaker (Xinkang, China) for 4 h. After extraction, one of the sealed ends was cut and connected to the needle of a BD Micro-Fine Syringe filled with air, and the other end was cut and put into a 100 μl pulled point conical glass vial. The syringe plunger was pushed to flush the acceptor into the conical glass vial. Approximately 8–12 μl of extracted solution could be got. Transferred 8 μl into another conical glass vial and added 10 μl 1 M HCl into the vial. Then 1 μl from the vial was injected into the GC system for analysis.

The efficiency of extraction was evaluated in terms of enrichment factor (*Ee*), which was defined as the fraction of the analyte concentration in acceptor to its initial concentration in donor and was calculated using the following equation:

$$Ee = C_A/C_S$$

where C_A was the concentration of analyte in acceptor after extraction and C_S was the initial concentration of analyte in donor solution.

2.5. Gas chromatographic analysis

Gas chromatographic analysis was carried out using the follow conditions. The temperature of the detector and injector were 240 °C and 200 °C, respectively. The carrier gas was nitrogen with a flow rate of 14.4 ml/min. The initial oven temperature programming was 100 °C isothermal for 0.5 min, increased to 180 °C at 8 °C/min, isothermal for 1.0 min at this temperature then increased to 200 °C at 20 °C/min and then held at 200 °C for 5 min. The flow rates of hydrogen, air and nitrogen were 30, 300 and 20 ml/min, respectively. Peak areas were used to quantify the analytes.

2.6. Evaluation of the method

Limits of detection (LOD) and quantification (LOQ) were estimated using the classical $3\sigma_{blank}$ and $10\sigma_{blank}$ approaches, respectively. σ_{blank} represented the standard deviation of blank measurements (Cruwys, Dinsdale, Hawkes, & Hawkes, 2002). To determine the recovery of the developed method, the standard mixture was added into the mutton slurry at three levels with known concentrations and subjected to treatment as described above and then analyzed