Development of Analysis Condition and Detection of Volatile Compounds from Cooked Hanwoo Beef by SPME–GC/MS Analysis

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Abstract

The current study was designed to optimize solid phase microextraction (SPME)-GC-MS conditions for extraction and analysis of volatile components for Hanwoo beef and to establish a tentative database of flavor components. Samples were taken from Hanwoo longissimus muscle (30 mon old steer, 1+B carcass grade) at 24 h post mortem. Results indicated that the optimum adsorption time for 75 µm CAR/PDMS fiber was 60 min at 60°C. Thermal cleaning at 250°C for 60 min was the best practice for decontamination of the fiber. A short analysis program with a sharp oven temperature ramp resulted in a better resolution and higher number of measurable volatile components. With these conditions, 96 volatile compounds were identified with little variation including 22 aldehydes, 8 ketones, 31 hydrocarbons, 12 alcohols, 8 nitrogen- and sulfur-containing compounds, 5 pyrazines and 10 furans. A noticeable observation was the high number of hydrocarbons, aldehydes, ketones, alcohols and 2-alkylfurans which were generated from lipid decomposition especially the oxidation and degradation of unsaturated and saturate fatty acids. This implies that these compounds can be candidates for flavor specification of highly marbled beef such as Hanwoo flavor.

Key words: beef, flavor, volatile component, solid phase microextraction, gas chromatography-mass spectrometry

Introduction

Aroma compounds in cooked beef are generated from the oxidation and degradation of lipids, the interaction of lipid-degraded products through the Maillard reaction and thiamine degradation during heating (Mottram, 1998; Macleod, 1994). Studies have identified a number of compounds associated with particular flavor characteristics in cooked beef such as 2-acetyl-2-thiazoline and 2-ethyl-3,5-dimethyl pyrazine (e.g., roasty, caramel-like burnt and earthy notes) (Cerny and Grosch, 1992), and 2-ethyl-3,6-dimethylpyrazin, 2,3-diethyl-5-methylpyrazine and ethenylbenzene (e.g., burnt, pungent, roasty notes) (Specht and Baltes, 1994). Odor in stewed beef included 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 12 methyl-tridecanal, methional, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, and 2-furfurylthiol (Guth and Grosch, 1993).

Gas chromatography-mass spectrometry (GC-MS) has been widely used in identification of volatile components in beef samples (Machiels et al., 2003; Moon et al., 2006; Raes et al., 2003; Stetzer et al., 2008). However, identified components varied depending on analysis conditions such as oven temperature, carrier gas flow rate and EM volts (Elmore et al., 2004; Machiels et al., 2003; McMaster, 2007; Gorraiz et al., 2002; Moon et al., 2006). Moon et al. (2006) identified 79 volatile compounds in simulated beef, but only 40 and 33 compounds were identified for roasted and boiled beef samples, respectively, under the same analysis condition. This suggested that heating condition interacts with analysis condition in terms of number and species of identified components in beef samples.

A number of techniques such as dynamic headspace, simultaneous steam distillation-solvent extraction (SDE), solid phase microextraction (SPME) have been developed to extract volatile compounds (Zhang and Pawliszyn, 1993). In recent years, SPME technique has been widely adopted to isolate aroma volatile compounds in cooked meat (Moon et al., 2006; Schilling et al., 2009; Stetzer et al., 2008; Yancey et al., 2006), because the other methods (i.e., dynamic headspace and SDE) require multi-step preparation, prolonged extraction time and high cost. On the other hand, previous studies showed that the extraction efficiency of volatile compounds in test samples by
SPME fibers is significantly affected by several factors such as stirring condition, adsorption temperature, adsorption times, size of sample, the nature of the SPME fiber and salt addition (Kataoka et al., 2000; Lee et al., 2003; Moon and Li-Chan, 2004).

It is well documented that SPME fiber absorbs volatile components on the theory of equilibrium partitioning of the analytes between the solid phase of the fiber and volatile components of the specimen (Zhang and Pawliszyn, 1993). For these reasons, SPME fiber required various optimum conditions for different matrix; orange juice (55 min/25°C) (Jia et al., 1998), palm oil (10 min/50°C) (Beltran et al., 2005; Ho et al., 2006), dry-cured hams (180 min/40°C) (Garcia-Gonzalez et al., 2008), simulated beef (60 min/60°C) (Moon and Li-Chan, 2004; Moon et al., 2006). The previous studies demonstrated the importance of extraction conditions for specific specimen. Although there are ample literatures available on analysis conditions of the above mentioned matrix, there are limited (if any) accessible reports on the analysis conditions for the highly marbled beef samples such as Hanwoo beef using SPME-GC/MS technique.

Cho et al. (2008) reported that Hanwoo beef have different fatty acid composition from imported beef, and that was a significant factor for Korean consumers’ preference. Similarly, Hwang et al. (2004) reported that Hanwoo and imported beef can be discriminated by fatty acid composition. In a previous study, Hwang et al. (2004) concluded that Korean consumers preferred Hanwoo beef to imported one although toughness of Hanwoo was greater. It is well documented that flavor components of cooked meats are significantly influenced by pre-harvest (diets, sex, breed and stress) (Elmore and Mottram, 2000; Elmore et al., 2004; Enser et al., 1998) and post-harvest (chiller ageing, cooking methods and pH) (Jame and Kalkin, 2008; Stetzer et al., 2008; Young et al., 1993) factors. Given these, flavor components of Hanwoo beef can be another discriminator versus imported one, but we are unaware of any information on flavor components of highly marbled Hanwoo beef.

The current study was designed to optimize SPME-GC/MS conditions for extraction and analysis of volatile components, and to establish a tentative database of flavor components for Hanwoo beef.

### Materials and Methods

#### Sample preparation and experimental design

Muscle tissue samples were obtained from beef longissimus muscle of Hanwoo steers (30 mon old, 1'B carcass grade) at 24 h postmortem. Subcutaneous fat and connective tissues were trimmed off. Samples were vacuum-packed and stored at -20°C until use. Approximately 50 g of frozen samples were cut into small pieces, powdered in liquid nitrogen and stored at -80°C. Three separate experiments were designed to optimize 1) length of adsorption (30, 45, or 60 min) into the SPME fiber (Carboxen/PDMS, 75 im, Supelco Co.), 2) two GC-oven programs (36 or 50 min with different temperature ramps), and 3) conditions of thermal ageing (60 min-separate, 60 min-simultaneous, or 80 min-simultaneous) for the SPME fiber as these conditions are one of the most critical aspects for extraction and separation of volatile components in meat samples (i.e., Alpendurada, 2000). All experiments were replicated three times.

#### Experimental procedures

**Experiment 1**

One gram powdered sample was placed in a 40 mL headspace vial sealed with PTFE-faced silicone septum (Supelco Co., USA) and cooked in an autoclave at 132°C for 30 min. The cooked sample was immediately cooled in an ice bath to prevent further aroma development. An octagonal magnetic stirring bar was put into the vial and the vial was equilibrated at room temperature for 15 min if it was stored at -20°C after cooking. Thereafter, it was placed into the 60°C water bath contained in a water jacket and allowed to equilibrate to 60°C for 10 min. Water bath temperature was monitored by a Thermo recorder TR-52 (T & D Corp., Japan). The SPME needle (Carboxen/PDMS, 75 im, Supelco Co., USA) was inserted into the PTFE/silicone septum and the fiber was exposed after the 10 min-equilibration period. The extraction process for volatiles was carried out at 30, 45 or 60 min. At the end of extraction, the fiber was retracted and immediately inserted into the GC-MS machine (Agilent Technologies 6890N, 5973 MSD, USA) for the analysis of volatile compounds. The fiber was exposed for 10 min in the GC injector (250°C) and then retraction of the fiber into the needle (i.e., fiber holder). The needle was kept in the GC injector for an additional 50 min. This was done separately from the sample analysis in the GC machine.

A DB-5MS capillary column, 30 m×0.25 mm i.d.×0.25 μm film thickness (Agilent J & W Scientific, Model No. 122-5532, Folcom, USA) was aged overnight at 230°C. The SPME Carboxen/PDMS fiber was desorbed at 250°C at the injection port for 5 min with a split ratio of 10:1 and split flow of 10 mL/min. Helium was the carrier gas.