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SPME-GC/MS ANALYSIS OF KEY VOLATILE COMPOUNDS GENERATED FROM UNHEATED AMINO ACID AND REDUCING SUGAR MIXTURES

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Introduction

Flavor is essential for consumer satisfaction and the commercial success of meat products. Dry-cured meat products of excellent flavor are popular to consumers. Only through a long ripening period (12 22 °C for about six months or more), the typical flavor of dry-cured meat products can be developed (Toldrá, Flores and Sanz, 1997; Martín, Córdoba, Antequera, Timón and Ventanas, 1998). Ventanas, *et al.* (1992) reported that there were Maillard reactions during dry-cured ham processing. The Strecker degradation of amino acids, a minor pathway of the Maillard reaction, is very important in relation to the formation of a series of volatile organic flavor compounds: the Strecker aldehydes. This seems to be the important pathway for the development of dry-cured ham flavor.

To understand the contribution of Maillard reaction to meat flavor, the reactions between heated amino acids and sugar have frequently been studied in order to identify volatile compounds formed (Meynier and Mottram, 1995; Venskutonis, Vasiliauskaite, Galdikas and Šetkus, 2002; Hofmann and Schieberle, 1998; Adamiec, Rössner, Velíšek, Karel and Jan, 2001; Cremer, Vollenbroeker and Karl, 2000), but the volatile compounds obtained from unheated amino acids and reducing sugar mixtures have not been extensively studied, which may be a significant contributor to the flavor formation of dry-cured meat products during long-term ripening.

Objectives

The purpose of this study was to use HS (headspace) SPME (solid phase microextraction) coupled to GC/MS to investigate the generation of volatile compounds under a controlled temperature of 20 ± 2 °C which mimics the dry-cured meat ripening condition.

Methodology

Materials

L- Alanine, L- Arginine, L- Asparagines, L- Aspartic acid, L- Cysteine, L- Glutamine, L- Glutamic acid, L- Glycine, L- Histidine, L- Isoleucine, L- Leucine, L- Lysine, L- Methionine, L- Phenylalanine, L- Proline, L- Serine, L- Threonine, L- Tyrosine, L- Valine, D- Ribose were purchased from Beijing Biodee Biotechnlogy Co., Ltd (Beijing, China); D- Glucose, NaH_2PO_4, Na_2HPO_4 were purchased from Beijing Chemical Reagents Company (Beijing, China).

Preparation of Maillard reaction products (MRPs) in model system

Equimolar mixture of each of two sugars and each of 19 amino acids were dissolved in a 0.2 M sterilized phosphate buffer (pH 6.5). At the same time, solutions containing only D- Glucose or D- Ribose or L- Lysine were made as the control, and the final concentration of each substance was 0.02 M. Test tubes (15 × 200 mm) containing 15 ml of each mixed solution were sealed and placed in an incubator with the temperature of 20 ± 2 °C for 10 d and 30 d. Equimolar mixtures of the selected sugar and amino acids were prepared as the above method.

Sampling

In order to select model systems, volatile compounds of MRPs generated from equimolar mixture of each of two sugars and each of 19 amino acids were detected by HS-SPME coupled to gas chromatography respectively on 10 d and 30 d.

For the selected model systems, volatile compounds were detected on 15 d by coupling HS-SPME to gas chromatography-mass spectroscopy and the color intensities were determined on 0 d, 10 d, 15 d, 25 d and 30 d.

Volatile compounds analysis

SPME method

The SPME device and fiber [Carboxen/ Polydimethylsiloxane (CAR/PDMS), 75 μm film thickness] used were purchased from Supelco (Bellefonte, PA, USA). The fiber was exposed to the splitless/split injection port of an Agilent 6820 gas chromatograph (Aglient Technologies, USA) under nitrogen flow and conditioned before use as recommended by the manufacturer. Fifteen-ml screw-top clear vials, hole cap with PTFE/Silicone septa (Supelco, Bellefonte, PA, USA) containing 5 ml of liquid MRPs were introduced onto the sample table maintained at room temperature for 20 min to form the headspace and a 15 min adsorption of the volatile compounds onto the fiber were chosen.

Gas Chromatography

After extraction, analyses were performed using an Agilent 6820 gas chromatograph (Aglient Technologies, USA). SPME fiber was thermally desorbed at 220 °C in the injector port for 10 min, the spilt valve being opened after 1 min (split raito15). A DB-1701 column (30 m × 0.32 mm i.d.) coated with cyanoproyl-phenyl (1.0 μm film

thickness) (Agilent Technologies, USA) was used to separate the volatile compounds of the model systems, the solutions containing only D- Glucose or D- Ribose or L- Lysine were made as the controls. The oven was held at 38 °C for 2 min, heated to 90 °C at 3 °C /min, then raised to 220 °C at 10 °C /min and held at 220 °C for 3 min, the program used 34.33 min. Nitrogen was used as carrier gas with a constant flow of 1.0 ml/min. The temperature of FID was 250 °C.

Gas Chromatography / Mass Spectrometry

After extraction, analyses were performed using an Agilent 5973 ion-trap mass spectrometer (mass selective detector) (Aglient Technologies, USA) fitted with an Agilent 6890 gas chromatograph (Aglient Technologies, USA). SPME fiber was thermally desorbed at 220 °C in the injector port for 10 min, the spilt valve being opened after 1 min. The above column was used to separate the volatile components of the model systems. The oven was held at 35 °C for 2 min, heated to 90 °C at 3 °C/min, then raised to 220 °C at 10 °C/min and held for 3min and finally by 20 °C to 250 °C and held for 2min at this temperature, the program used 39.83 min. Helium was used as carrier gas with a constant flow of 1.4 ml/min. Transition – line temperature was 250 °C. The mass spectrometer scanned from 12 450 amu. Ionization energy was set at 70 eV. Identification of peaks was based on the comparison with the MS computer library NIST98. L – Software package and the volatile standards when they are available.

Determination of color intensity

The color intensities of each of the selected model MRPs were determined with a 752 UV spectrophotometer (Shanghai analytical equipment factory, China) as the absorbance at 420 nm against phosphate buffer (c.f. Kwak and Lim, 2004).

Statistical analysis

Data were analyzed using ANOVA (The SAS System for Windows V8).

Results & Discussion

The results of color change and SPME-GC determination showed that D- Glucose did not yield detectable browning with any of the amino acids under conditions used in this study (data were not given). D- Ribose could react with 19 amino acids resulting in a color change, yet the SPME-GC determination showed that only the reactions of D-Ribose with L- Leucine, L- Isoleucine, L- Valine and L- Methionine generated detectable volatile compounds. Therefore, these four model systems were chosen to study the color change at different reacting periods and to identify volatile compounds produced.

The identified key volatile compound obtained from the Leu+R model system was 3-methylbutanal and that from the Ile+R model system was 2-methylbutanal (Table 1). Volatiles from the Val+R were 2-methyl-2-propenal and 2-methylpropanal, and from the Met+R system was dimethyl disulfide. These volatile compounds were found in Parma ham (Hinrichsen and Pedersen, 1995) and Serrano ham (Dirinck, Van Opstaele and Vandendriessche, 1997) during processing.

The volatile compounds generated from unheated amino acids (L- Leucine, L-Isoleucine, L- Valine and L- Methionine) and reducing sugar mixtures under acidic

conditions in this study probably were *via* the generally accepted Strecker degradation pathway in thermal treated alkali conditions (Mottram and Donald, 1998; Cremer, Vollenbroeker and Karl, 2000). Considering the difference of the pH, the mechanisms for the formation of these volatile compounds should be studied in details in the future.

Figure 1 shows changes of color intensity of the four model MRPs at 0 d, 10 d, 15 d, 25 d and 30 d. The browning of the four model MRPs increased significantly ($P<0.05$) along with the reacting time. At 10 d and 25 d, the color intensities of the Val+R and Met+R model systems were significantly deeper ($P<0.05$) than that of Ile+R and Leu+R mixture.

Conclusions

Key volatile compounds were generated from the reaction of D- Ribose with four amino acids L- Leucine, L- Isoleucine, L- Valine and L- Methionine at 20 ± 2 °C. These compounds might be important in the flavor development of dry-cured meat products.

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Tables and Figures

Table 1. Key volatile compounds identified from the four model systems by SPME-GC/MS

Model system*	Key volatile compounds
Leu+R	3-methylbutanal
Ile+R	2-methylbutanal
Val+R	2-methylpropanal, 2-methyl-2-propenal
Met+R	dimethyl disulfide

* Model system D- Ribose and L- Leucine mixture (Leu+R), D- Ribose and L- Isoleucine mixture (Ile+R), D- Ribose and L- Valine mixture (Val+R), D- Ribose and L- Methionine mixture (Met+R).

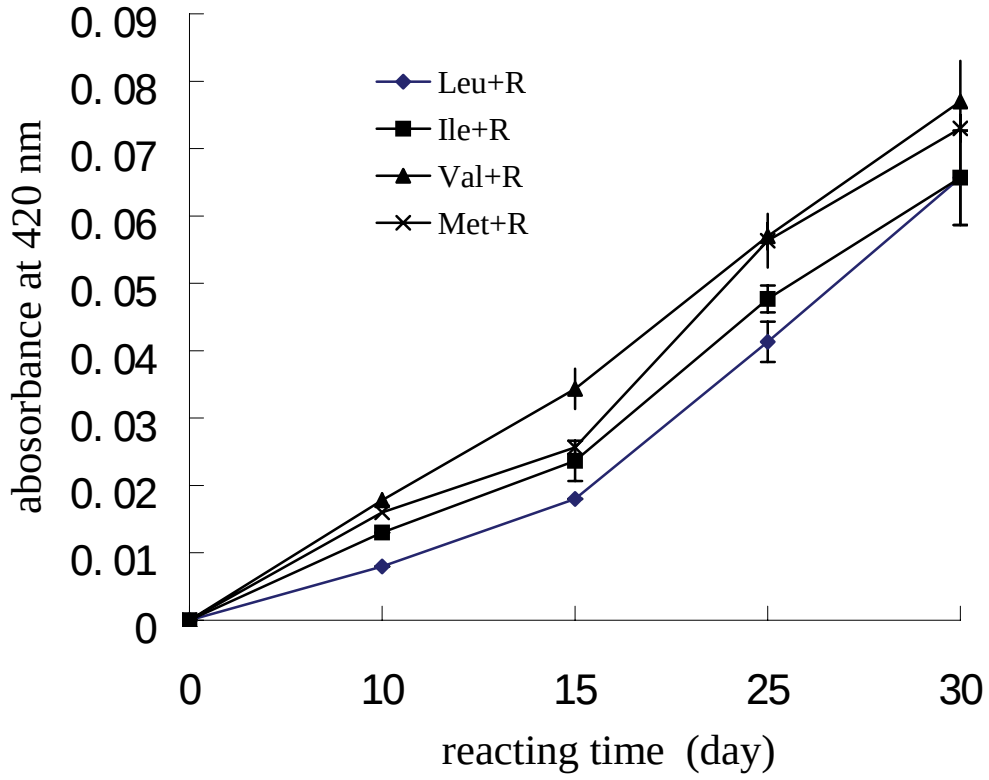

Fig.1 Changes of color intensity of four model MRPs prepared by D-Ribose with four amino acids at 20 ± 2 °C during reacting (n = 3).