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Contribution to aroma characteristics of mutton process flavor from the enzymatic hydrolysate of sheep bone protein assessed by descriptive sensory analysis and gas chromatography olfactometry

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ABSTRACT

Changes in the aroma characteristics of mutton process flavors (MPFs) prepared from sheep bone protein hydrolysates (SBPHs) with different degrees of hydrolysis (DH) were evaluated using gas chromatography–mass spectrometry (GC–MS), gas chromatography–olfactometry (GC–O), and descriptive sensory analysis (DSA). Five attributes (muttony, meaty, roasted, mouthful, and simulate) were selected to assess MPFs. The results of DSA showed a distinct difference among the control sample MPF0 and other MPF samples with added SBPHs for different DHs of almost all sensory attributes. MPF5 (DH 25.92%) was the strongest in the muttony, meaty, and roasted attributes, whereas MPF6 (DH 30.89%) was the strongest in the simulate and roasted attributes. Thirty-six compounds were identified as odor-active compounds for the evaluation of the sensory characteristics of MPFs via GC–MS–O analysis. The results of correlation analysis among odor-active compounds, molecular weight, and DSA further confirmed that the SBPH with a DH range of 25.92–30.89% may be a desirable precursor for the sensory characteristics of MPF.

1. Introduction

Mutton is widely consumed and increasingly popular in Western countries because of its nutritional and special flavor qualities [1]. Clearly, opportunities exist for the expansion of the mutton market. Meanwhile, much attention has been given to the development of simulated mutton flavor to meet different consumer demands (e.g., meatless products with special meat flavor) and different applications in food.

Abundant information on meat flavors from beef, chicken, pork, and other domestic red meat species is available. However, published data, particularly on mutton flavor, are limited. Although a few papers described the volatile compounds of cooked goat meat [2–4], the effect of precursors on mutton flavor formation remains unknown. No study that describes simulated mutton flavor has been found in the literature.

Meat flavor precursors can be divided into two categories: water-soluble components and lipids [5]. The former provides the "basic meat flavor" through Maillard reaction and, together with the latter, imparts the "characteristic flavor" through the coordination of Maillard reaction and lipid oxidation. In general, water-soluble precursors include free amino acids, peptides, and reducing sugars [6]. The different compositions of these precursors, which can be derived by controlling the degree of hydrolysis (DH), generate a significant difference in flavor because of the different levels of volatile formation under thermal reaction conditions [2]. Therefore, precursors play an important role in thermal reaction flavor. To obtain desirable flavor precursors, especially free amino acids and peptides, enzymatic proteolysis of the protein from various sources has been studied extensively. For many years, enzymatic proteolysis of hydrolyzed vegetable protein (HVP) and meat protein has been used to produce precursors of meat flavor [7–9]. Moon et al. [10] showed that simulated beef flavor could be obtained by adding soy protein isolates. These simulated meat flavors were favored by vegetarian and health-conscious consumers. Song et al. [11] reported that beef base prepared by beef hydrolysis with a suitable DH is useful in accentuating or extending the characteristic meat flavor. However, meat flavors based on HVP only partially simulate natural meat flavor and, from an economic point of view, meat flavor based on meat protein has negligible economic advantage. In contrast to the above two sources, enzymatic proteolysis of the protein of meat by-products does not only simulate realistic meat aroma but also reduce costs and increase their value.

As one of the by-products of the mutton processing industry, sheep bone is a rich resource of protein, amino acids, and other useful biological substances. In a previous study, sheep bone has

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a similar of protein and amino acid content to mutton. Collagen and chondroitin, which comprise approximately 90% of the protein in sheep bone, have higher physiological activities than common peptides. They could be converted into collagen peptides and amino acids by moderate enzymatic hydrolysis [12]. These moderate enzymatic hydrolysates can be used as the precursors for preparing mutton process flavors. Methods for controlling the DH to accomplish moderate enzymatic hydrolysis require further investigation. Therefore, the effects of the different compositions of amino acids and the different molecular weight (MW) distribution of peptides on the aroma characteristics of simulated mutton flavor should be determined. However, systematic studies on the effects of bone hydrolysate with different DHs on the aroma characteristics of simulated mutton flavor are lacking.

The primary objectives of this study are to evaluate the changes in the aroma characteristics of mutton process flavors (MPFs) prepared from sheep bone protein hydrolysates (SBPHs) with different DHs using descriptive sensory analysis (DSA) and to analyze the corresponding volatile odor-active compounds by gas chromatography–mass spectrometry–olfactometry (GC–MS–O). Through a correlation analysis of the DSA data, odor-active compounds, and MW of peptides, desirable SBPHs with the appropriate DH were recommended for mutton flavor, aiming to produce a desirable precursor for the sensory characteristics of mutton flavor. This study also aims to change waste products into useful materials and increase the utilization of sheep bone protein. The results of this study serve as a theoretical basis for the utilization of livestock bone.

2. Materials and methods

2.1. Materials

Lean mutton and sheep bone were purchased from Wal-Mart supermarket in Wuxi, China. HVP was provided by Tianning Flavor Fragrance Co., Ltd. (Shanghai, China). Serial n-alkanes (C_6–C_{26}), L-cysteine, alanine, glucose, thiamine, and taurine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). I + G (5′-IMP:5′-GMP = 50%:50%) was provided by Guangdong Dinghu Biotechnology Co. (Guangdong, China). Refined suet was purchased from Tianjin Tianyuan Oil and Fats Co., Ltd. (Tianjin, China). Alcalase and Flavorzyme were provided by Novozymes (Bagsvaerd, Denmark). Pure standards hexanol, heptanol, sulfurol, hexanal, heptanal, octanal, nonanal, benzaldehyde, acetic acid, pentanoic acid, hexylic acid, nonanoic acid and decanoic acid were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pure standards decanal, tetradecanal and 2-tridecanone were purchased from Tokyo Chemical Co. (Tokyo, Japan). Pure standards (E,E)-2,4-octadienal, (E,E)-2,4-dodecadienal, 12-methyltridecenal, 4-methylnonanoic acid, butyl 2-decenoate, 2,3-dimethyl pyrazine and benzyl methyl sulfide were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

2.2. Sample preparation

2.2.1. Preparation of SBPH

Fresh sheep bones were washed with tap water to clean the bloodstain and other impurities and autoclaved at 121 °C for approximately 4 h. The attached meat, fat, bone marrow, and fascia were removed, and the bones were washed repeatedly in hot water to eliminate residual fat. Subsequently, the bones were dried in an oven at 55 °C for 5 h and then ground into powder (80 orders) using a grinder. Bone powder (20 g) was mixed with 80.0 mL of deionized water. Afterward, the mixture dispersion was heated at 95 °C for 10 min to deactivate endogenous enzymes and denature bone protein. Enzymatic hydrolysis of bone powder was performed in two steps. Alcalase was utilized in the first step, and Flavorzyme was used in the second step. Hydrolysis was conducted using Alcalase at 55 °C, pH 8.5 (1.0 mol/L NaOH) with an E/S of 0.20 (LAPU/g based on protein content) for 2 h and then treated at 50 °C using Flavorzyme with an E/S of 0.20 (LAPU/g protein content). The pH was adjusted to 7.0 using HCl (1.0 mol/L) for 1, 2, 3, 4, 5, 6, and 7 h to prepare SBPHs with different DHs. After hydrolysis, the reactions were terminated by immersing the reaction vessel into a 95 °C water bath for 10 min with stirring to ensure the inactivation of the enzyme. The sample was then centrifuged at 4000 rpm for 20 min to remove the insoluble residue. The supernatant liquid was used for further analysis.

2.2.2. Preparation of MPF

A mixture of HVP (0.5 g), L-cysteine (1 g), glucose (0.5 g), thiamine (0.5 g), taurine (0.5 g), alanine (1 g), I + G (0.5 g), and oxidized suet (4 g) was dissolved in 81.5 g solution of SBPH. The solution was transferred into 50 mL screw-sealed tubes. The pH was adjusted to 6.5 with 6 mol/L NaOH, and the tubes were tightly capped and then heated in a thermostatic oil bath with magnetic stirring (150 rpm) at 120 °C for 120 min. After the reaction, the tubes were immediately cooled in ice-water, and the thermal reaction products named MPFs were obtained for further use.

2.3. Analysis of SBPH properties

2.3.1. MW distribution

To quantify the MW distribution of the hydrolysates, the SBPH samples were analyzed in triplicate using liquid chromatography. The SBPH samples were centrifuged at 3500 rpm for 30 min, and the supernatant liquid was stored at 4 °C prior to injection.

The MW distribution of the hydrolysate was determined using a Waters™ 600E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA) with a 2487 UV detector and Empower work station. A TSK gel, 2000 SWXL (300 mm × 7.8 mm) column (Tosoh Co., Tokyo, Japan) was used with 0.1% trifluoroacetic acid and acetonitrile (45:50) as the mobile phase. The flow rate was 0.5 mL/min. The column temperature was 30 °C, and 10 μL of sample was injected into the HPLC system. A MW calibration curve was obtained using 0.5 mg/mL each of the following standards from Sigma Chemical Co. (St. Louis, Mo, USA): cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYR (451 Da), and tripeptide GGG (189 Da). SBPH samples prepared according to the above-mentioned method were diluted 25 times with water and filtered by microporous filtering film before injection. Absorbance was monitored at 220 nm. The results were obtained and processed with the aid of Millennium 32 Version 3.05 software.

2.3.2. Amino acid analysis

An appropriate pretreatment of the SBPH samples was conducted before free amino acid analysis. An equivalent volume of trichloroacetic acid was added to the SBPH sample to precipitate the peptides and proteins. After incubation for 2 h at room temperature, the solution was filtered through Whatman filter paper No. 4. The filtrate was centrifuged at 7000 × *g* for 10 min, and the supernatant liquid was stored at 4 °C before injection.

The amino acid composition of the sheep bone protein was determined by RP-HPLC (Agilent 1100, Palo Alto, CA, USA) with a UV detector operated at 338 nm using a Hypersil ODS C18 column (4 mm × 125 mm, Thermo Co., NY, USA). The mobile phase consisting of 20 mM sodium acetate and 1:2 (v/v) methanol–acetonitrile was delivered at a flow rate of 1 mL/min. The column temperature was 40 °C, and 1 μL of sample was injected into the HPLC system. A calibration curve was obtained using 0.25–2 mM standard amino