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Toxicological analysis of roast duck flavor components

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ABSTRACT

The aim of the study was to investigate toxicity of the synthesized roast duck flavor through animal experiment (mice feeding with the flavor for 35 days), and the major toxic compounds (acrylamide and 3,4-benzopyrene) were detected by high performance liquid chromatography. Compared with the control group, the blood biochemical indexes including protein content, bilirubin content, activity of alkaline phosphatase, activity of aspartate transaminase (AST) and alanine transaminase (ALT), cholesterol content, high density lipoprotein (HDL) and low density lipoprotein (LDL) content, triglycerides content, activity of creatine kinase (CK) and CK-MB, activity of cholinesterase (CHE) and lactate dehydrogenase (LDH), total bile acid (TBA) in high dose feeding group were significantly different. And body weight of mice fed by the flavor was decreased distinctly, and the heart weight was also decreased, while the liver weight was increased obviously. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in liver and heart were significantly decreased, while methane dicarboxylic aldehyde (MDA) contents were increased evidently. Acrylamide was detected by high performance liquid-mass spectrum (HPLC-MS), and the content was 5.21 mg/kg 3,4-benzoapryene was calculated by HPLC with fluorescence detector, and the content was 21.13 μg/kg. Thus, the roast duck flavor was considered to be potential toxicity to human being.

1. Introduction

In recent years, meat flavors are used widely in food industry as food additives to improve the flavor of some foods or substitute the real meat. It is known that the most used meat flavors are beef flavor, pork flavor and chicken flavor (Yuasa and Tsurata, 2004). The meat flavors are mainly man-made, and three methods have been developed to produce them. The first method is using hydrolytic plant proteins as basic material, and adding some sauce, natural and synthetical flavors; the second is employing Maillard reaction products as the basic, and adding some flavor; the third is absolutely allocated by sauce, natural and synthetical flavors. The second method is frequently used in food industry to prepare meat flavors, since economic benefit and practicability (Sun, 1998).

Maillard reaction is one kind of non-enzymatic browning or glycation reaction (Silván et al., 2006). It occurs between amino groups (from amino acids, peptides or proteins) and carbonyl groups of reducing sugars during food storage and processing, including frying, baking or roasting (Wang et al., 2011). In those processes, Maillard reaction is favorable for it can improve food color and flavor. But it is unfavorable during food drying, because pasteurization and sterilization can cause nutritional losses of essential amino acids as well as formation of some undesired compounds (Jaeger et al., 2010). In the last decades, a lot of researches have been conducted in controlling Maillard reaction products in food industry to reduce the nutritional losses, and obtain the desired color and flavor. The Maillard reaction has gained considerable importance in flavour chemistry and human pathology, and has important medical and technological implications (Troise and Fogliano, 2013; Yaylayan, 1997). Some meat flavors have been produced in this phenomenon. However, some papers have reported that MRPs are toxic. Skog et al. (1998) reviewed carcinogenic heterocyclic amines in model Maillard reaction, including 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline and 2-amino-1-methyl-6-imidazo[4,5-*b*] pyridine. Tessier and Birlouez-Aragon (2012) demonstrated that MRPs contribute to the development of diabetes and related complications.

Roast duck is one of the most well-known Chinese dishes, and one kind of representative roast foods. Chen et al. (2009) have researched the aroma-active compounds of Beijing roast duck by using aroma extract dilution analysis, dynamic headspace dilution analysis and gas chromatography-olfactometry-mass spectrometry. The identified compounds were derived from degradation of fatty acids and amino acids, Maillard/Strecker and other associated reactions, which were considered to account for the typical aroma of Beijing roast duck. Lin et al. (2011) have assessed food safety of roast duck by determining content of benzo(*a*)pyrene or the total amount of major PAHs, which are potentially carcinogenic for humans. In view of universal application of

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meat flavor in food industry, its safety should be considered. Hence, roast duck flavor was prepared and its toxicity was evaluated.

In the present study, roast duck flavor was prepared by using Maillard reaction according to the condition of roast duck. The objective of this study was to assess toxicological safety of the roast duck flavor through toxicity test, including blood biochemical indexes and organ indexes in the mice, and determine the major toxicological components in the flavor.

2. Materials and methods

2.1. Chemicals

L-Cysteine, L-Glycine, glucose, D-Xylose, methanol (HPLC grade) tetrahydrofuran (HPLC grade) and acetonitrile (HPLC grade) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Duck meat was obtained from local market in Shanghai, China. 3,4-benzopyrene and acrylamide was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of roast duck flavor

Duck meat was frozen dried to powder by a freeze dryer (FD-80, Beijing Boyikang Instrument Co., Ltd., China). 20 g of the powder was weighted into the cylinder, and added L-Cysteine (0.5 g), L-Glycine (0.5 g), glucose (3 g), D-Xylose (1.0 g) and water (25 ml). The reaction was started in a compact reactor (PARR 5500, Parr Instrument Company, USA) at 125 °C for 40 min(Yang et al., 2010).

2.3. Animal experimental procedure

Male Kunming mice (30 days old, weight 20–25 g) were purchased from Shanghai Slakey, laboratory animal Co., Ltd. The mice were keep in the temperature and humidity controlled room (50%, 25 °C), and free access to standard water and food. After 7 days of adaptation, the mice were divided into 4 groups randomly, the control group and the test groups, including low, medium and high dose. Mice in the test groups were introgastric gavage administered with the flavor (1.0, 2.0, and 4.0 g/kg body weight) every seven days for 35 consecutive days. The control group was accessed to standard water and food. Before administered, body weight of the mice was weighted.

After three days of the last gavage, the mice were decapitated and blood was collected. Biochemical indexes (protein content, bilirubin content, activity of alkaline phosphatase, activity of AST and ALT, cholesterol content, HDL and LDL content, triglycerides content, activity of CK and CK-MB, activity of CHE and LDH, TBA) of the blood were analysis by Analysis Center of Central Hospital in Fengxian District, Shanghai, China.

The heart and liver were removed rapidly and added normal saline to a concentration of 10% (w/w). A cross homogenate was used to homogenize them, then removed the cell debris by centrifugation (4000 r/min, 10 min) at 4 °C. The supernatant was collected and conserved for analysis. According the methods and steps described in specification of the test kit (Nanjing Jiancheng Bioengineering Institute) to measure activities of SOD, MDA and GSH-Px.

2.4. Analysis by HPLC-MS of acrylamide in the flavor

The analysis of acrylamide by HPLC-MS was performed with a modified method applied for the analysis of acrylamide in heated foodstuffs (Tareke et al., 2002). The flavor was diluted with distilled water, and filtered through a syringe filter (0.22 μm micropore film). The filtrate was collected for HPLC analysis.

Chromatographic analysis was performed on a Shimadzu (Kyoto, Japan) HPLC system, consisting of an SIL-20A auto sampler, a CTO-20A column oven, an SPD-M20A PDA and LC-20AD binary pump. Chromatographic separation was executed at 25 °C on an INERTSIL ODS-SP column (4.6 × 250 mm, 5 μm). The mobile phase consisted of ultrapure water (A) and methanol (B), and carried out in a linear gradient, 0 min (5% A, v/v)-10 min (5% A)-25 min (35% A)-50 min (65% A). The flow rate was kept at 0.4 mL/min with detection at 284 nm, and the injecting volume was set at 10 μL.

The mass spectrometry was analyzed by the MS-2020 Shimadzu (Kyoto, Japan) with electrospray positive ionization (ESI^+) and Quadrupole Mass Analyzer. The MS analysis worked using full scan mode and mass range was set at m/z 50–400 in both positive and negative modes. The $[M+H]^+$ ion for the target analysis of acrylamide was at m/z 72. The flow rate of drying gas was at 15.0 L/min, and temperature was at 400 °C.

2.5. Analysis of 3,4-benzopyrene in the flavor by HPLC

The analysis of **3,4-benzopyrene** was conducted using a LC-10AT liquid chromatography, consisting of a RF-10AXL Fluorescence Detector, a LC-10AT VP Plus Pump, a CTO-10AS VP Plus Column Oven, and a CBM-20A system controller.

2 g of the flavor was weighted into a tube of 10 ml, and added 5 ml tetrahydrofuran. The acetonitrile was used to reach the total volume of 10 ml. The solution was filtered through a syringe filter (0.22 μm micropore film), and the filtrate was collected. Chromatographic separation was executed at 25 °C on an INERTSIL ODS-SP column (4.6 × 250 mm, 5 μm). The mobile phase consisted of ultrapure water and acetonitrile (12:88, v/v), and the flow rate was at 1.0 ml/min. The injection volume was at 10 μl. The excitation wavelength was at 384 nm, and the emission wavelength was at 406 nm (Doremire et al., 1979).

2.6. Statistical analysis

Toxicological experiments were conducted at least in triplicate. Data were analyzed by analysis of variance and using SPSS software (SPSS for Windows, 16.0, 2007, SPSS Inc., USA), and reported as mean ± standard deviation (SD). Significant differences were determined using Duncan's multiple-range test at $P < 0.05$.

3. Results and discussion

3.1. Variation of body weight

As shown Fig. 1, body weight was increasing with the feeding time increased in the control group. Body weights of mice exposed to the flavor were increasing with the feeding time increased before the

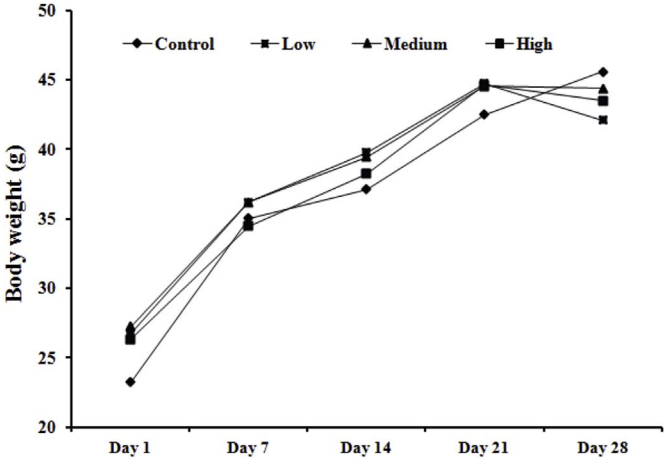

Fig. 1. Variations of body weight in the process of the mice fed by low, medium, high dose roast duck flavor, and normal diet, respectively.