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Monitoring the contents of biogenic amines in sufu by HPLC with SPE and pre-column derivatization

Tao Tang [a], Kun Qian [a], Tianyu Shi [a], Fang Wang [a], Jianqiang Li [a], Yongsong Cao [a,*], Qiongbo Hu [b,**]

[a] College of Agriculture and Biotechnology, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, PR China
[b] College of Natural Resources and Environment, South China Agricultural University, 483#, Wushan Road, Tianhe, Guangzhou 510642, PR China

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ABSTRACT

Sufu is a nutritious fermented food whose consumption is currently increasing in many countries. The purpose of this study was to develop an HPLC method with SPE and pre-column derivatization for monitoring the content of biogenic amine in sufu and to understand the differences in the contents of biogenic amines with different processing technologies. Biogenic amines in sufu were extracted with 6% trichloroacetic acid, derivatized with 4-chloro-3,5-dinitrobenzotrifluoride, purified with Waters Oasis MCX cartridge, separated by HPLC using gradient elution, and detected by spectrophotometric UV at 254 nm. The method linearity, calculated for each biogenic amine, has a correlation coefficient higher than 0.9915. Detection limits of the biogenic amines were ranged from 0.016 mg/kg for putrescine to 0.067 mg/kg for spermine. Spiking experiments showed that the overall recoveries ranged between 83.6% and 103.5% with relative standard deviations of 1.26–5.24%. Of the seven biogenic amines under study, at least four biogenic amines were detectable in different kinds of sufu. Tryptamine, putrescine, histamine, and tyramine were the most prevailing biogenic amines in all sufu samples. The mean values of histamine, tryptamine, phenylethylamine, tyramine, putrescine, spermidine and spermine determined in samples were found to be 18.2 mg/kg, 39.0 mg/kg, 1.1 mg/kg, 21.7 mg/kg, 34.6 mg/kg, 11.1 mg/kg, and 8.7 mg/kg, respectively. The total contents of biogenic amines in bacteria fermented sufu samples (129.0 mg/kg) were slightly lower than those in enzymatically ripened sufu samples (137.7 mg/kg). The concentrations of biogenic amines detected in sufu samples were below the maximum permissible limits.

1. Introduction

In recent years, the demand of consumers for food safety as well as healthier products has promoted the investigation for food with harmful compounds. Among these toxic compounds, the presence of biogenic amines (BAs) in fermented foods, such as beer (Loret, Deloyer, & Dandrifosse, 2005), cheese (Komprda et al., 2007), aged meat (Lorenzo, Martínez, Franco, & Carballo, 2007), wine (Marques, Leitão, & San Romão, 2008) and fishery products (Anderson, 2008; Özogul, Özogul, & Kuley, 2008), have received considerable interest due to their undesirable physiological effects in humans. Biogenic amines in low concentrations are essential for many physiological functions; while at high concentrations can cause some deleterious effects. For this reason, US Food and Drug Administration (FDA) established a tolerance level of histamine (500 mg/kg) in canned albacore, skipjack, and yellow fin tunas as an indication of potential health hazard danger to health (Food and Drug Administration, 1995). The levels of histamine in food were restricted by the European Community (100 mg/kg), South Africa (100 mg/kg), and Australia (200 mg/kg) (Auerswald, Morren, & Lopata, 2006; Carelli, Centonze, Palermo, Quinto, & Rotunno, 2007; Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997). The acceptable levels of histamine, tyramine, and phenylethylamine in fermented foods were 50–100 mg/kg, 100–800 mg/kg, and 30 mg/kg, respectively (Nout, 1994). The maximum concentration of histamine is about 2–20 mg/L in alcoholic beverages and 100–200 mg/kg in fish (García-Villar, Hernández-Cassou, & Saurina, 2009; Park et al., 2010), whereas the potentially toxic concentration of tyramine ranges between 100 mg/kg and 800 mg/kg (Silla Santos, 1996). In view of the possible harmful effects of biogenic amines, their concentration levels in foods deserve careful investigation.

Sufu is known as tofuyo, nyu-fu or fu-nyu in Japan, chao in Vietnam, ta-huri in Philippines, taokaoan in Indonesia and tao-hu-yi in Thailand (Beuchat, 1995). In China, sufu is one of the most important traditional fermented soybean foods and is a popular

* Corresponding author. Tel.: +86 10 62734302 (Office); fax: +86 10 62734302.
** Corresponding author. Tel.: +86 20 85280308 (Office).
E-mail addresses: caoysong@126.com (Y. Cao), hqbscau@126.com (Q. Hu).

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side dish consumed mainly with breakfast rice or steamed bread. Two types of sufu can be distinguished according to the processing technologies. One is the bacteria fermented sufu that five steps are involved in making this type of sufu; preparing tofu, pre-salting, preparing pehtze with a pure culture bacterial fermentation, salting, and ripening. The ripening time normally takes less than 3 months (Chou & Hwan, 1994). The other is enzymatically ripened sufu that three steps are involved in making this type of sufu; preparing tofu, salting, and ripening. Because there is no fermentation before ripening, some koji is added in the dressing mixture for enzymatic ripening. The ripening time takes 6–10 months (Huang, 1991). The annual production was estimated over 300,000 metric tons in China (Han, Rombouts, & Robert Nout, 2001). It is necessary to monitor the contents of biogenic amines in sufu considering the amount consumed in China.

The difficulties in the analysis of biogenic amines in food are the structure and low concentration present in complex matrices. Biogenic amines do not possess absorption moieties in the visible, ultraviolet, or fluorescent wavelength ranges. For that reason, many derivatization agents is usually used to reach the levels of selectivity and sensitivity required for determination, such as 3,5-dinitrobenzoyl chloride (Kirschbaum, Rebscher, & Brückner, 2000), benzoyl chloride (Paleologos, Chytiri, Savvaidis, & Kontominas, 2003), N-(9-fluorenylmethoxycarbonyloxy) succinimide (Lozanov, Petrov, & Mitev, 2004) *o*-phthalaldehyde (Pereira, Pontes, Câmara, & Marques, 2008), dansyl chloride (Dugo, Vilasi, Torre, & Pellicanò, 2006; Gosetti, Mazzucco, Gianotti, Polati, & Gennaro, 2007; Proestos, Loukatos, & Komaitis, 2008), and 4-chloro-3,5-dinitrobenzotrifluoride (Tang et al., 2009). Although the derivatization can improve the selectivity and sensitivity, the matrix interferences in complex samples cannot be eliminated efficiently. Therefore, sample pretreatment and preconcentration steps are required to make the detection more accurate. Solid-phase extraction (SPE), as a commonly used sample preparation technique, has been widely used for the determination of biogenic amines or as a means to enrich biogenic amines in most matrices (Loukou & Zotou, 2003; Proestos et al., 2008). The Waters Oasis MCX cartridge, containing a mixed mode polymeric sorbent with reversed-phase and cation exchange functionalities is reported to be very similar to the C_{18} sorbent while has higher selectivity for basic compounds in the elution solutions. These characteristics of MCX cartridge favor the removal of fat or protein and improve the extraction efficiency for some specific compounds.

To the best of our knowledge, no information is available on quantitative determination of biogenic amines in traditional Chinese sufu. The purpose of this study was to develop an HPLC method with SPE and pre-column derivatization for determination of the biogenic amine in sufu samples, and to understand the differences in the contents of biogenic amines with different processing technologies.

2. Materials and methods

2.1. Standard and reagents

Seven biogenic amines (putrescine (PUT), histamine (HIM), tyramine (TYR), phenylethylamine (PHE), spermine (SPM), spermidine (SPD), tryptamine (TRYP); ≥99.0%) were purchased from J&K Chemical Co. Ltd. (Beijing, China). 4-Chloro-3,5-dinitrobenzotrifluoride (CNBF) was obtained from Alfa Aesar (Ward Hill, MA, USA). Acetonitrile and methanol were HPLC grade and purchased from J.T. Baker (USA). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA). All other chemicals and solvents were analytical grade and from the local commercial sources.

2.2. Apparatus and HPLC conditions

The cleanup and preconcentration of the biogenic amine derivatives were carried out on Oasis MCX cartridges (6 mL, 500 mg, 60 μm) were from Waters (Milford, MA, USA). The quantification of the biogenic amine derivatives was carried out by using a Shimadzu HPLC system consisting of two LC-10ATvp pumps and an SPD-10Avp ultraviolet detector, an SCL-10AVP system controller and two DGU-14A degassers. The concentration of biogenic amine derivatives was obtained from data processed with Shimadzu software Class-VP 5.03. The chromatography column was a kromasil ODS C_{18} column (250 × 4.6 mm) packed with 5 μm particles and connected with a guard cartridge (kromasil easyguard C_{18}-6201). Before the analysis, the column was pre-equilibrated with the mobile phase for 30 min. HAc–NaAc buffer (10 mmol/L, pH 6.2) (eluent A) and acetonitrile (eluent B) were used as mobile phase. All the solvents were filtered with a 0.45 μm membrane filter. The gradient elution was set for a liner gradient starting from 70% of solvent B to 100% of the solvent at 22 min the injection volume was 20 μL. The detection wavelength was at 254 nm. The flow rate was constant at 1.0 mL/min and the column temperature was at room temperature.

2.3. Preparation of solutions

Stock solutions of the selected biogenic amines were prepared separately by adding an accurately 100 mg of each amine to a 100 ml volumetric flask and brought to the mark with 0.1 M HCl. Working solution was prepared by mixing aliquot of the stock solution with distilled water. CNBF (5 mg/mL) solution was prepared by dissolving 50 mg in 10 ml of methanol and filtered through a 0.45 μm nylon membrane filter. H_3BO_3–$Na_2B_4O_7$ buffer was prepared by mixing 0.2 mol/L H_3BO_3 solution with 0.05 mol/L $Na_2B_4O_7$ solution to the required pH value. HAc-NaAc buffer was prepared by mixing 0.1 mol/L HAc solution with 0.1 mol/L NaAc solution to the required pH value. All the solutions were stored at 4 °C until the use.

2.4. Sufu samples and extraction

Ten samples including five bacteria fermented sufu and five enzymatically ripened sufu from different manufactures were purchased at local supermarkets. A list of all sufu samples analyzed in this study is presented in Table 1. Sufu sample was ground in a blender for 3 min. The ground sample (5 g) was homogenized and extracted twice with 20 mL of 6% trichloroacetic acid (TCA). The homogenate was centrifuged (10,000 g, 10 min, 4 °C) and filtered through a 0.45 μm membrane millipore filter. The filtrate was then placed in volumetric flask, and 6% TCA was added to bring to a final volume of 50 mL.

Table 1
List of sufu samples analyzed in this work.

Sample No.	Type	Varieties	Areas (province)
1	BFs[a]	Kedong	Heilongjiang
2	BFs	Beikang	Jilin
3	BFs	Wangzhihe	Beijing
4	BFs	Laocaicheng	Beijing
5	BFs	Dexinzhai	Shandong
6	ERs[b]	Huaqiao	Guangxi
7	ERs	Xianheng	Zhejiang
8	ERs	Guanghe	Guangdong
9	ERs	Dingfeng	Shanghai
10	ERs	Xinzhong	Jiangsu

[a] Bacteria fermented sufu.
[b] Enzymatically ripened sufu.