THE DETERMINATION OF BIOGENIC AMINES IN CHINESE FERMENTED SAUSAGES

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Key Words: Biogenic amines, HPLC, Chinese fermented sausages

Introduction

Biogenic amines are toxic and often associated with the outbreaks of food poisoning, particularly histamine poisoning as well as genetic predisposition, gastrointestinal diseases and inhibiting amino oxidase activity to human and so on .Biogenic amines may also be considered as carcinogens because of their ability to react with nitrites to form potentially carcinogenic nitrosamines(Shalaby, 1996). Histamine, putrescine, cadaverine, tryptamine,tyramine,β-phenylethylamine, spermine and spermidine are considered to be the most important biogenic amines occurring in foods. HPLC is most often used for the analysis method of BAs (Önal, 2007).

BA, such as histamine, putrescine, cadaverine, tyramine,β-phenylethylamine, spermine and spermidine are widely reported in different type of meat and meat products particularly in dry fermented sausages. Dry fermented sausages can potentially support the accumulation of BA.In fact, the high amounts of proteins present in these products and the proteolytic activity during ripening provide the precursors for decarboxylase activity of starter cultures and wild microflora (Suzzi and Gardini, 2003).Excessive consumption of these amines can be of health concern because their not equilibrate assumption in human organism can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure(Silla Santos, 1996). For these reasons, it is important to monitor biogenic amines levels in fermented sausage. Chinese fermented sausages are popularly consumed throughout Southeast China .The objective of this paper is to determine the major biogenic amines and their concentrations of commercial fermented sausages produced in Zhejiang and Jiangsu province.

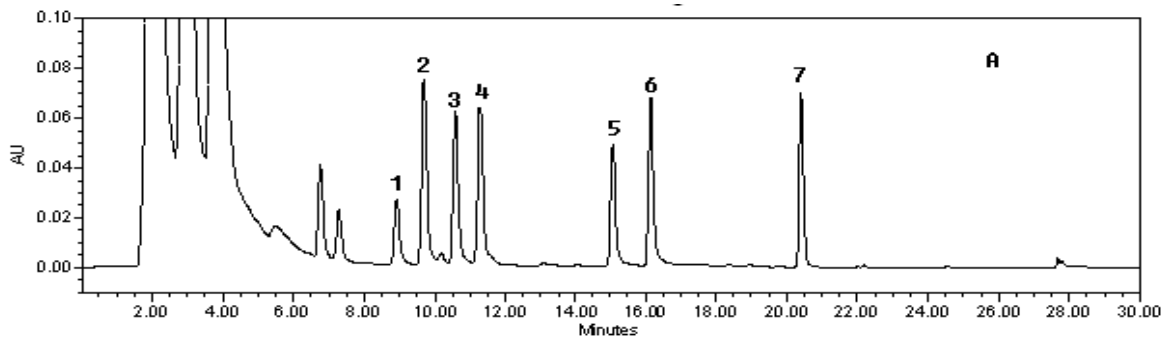
Materials and Methods

Twenty-four samples of eight types of sausages from four different manufacturers in Zhejiang and Jiangsu province were purchased at local supermarkets. Seven amines standards(Histamine, putrescine, cadaverine, tyramine,β-phenylethylamine, spermine and spermidine) were prepared in 0.4M perchloric acid to a final concentration of 1 mg/ml for each amine.Amine standard solutions and sample treatments used in this paper was described by Vincia and Antonelli (2002). The dansylationreaction proceedes at 40℃ for 30min (Buteau, et al., 1984) in darkness . One hundred μl of NH_4OH were added to stop the reaction and to remove residual dansylchloride. The final volume was adjusted to 5 ml by adding acetonitrile. The dansylatedamine solution obtained was filtered with 0.22 μm syringe filter and injected into the chromatograph.

Chromatographic conditions:

Waters Alliance 2695 Separations Module, connected to Waters 2487 Dual λ Absorbance Detector, with an Agilent ZORBAX SB-C18 (4.6×250mm^2, 5μm) column were employed. Column temperature (T_{col})＝30℃; flow rate＝1ml/min; injected volume＝20μl; detection wavelength (λ)＝254 nm were used. The mobile phase was a gradient elution program with water (solvent A) and acetonitrile (solvent B) as follows: The gradient elution procedure was 65%B at 0 min, 30%A + 70%B at 5 min,100%B at 20 min and 35%A + 65%B at 25 min. SPSS software was used to perform all statistical analyses. All data were expressed as means ± SD (n = 3).

Results and Discussion



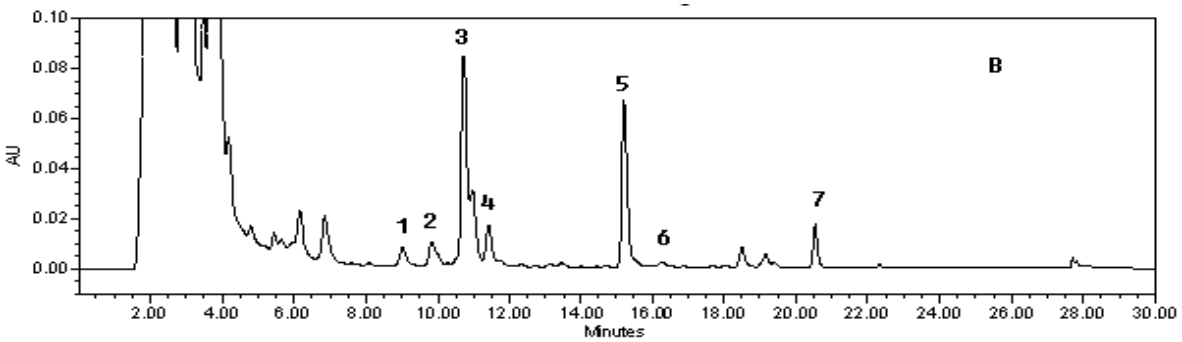


Figure 1. Chromatographic profiles relative to: (A:standard solution of seven biogenic amines:1:β-phenylethylamine(PHE),2:putrescine(PUT), 3:cadaverine(CAD), 4:histamine(HIS), 5:tyramine(TYR), 6:spermidine(SPD), 7 spermine(SPM); B:sausage samples)

The proposed method for BA determination in sausages, by means of chromatographic separation of their dansylchloride derivatives ,can be observed that this was a good resolution for peaks relating to all the amines examined in a quite short analysis time (about 22 min). In the chromatograms of fermented sausage samples we got satisfactory results with the seven biogenic amines under examination.

Table 1 Concentration of biogenic amines (mg/100g) in commercial fermented sausage

Sample	PHE	PUT	CAD	HIS	TYR	SPD	SPM	Total
01A	5.54±0.02	3.34±0.02	1.56±0.18	1.27±0.01	1.14±0.01	0.23±0.01	3.32±0.06	16.4
02A	3.56±0.16	1.41±0.08	0.85±0.07	0.15±0.01	1.01±0.18	0.24±0.04	2.78±0.08	9.99
03B	3.72±0.02	1.34±0.02	0.58±0.07	0.16±0.02	8.37±0.31	0.28±0.02	2.61±0.15	17.05
04B	4.73±0.05	2.02±0.12	0.72±0.08	0.46±0.04	8.09±0.45	0.26±0.01	2.85±0.13	19.13
05C	4.64±0.03	1.51±0.18	1.34±0.02	0.38±0.07	0.36±0.02	0.35±0.02	2.01±0.06	10.58
06C	6.09±0.01	2.99±0.22	1.79±0.04	0.56±0.06	0.47±0.06	0.43±0.04	2.74±0.02	15.07
07D	2.93±0.05	0.48±0.01	1.08±0.05	0.24±0.01	0.52±0.01	0.41±0.04	2.17±0.07	7.83
08D	4.31±0.07	1.87±0.02	1.30±0.03	0.35±0.03	0.55±0.06	0.38±0.01	2.36±0.06	11.12

A,B,C: manufactures in Zhejiang province; D:manufacture in Jiangsu province ; n=3 ;

The concentrations (mean±standard deviation) of biogenic amines and total biogenic amine levels in fermented sausages from manufacturers in Zhejiang and Jiangsu province are listed in Table 1 .Table 1 shows that each kind of sausage samples examined contains seven biogenic amines, respectively. In the sausages studied, the levels of seven amines were 2.93-6.09 mg/100g for β-phenylethylamine, 0.48-3.34 mg/100g for putrescine, 0.58-1.79 mg/100g for cadaverine, 0.15-1.27mg/100g for histamine,0.36-8.37 mg/100g for tyramine ,0.23-0.43 mg/100g for spermidine and 2.01-3.32 mg/100g for spermine. Overall, The mean total level of biogenic amines in samples was 13.40 mg/100g with a range from 7.83 to 19.13 mg/100g.

Conclusions

The proposed method for biogenic amines determination in fermented sausage samples showed valid. Using the proposed method, we determined the contents of biogenic amines of 8 different produced in Jiangsu and Zhejiang province. The results showed that Chinese commercial fermented sausages contain at least seven kinds of biogenic amines. The mean total level of biogenic amines in samples was 13.40 mg/l with a range from 7.83 to 19.13 mg/100g. These levels are below the level that may elicit direct adverse reactions for most consumers.

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