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A survey of biogenic amines in Chinese rice wines

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Abstract

Fourteen Chinese rice wines from four rice wine-making regions of China were analyzed for the first time, using HPLC with diode array detection after pre-column derivatization with dansyl chloride. The results showed that Chinese rice wines contained the five biogenic amines histamine, tyramine, cadaverine, spermine and spermidine. Histamine was detected in all samples (100%), followed by spermine (93%), cadaverine (87%), tyramine (79%) and spermidine (79%). The mean total level of biogenic amines in samples was 107 mg/l with a range from 39.30 to 241 mg/l. These levels are below the level that may elicit direct adverse reactions for most consumers. However, patients being treated with monoamine oxidase inhibitors should be aware of the presence of amines in rice wines and limit their consumption.
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Keywords: Biogenic amines; Chinese rice wines; Histamine; Tyramine; HPLC

1. Introduction

Biogenic amines are low molecular weight organic compounds which occur in fermented foods. They are mainly produced through microbial decarboxylation of amino acids (ten Brink, Damink, & Joosten, 1990). In recent years, more concern about food safety together with the consumer's demand for safe and healthier products have promoted the studies for compounds with harmful effects on human health. Among them, the presence of biogenic amines in fermented foods, such as cheese and other dairy products (Fernández-García, Tomillo, & Núñez, 1999), sauerkraut (Kalac, Spicka, & Krizek, 2000), fish (Ruiz-Capillas & Moral, 2002), sausages and meat (Kaniou, Samouris, & Mouratidou, 2001) and beer (Izquierdo-Pulido, Mariné-Font, & Carmen Vidal-Carou, 2000), have received considerable interest, owing to their undesirable physiological effects on sensitive humans. The harmful results will be more severe when they are taken together with ethanol because ethanol is a monoamine oxidase inhibitor (Blackwell & Mabbit, 1965). Most foodborne biogenic amines may cause headaches, hypotension and digestive problems, whereas aromatic amines, tyramine and phenylethylamine, have been associated with migraines and hypertension (Silla Santos, 1996). After some years of controversy about the origin of biogenic amines in wines, numerous researchers have presented evidence that in winemaking, amines are mainly formed during malolactic fermentation (Soufleros, Barrios, & Bertrand, 1998), by the action of lactic acid bacteria, causing decarboxylation of free amino acids (Victoria Moreno-Arribas, Carmen Polo, & Jorganes, 2000, 2003).

Rice wine is a traditional Chinese alcoholic beverage, which is consumed widely in south China and is also used as an ingredient in traditional Chinese medicine. Traditional rice wines are made from sticky rice, wheat and some medicinal plants or herbs, fermented by Chinese koji. The fermentation and ageing period varies from 6 months to 5 years, depending on the rice wine variety and fermentation process (Li Jianrong, 2005). During the fermentation and ageing, the favorable taste and flavor may develop gradually by several enzymatic reactions and microbial degradation processes. Meanwhile, rice wines contain relatively high amounts of free amino acids (Fan Huaide & Qiao

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Zilin, 2000), which could be potentially a source of biogenic amines. However, there is no report on the occurrence of biogenic amines in Chinese rice wines. The objective of this paper is to determine the major biogenic amines and their concentrations in some traditional rice wines from different regions and manufacturers.

2. Materials and methods

2.1. Standards and reagents

Biogenic amines standards (cadaverine, histamine, tyramine, spermidine and spermine) were from Sigma Chemical Co.; acetonitrile, acetone and dansyl chloride for HPLC from Merck; ultrapure water was obtained with a Milli-Q system (Millipore). Other reagents used in this study were HPLC grade.

2.2. Biogenic amines determination

2.2.1. Chromatographic system and chromatographic conditions

Agilent 1100 HPLC system was used, C18 column, 7 μm, $4.6\,cm \times 150\,mm^2$, with diode array detector. A gradient elution system with a mixture of acetonitrile (A) and water (B) was used. The gradient elution procedure was 35%A + 65%B at 1 min, 20%A + 80%B at 5 min, 10%A + 90%B at 6 min and 8%A + 92%B at 16 min.

2.2.2. Preparation of standard amine solution

Amine standard solutions were prepared in 0.4 M perchloric acid to a final concentration of 1 mg/ml for each amine. Solutions in 0.4 M perchloric acid to final concentration of 2.0, 5.0, 10, 20 and 40 μg/ml for each amine were prepared.

2.2.3. Derivatization procedure

The derivatization procedure used in this paper was described by Buteau, Duitschaever, and Ashton (1984). Standard amine solution or rice wine sample (100 μl) was pipetted into a screw-capped vial. To the vial, 0.2 ml of 2 M NaOH solution, 0.3 ml of saturated $NaHCO_3$ solution and 1.0 ml of dansyl chloride solution (10 mg/ml) were added, and the mixture was stirred in the dark at 40 °C for 0.5 h. Dansyl chloride was then precipitated with ammonia. The supernatant was placed in a volumetric flask and the volume was made up to 5 ml with acetonitrile. The complete extract was then filtered through a Millipore filter (pore size 0.45 μm) before HPLC analysis.

2.2.4. Preparation of samples

Forty-two samples of 14 rice wine varieties from eight different manufacturers in four typical rice wine-making areas were purchased at local supermarkets.

About 5 ml of each rice wine was homogenized for 30 min at 15 °C in an ultrasonic bath (UP3200HF, Panda Group, China). For analyses, 5 ml of each stock solution were diluted to 50 ml with 0.4 M perchloric acid. Dansylation was conducted in duplicate, following the procedure for the amine standards above. HPLC analysis of dansylated samples was carried out as described previously.

2.3. pH and ethanol concentration determination

The methods used for pH and ethanol concentration of the samples are described by ASBC (1987).

2.4. Statistical analysis

SPSS software was used to perform all statistical analyses. All data were expressed as means ± SD (mean of at least three determinations for each sample, n = number of samples).

3. Results and discussion

3.1. Determination of biogenic amines by HPLC

Fig. 1(a) and (b) show typical chromatograms of biogenic amines in standard solution and in a rice wine sample, respectively.

It can be observed that there was good resolution for the peaks related to the biogenic amines short analysis time (about 15 min). In the chromatograms of rice wine samples, there were free amino acid peaks, which did not interfere with those of the amines under examination, since they eluted before the amines (Moret, Smela, & Populin, 2005).

The reliability of the chromatographic method was studied in terms of repeatability, sensitivity, linearity and recovery. The repeatability of the method was estimated by the relative standard deviation (RSD) of the areas for five consecutive injections of a standard solution prepared in 0.4 M $HClO_4$ solution. The values obtained for this parameter were 0.7 for spermidine, 0.9 for spermine, 2.8 for cadaverine, 1.1 for histamine and 2.7 for tyramine. Detection limits were estimated from the area corresponding to three times the system noise (IUPAC, 1978) which was calculated as the mean of the area of the noise of seven injections of a 0.4 M perchloric acid solution. The values obtained were 0.05 mg/l for cadaverine, histamine and spermidine, 0.1 mg/l for tyramine and 0.25 mg/l for spermine.

The mean values of recovery are shown in Table 1. Recovery has been estimated as 100 * (amount found in the spiked sample – amount found in the sample)/amount added (Massart, Vandeginste, Deming, Michotte, & Kaufman, 1988). The mean values correspond to the individual values obtained from the recovery experiments and also to the values obtained for two more different rice wine samples. The mean values of recovery range from 96.6% for cadaverine to 107.4% for spermine.

Linear regression analysis of area versus concentration of biogenic amines in the standard solutions (Table 1). A linear relationship was obtained over the range of concentrations between 1.0 and 75 mg/l. The values of the

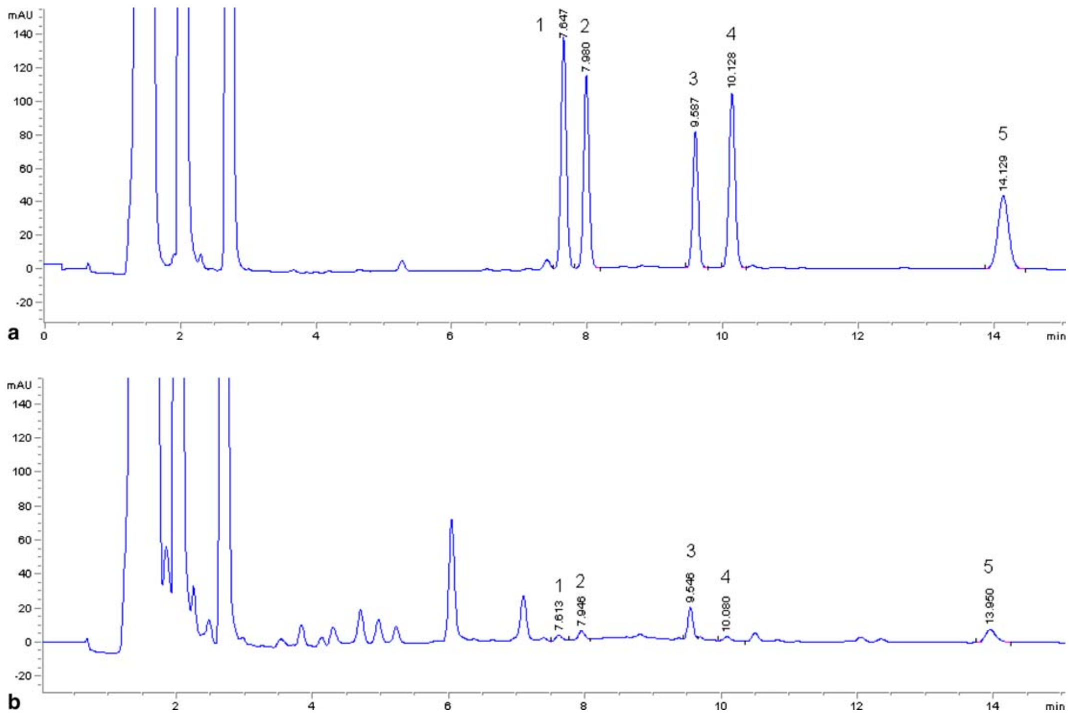

Fig. 1. HPLC chromatographic profiles of the dansyl chloride-derivatives of a biogenic amine standard solution (a); HPLC chromatographic profiles of the dansyl chloride-derivatives of biogenic amines in a rice wine sample (b). Peak identities: cadaverine (1), histamine (2), tyramine (3), spermidine (4), spermine (5).

Table 1
Regression equation for area versus concentration for standard solutions and recovery of the method

Biogenic amine	Regression equation	R^2	Recovery (mean values, %)
Cadaverine	$y = 48.9x$	1.00	96.6
Histamine	$y = 36.2x$	0.999	101.9
Tyramine	$y = 29.4x$	0.990	101.9
Spermidine	$y = 46.7x$	0.997	98.7
Spermine	$y = 31.2x$	0.993	107.4

R^2, coefficient of determination. Recovery, 100 * (amount found in the spiked sample − amount found in the sample)/amount added.

coefficient of determination (R^2 in Table 1) were all higher than 0.99.

3.2. Biogenic amines in rice wine

The concentrations (mean ± standard deviation) of biogenic amines in the rice wines from different producing areas and manufacturers are indicated in Table 2 Total biogenic amine levels, pH and alcohol content of rice wines are also shown in Table 2.

Table 2 shows that each kind of rice wine examined contains at least three biogenic amines. Histamine was detected in all samples. Spermine was detected in 93% of the rice wines: cadaverine, tyramine and spermidine occurred in 87%, 79% and 79% of the rice wines examined, respectively. In the rice wines studied, the levels of five amines were 0–101 mg/l for tyramine, 5.02–78.5 mg/l for histamine, 0–121 mg/l for cadaverine, 0–22.5 mg/l for spermidine and 0–33.6 mg/l for spermine. Overall, the average amount of the total biogenic amine content was 107 mg/l. These levels were higher than values reported in wines. In France, 54 red, 15 rose and 15 white commercial bottled wines from Vallée du Rhône were analyzed by HPLC (FMOC derivatization) to determine their amine content such as histamine, tyramine, phenylethylamine, putrescine and cadaverine. Only agmatine and putrescine levels were found to be higher than 1 mg/l in all samples (8% of the samples contained more than 20 mg/l of putrescine, 1.2% more than 10 mg/l of histamine and tyramine) (Bauza, Blaise, & Daumas, 1995). The biogenic amines contents of 109 different commercial Rioja DOC wines were determined with HPLC (OPA derivatization). The highest levels in the wines were 33.1 mg/l for putrescine, 1.74 mg/l for cadaverine, 5.98 mg/l for tyramine and 8.72 mg/l for histamine in analysed red wines(Vazquez-Lasa, Iniguez-Crespo, Gonzalez-Larraina, & Gonzalez-Guerrero, 1998). Thirty Portuguese wines (including fortified wines such as port) were analyzed with HPLC (OPA derivatization); the maximum content of histamine was 1.7 mg/l. Putrescine and cadaverine levels varied between 0.2 and 0.6 mg/l (Mafra, Herbert, & Santos, 1999).

The samples from Zhejiang province had higher level of biogenic amines than the samples from other regions, reaching to 100–241 mg/l. The highest amount of total amines was observed in Shaoxing rice wines from Zhejiang region, produced by traditional craft. During Shaoxing rice wine production procedure, raw rice was soaked for a long time (about 15 d) and soak water (sour liquor) was added

Table 2
Concentration of biogenic amines (mg/l), pH and ethanol content of rice wine

	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	Total amine content	pH	Ethanol content (v/v)
Zhejiang								
01A	6.46 ± 0.05	78.5 ± 0.42	10.04 ± 0.19	ND	15.4 ± 0.36	110	4.27	16.0
02A	3.5 ± 0.14	10.7 ± 0.25	100.8 ± 0.22	6.69 ± 0.16	24.1 ± 0.38	146	4.14	16.5
03A	6.50 ± 0.35	10.5 ± 0.18	82.7 ± 0.36	1.99 ± 0.41	14.5 ± 0.45	116	4.16	16.5
04B	93.9 ± 0.43	44.7 ± 0.35	ND	27.1 ± 0.17	32.7 ± 0.33	198	4.22	16.8
05B	8.45 ± 0.25	12.9 ± 0.13	61.3 ± 0.33	ND	17.7 ± 0.36	100	4.09	16.5
06C	98.7 ± 0.15	12.6 ± 0.19	ND	5.16 ± 0.18	ND	117	4.16	12.1
07C	ND	10.5 ± 0.15	53.6 ± 0.15	22.5 ± 0.15	14.2 ± 0.15	101	4.18	16.5
08C	121 ± 0.15	65.7 ± 0.15	ND	20.7 ± 0.15	33.6 ± 0.15	241	4.33	16.5
Shanghai								
09D	4.04 ± 0.16	7.09 ± 0.11	41.0 ± 0.17	10.8 ± 0.32	26.9 ± 0.05	89.9	4.26	14.0
10D	ND	12.43 ± 0.23	20.4 ± 0.08	ND	17.6 ± 0.15	50.4	4.30	14.5
Jiangsu								
11E	2.80 ± 0.15	5.32 ± 0.15	9.15 ± 0.15	2.00 ± 0.15	20.0 ± 0.15	39.3	4.16	11.2
12F	5.61 ± 0.01	6.71 ± 0.34	28.1 ± 0.03	5.44 ± 0.34	18.51 ± 0.05	64.4	4.28	17.0
13G	2.23 ± 0.41	5.02 ± 0.50	28.5 ± 0.08	4.78 ± 0.17	6.37 ± 0.22	46.9	4.04	0.3
Hubei								
14H	2.65 ± 0.23	26.3 ± 0.33	40.2 ± 0.47	5.71 ± 0.25	3.90 ± 0.46	78.7	4.12	0.13

* A,B,C,D,E,F,G,H: same letters signify same wineries of each region; $n = 3$; ND below detection limits.

into the wort as a fermentation substrate, the latter containing a large amount of lactic acid bacteria with strong decarboxylase activity (Li Chao & Dong MingSheng, unpublished) and free amino acids. In view of this fact, we suggest that there is a possible correlation between higher total biogenic amines content and the specific traditional procedure.

The threshold levels for intoxication in humans by amines are very difficult to establish, because they depend on individual responses and the presence of other amines (Halász, Baráth, & Simon-Sarkadi, 1994; ten Brink et al., 1990). Askar and Treptow (1993) have suggested histamine at a concentration of 500 mg/kg to be hazardous for human health. On the other hand, ten Brink et al. (1990) reported that 100–800 mg/kg of tyramine in foods are toxic; while Silla Santos (1996) suggested that more than 1000 mg/kg (total amines in food) was dangerous for health. The total amine levels of Chinese rice wines tested were lower than values considered as dangerous for health. However, patients being treated with monoamine oxidase inhibitors should limit the consumption of rice wines.

Values of pH ranging from 4.04 to 4.33 were found in rice wines from the different origin areas studied. No significant differences were detected for any of these values. pH may influence growth of lactic acid bacteria and metabolic activity (Lonvaud-Funel, 2001) and according to the study of Gerbaux, Villa, Monamy, and Bertrand (1997), pH is one of the most important enological factors influencing the production of biogenic amines, particularly histamine, tyramine and putrescine. Nevertheless, in this work no significant correlation was found between pH and the amount of any of the amines detected. Similar results were reported in studies of Spanish wines by Marcobal, Polo, and Martín-Álvarez (2005).

4. Conclusion

The use of dansyl chloride as a derivatizing reagent for the determination of biogenic amines yielded satisfactory results. The proposed method for biogenic amines determination in rice wine samples showed good recoveries. Using the proposed method, we determined the contents of biogenic amines of 14 different rice wines produced by different manufacturers. The results showed that Chinese rice wines contain at least five kinds of biogenic amines. Quite large differences may occur among samples from different manufacturers.

However, the concentration of biogenic amines detected in Chinese rice wines are below the critical concentration, which may lead to direct adverse effects in consumers. However, the relative contribution of other pressor agents, polyamines and ethanol are not well established. Therefore, patients being treated with monoamine oxidase inhibitors should be aware of the danger of amines in rice wines and control their consumption of rice wine.

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