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A survey of biogenic amines in chinese red wines

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Abstract

Eight biogenic amines were analyzed in 38 samples of red wine from five wine-making regions of China. The analysis was carried out by reverse phase high performance liquid chromatography with fluorescence detector and dansyl chloride precolumn derivation. Tryptamine was not found in any of the samples. Putrescine was detected in all samples (100%), followed by phenylethylamine (84.2%), spermidine (60.5%), histamine (57.8%), tyramine (57.8%), cadaverine (47.4%), and spermine (36.8%). In all the samples, the levels of aromatic and heterocyclic amines with toxicological effects were: 0–4.58 mg/l for phenylethylamine, 0–10.51 mg/l for histamine, and 0–9.13 mg/l for tyramine. The amount of histamine and tyramine in most of the samples (94.7%) was less than 8 mg/l. The amines associated with sanitary conditions were also found to be present in a very low range, between 0 and 12.98 mg/l for putrescine, 0 and 19.01 mg/l for cadaverine, respectively. In the case of other amines such as spermine and spermidine, they yielded very low levels varying between 0 and 2.64 mg/l for spermine, 0 and 3.82 mg/l for spermidine, respectively.

Keywords: HPLC; Dansylation; Biogenic amines; Histamine; Red wine

1. Introduction

Biogenic amines are derived from microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes by amino acid transaminases (Zolou, Lokou, Souflero, & Stratis, 2003). It is well known that consumption of food containing high amounts of biogenic amines may cause headaches, nausea, cardiac palpitations and digestive problems (Aerny, 1990; Silla Santos, 1996). The toxic limits for biogenic amines are given as follows in terms of histamine (HIM): 8–40 mg, slight poisoning; 40–100 mg, intermediate poisoning; over 100 mg, intensive poisoning. Consumption of over 100 mg of tyramine (TYR) can cause migraines (Ayhan, Kolsarici, & Ozkan, 1999). For this reason, some countries such as the USA, Sweden, Austria and the Netherlands, have established regulations and legal requirements for the maximum limits of biogenic amines (mainly histamine) in various foods. The lack of legislation on the tolerated contents of biogenic amines in wine makes it difficult to import and export this product (Anli, Vuralb, Yimaza, & Vural, 2004).

Biogenic amines are produced during and after wine-making, although some are originally present in small amounts in grape juice. Biogenic amines can be present in the must or formed by yeasts during alcoholic fermentation. In some studies (Haláse, Baráth, & Holzapfel, 1999; Zee, Simard, L'Heureux, & Tremblay, 1983), biogenic amines have been suggested as indicators of hygienic quality or manufacturing practices. Some amines such as putrescine (PUT) and cadaverine (CAD) (Baucom, Tabacchi, Cottrell, & Richmond, 1996) are associated with poor sanitary conditions of grapes. (Marcobal, Martín-Álvarez, Polo, Muŭoz, & Moreno-Arribas, 2006) reported that some amines such as PUT, CAD and phenylethylamine (2-PHE) can be produced in grapes or the must or formed by yeasts during alcoholic fermentation (ethylamine and

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2-PHE), although quantitatively only very low concentrations are reached during these early stages. However, biogenic amines, such as TYR, PUT and HIM, are mainly produced during malolactic fermentation (Soufleros, Barrios, & Bertrand, 1998), by the action of lactic acid bacteria, causing decarboxylation of the corresponding free amino acids (Victoria, Carmen, & Jorganes, 2000, 2003). The main factors involved in the generation of these amines are malolactic fermentation and the pH of the wine. At high pH, biogenic amines are always produced in large amounts (Lonvaud-Funel, 2001). Thus red wines that are less acidic contain higher biogenic amine concentrations than white wines. Amino acids are the sequential precursors of biogenic amines, and as a consequence the higher the content of free amino acids, the higher the probability of biogenic amine production. The objective of this study is to determine the major biogenic amines and their concentrations in some red wines from different regions and manufacturers in China.

2. Materials and methods

2.1. Standard and reagents

Tryptamine (TRY), phenylethylamine (2-PHE), putrescine (PUT), cadaverine (CAD), histamine (HIM), tyramine (TYR), spermidine (SPD) and spermine (SPM) were obtained as hydrochloride salts from Sigma Chemical Co. (Germany). 1,7-diaminoheptane was purchased from Acros Organic (USA), and dansyl chloride (Dns-Cl) was obtained from Sigma. Methanol and acetone for HPLC was obtained from Fisher scientific (USA). Ultrapure water was obtained with Milli-Q system (Millipore). Other reagents were obtained from the Beijing Analytical Instruments Factory (China) and were of analytical or higher grade.

2.2. Biogenic amine determination

2.2.1. High performance liquid chromatography

The HPLC system consisted of an Agilent 1100 series equipped with a binary gradient pump, a fluorescence detector, an injection valve with a 20 μl loop, a reversed-phase Capcell PAK C18 MG (150 × 4.6 mm ID, particple size 5 μm) obtained from Shiseido Co., Japan. Prior to use, the eluents were filtrated through 0.45 μm filters and degassed under the vacuum. The column effluent was monitored with a fluorescence detector. The C18 column was equilibrated at 30 °C with a mobile phase consisting of 55% methanol and 45% water. The elution program consisted of a gradient system with a flow rate of 1.5 ml/min (Table 1). The eluted dansylamines were detected by monitoring at 350 nm (excitation) and 520 nm (emission).

2.2.2. Preparation of the standard solution

Amine standard solutions were prepared in 0.1 M hydrochloric acid to a final concentration of 1 mg/ml for each amine as a free base. The working standard solution was prepared daily because some biogenic amines are unstable. The internal standard solution was prepared by dissolving 10 mg of 1,7-diaminoheptane in 10 ml of 0.1 M hydrochloric acid and then diluted to a concentration of 100 μg/ml. Dns-Cl solution was prepared by dissolving 500 mg of Dns-Cl in 100 ml of acetone. They were kept at 4 °C.

Table 1
Gradient elution program for biogenic amines analysis

Time (min)	0	7	14	20	27	30	35	36	45
Methanol (%)	55	65	70	70	90	100	100	55	55
Water (%)	45	35	30	30	10	0	0	45	45

2.2.3. Derivatization procedure

The derivatization procedure was according to (Li et al., 2005). Briefly, under alkaline conditions, the standard amine solution was derivatized with dansyl chloride, and the mixture was extracted using diethyl ether. The final residue was dissolved in 1 ml methanol for HPLC analysis.

2.2.4. Preparation of sample

Thirty-eight samples of commercially available red wines from twenty different manufacturers in five typical wine-making areas (Beijing, Hebei, Shandong, Tianjin, Xinjiang) were purchased at local supermarkets.

A 5-ml aliquot of samples was accurately transferred into a centrifuge tube, and 200 μl of 1,7-diaminoheptane (100 μg/ml) was added. The samples were then saturated using sodium chloride and the pH was adjusted to 12.0 using sodium hydroxide. The samples were extracted three times with equal volume of *n*-butanol–chloroform (1:1, v/v). For each extraction, an equal volume of *n*-butanol–chloroform was added to the test tube, and the tube was vortexed for 5 min and then centrifuged for 10 min at 3600 rpm. The *n*-butanol–chloroform layer was aspirated, and the *n*-butanol–chloroform extracts were combined. A 3-ml aliquot of the extract was transferred into a 5-ml test tube, two drops of 1 M hydrochloric acid were added to the organic extract and then evaporated to dryness under a stream of nitrogen with heating at 40 °C. The residue was dissolved in 1 ml of 0.1 M hydrochloric acid for derivatization. Dansylation was conducted in triplicate, following the procedure for the amine standards above. HPLC analysis of the dansylated samples was carried out as described previously.

2.2.5. Statistical analysis

SPSS software was used to perform all statistical analyses. All data were expressed as means ± SD ($n = 3$).

3. Results and discussion

3.1. Evaluation of the method for biogenic amines analysis

The eight biogenic amines plus the internal standard were well resolved with the gradient elution pattern described in

Table 1. Fig. 1a and b showed typical chromatograms of biogenic amines in standard solution and in a red wine sample, respectively. Biogenic amines were identified on the basis of retention time by comparison with the standard solution.

The intra-day repeatability of the entire analytical procedure was tested by six parallel analyses of a spiked sample. For trace analysis, repeatability with an RSD $\leqslant$ 10% is considered acceptable (Tamim, Bennett, Shellem, & Doerr, 2002). With this method, intra-day relative standard deviation was 5.5%, 8.8%, 4.3%, 6.5%, 3.3%, 2.8%, 2.3% and 7.0% at mean concentrations 4.54, 4.71, 5.33, 5.16, 5.54, 6.41, 5.35 and 5.15 mg/l for TYR 2-PHE, PUT, CAD, HIM, TYR, SPM and SPD, respectively. The inter-day repeatability was evaluated by five days of analyses, and the inter-day relative standard deviation was 6.9%, 9.1%, 4.7%, 6.6%, 4.3%, 3.7%, 2.9% and 8.8% at mean concentrations 4.81, 4.63, 5.40, 5.19, 5.48, 6.33, 5.51 and 5.21 mg/l for TYR 2-PHE, PUT, CAD, HIM, TYR, SPM and SPD, respectively. The limits of detection calculated from the amount of amines required to give a signal-to-noise ratio of 3 were 0.06 mg/l for SPM, 0.08 mg/l for PUT and SPD, 0.1 for HIM, 0.15 mg/l for 2-PHE and CAD, and 0.20 mg/l for TRY and TYR. The limits of detection and repeatability are similar to those of others (Busto, Miracle, & Guasch, 1997; Zolou et al., 2003).

Table 2 summarizes the regression analyses for calibration and recoveries obtained for the eight biogenic amines. Linear regression analysis of area versus concentration of biogenic amines, in the standard solutions, was studied. The method was linear for the amines studied at concentrations ranging from 0.05 to 25 mg/l, except for SPD and SPM, which ranged from 0.05 to 15 mg/l. The values for the coefficient of regression (R^2 in Table 2) were all higher than 0.99. The recovery data was obtained from each biogenic amine spiked with concentration at 1 mg/l, 3 mg/l and 10 mg/l, respectively. The recovery for all individual amines was satisfactory (85.3–99.4%), except for PUT with 127.5%.

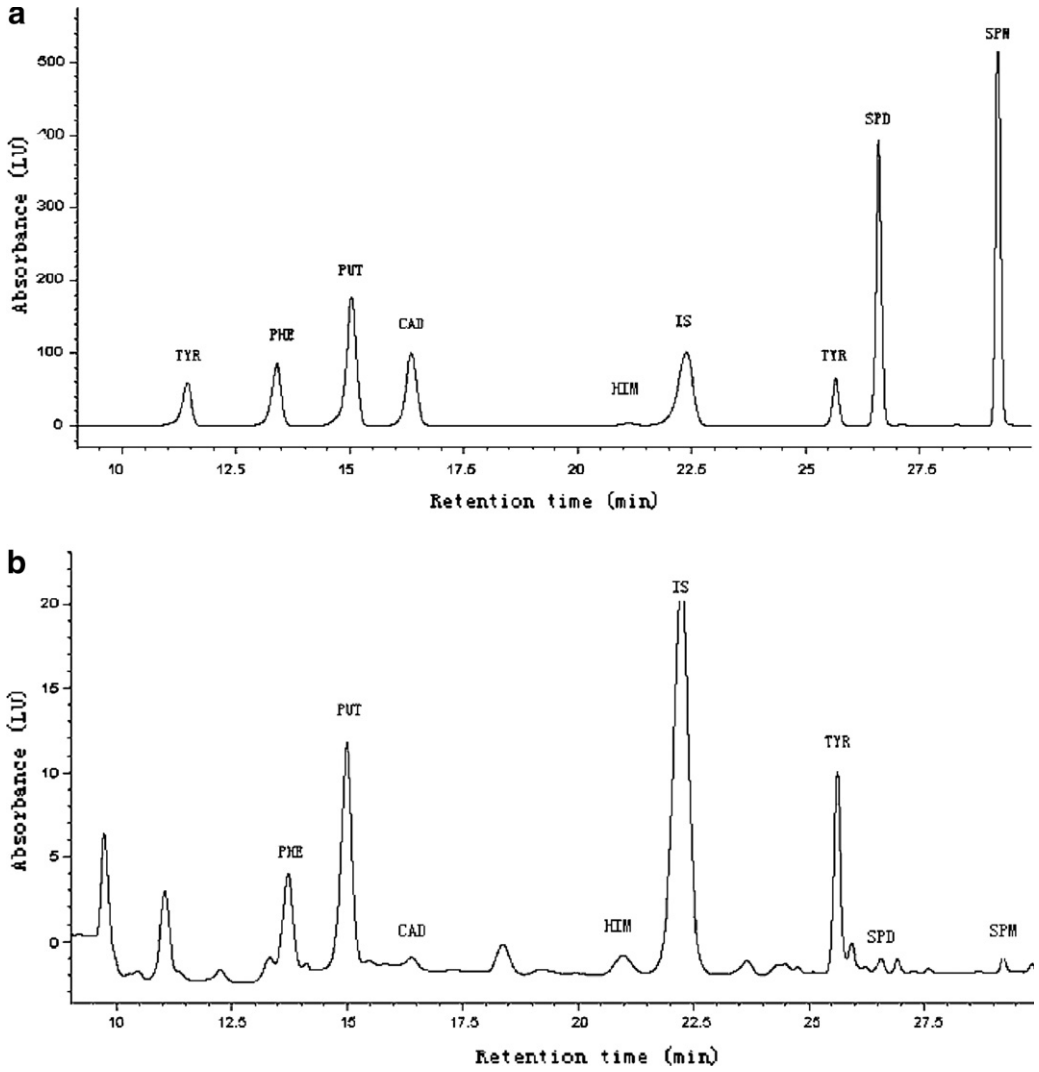

Fig. 1. HPLC chromatography profiles of the dansyl chloride-derivates of biogenic amine standard solutions (a) HPLC chromatography profiles of the dansyl chloride-derivates of biogenic amine in the red wine samples (b).

Table 2
Regression equations for area versus concentration for standard biogenic amine solutions and recovery of the method

Biogenic amine	Regression equation	R^2 (Correlation coefficient)	Recovery (Mean values (%))
Tryptamine	$y = 0.0373x - 0.0072$	0.9995	86.6
Phenylethylamine	$y = 0.0579x - 0.0031$	0.9989	91.2
Putrescine	$y = 0.0720x + 0.0343$	0.9990	127.5
Cadaverine	$y = 0.0686x + 0.006$	0.9996	99.4
Histamine	$y = 0.0138x - 0.0036$	0.9995	89.1
Tyramine	$y = 0.0286 - 0.0025$	0.9997	99.1
Spermidine	$y = 0.1038x - 0.0521$	0.9957	91.0
Spermine	$y = 0.0929x + 0.1093$	0.9951	85.3

3.2. Biogenic amines in red wine

Table 3 showed the results of the concentration of biogenic amines of Chinese red wines. 2-PHE, PUT, CAD, HIM, TYR, SPD and SPM were found in the red wine samples. Most of the red wines presented low concentrations (less than 8 mg/l). TRY was not found in any of the red wine samples, this agreed with the result of Romero, Sanchez-Vinas, Gazquez, and Bagur (2002). PUT was detected in all samples (100%), followed by 2-PHE (84.2%), SPD (60.5%), HIM (57.8%), TYR (57.8%), CAD (47.4%), and SPM (36.8%).

Table 3
Concentrations (mg/l) of biogenic amines of Chinese red wines calculated as mean ± standard error of the three determinations

	TRY	PHE	PUT	CAD	HIM	TYR	SPD	SPM	TAC[c]
Beijing									
1A[a]	ND[b]	ND	5.19 ± 0.0013	2.41 ± 0.0021	1.18 ± 0.0032	2.89 ± 0.0041	0.79 ± 0.0074	ND	12.5
2A	ND	0.91 ± 0.08	5.89 ± 0.05	1.99 ± 0.07	2.15 ± 0.01	0.44 ± 0.08	1.60 ± 0.011	ND	13.0
3A	ND	0.30 ± 0.03	2.94 ± 0.02	0.80 ± 0.004	6.44 ± 0.023	0.63 ± 0.04	1.78 ± 0.018	ND	12.9
4B	ND	ND	1.83 ± 0.07	ND	2.04 ± 0.06	ND	ND	ND	3.87
5C	ND	0.42 ± 0.02	2.84 ± 0.08	ND	1.18 ± 0.01	ND	0.54 ± 0.03	ND	4.98
6C	ND	0.33 ± 0.01	2.92 ± 0.09	0.24 ± 0.01	1.14 ± 0.04	ND	0.77 ± 0.02	ND	5.4
7C	ND	ND	0.37 ± 0.02	ND	ND	ND	ND	ND	0.37
8D	ND	1.78 ± 0.20	19.0 ± 0.14	0.54 ± 0.07	6.32 ± 0.11	1.06 ± 0.01	0.55 ± 0.02	ND	29.3
9D	ND	0.84 ± 0.03	1.46 ± 0.14	0.15 ± 0.13	1.07 ± 1.60	6.94 ± 0.21	0.44 ± 0.02	0.26 ± 0.03	11.2
10E	ND	0.22 ± 0.01	5.37 ± 0.25	0.13 ± 0.03	1.01 ± 0.08	0.88 ± 0.02	ND	ND	7.61
Hebei									
11F	ND	2.76 ± 0.31	0.90 ± 0.30	ND	10.5 ± 0.86	11.5 ± 1.39	1.20 ± 0.16	0.50 ± 0.09	27.4
12G	ND	4.58 ± 1.52	0.75 ± 0.21	ND	1.85 ± 1.61	6.94 ± 1.36	1.93 ± 0.30	0.56 ± 0.02	16.6
Shandong									
13H	ND	0.24 ± 0.09	1.84 ± 0.08	ND	ND	0.88 ± 0.05	0.14 ± 0.04	ND	3.1
14H	ND	0.16 ± 0.01	0.19 ± 0.02	ND	ND	ND	ND	ND	0.35
15I	ND	0.43 ± 0.09	0.26 ± 0.05	ND	0.49 ± 0.08	0.48 ± 0.04	0.66 ± 0.03	0.37 ± 0.08	2.69
16I	ND	ND	6.75 ± 0.15	ND	0.17 ± 0.17	0.45 ± 0.03	0.25 ± 0.02	0.27 ± 0.03	7.89
17I	ND	0.60 ± 0.12	0.30 ± 0.04	ND	ND	0.63 ± 0.05	0.66 ± 0.05	0.46 ± 0.05	2.65
18I	ND	1.21 ± 0.22	0.36 ± 0.05	1.01 ± 0.11	ND	0.72 ± 0.02	0.72 ± 0.10	0.45 ± 0.06	4.47
19J	ND	1.07 ± 0.27	0.34 ± 0.08	0.23 ± 0.21	ND	0.76 ± 0.26	1.12 ± 0.13	0.65 ± 0.30	4.17
20J	ND	0.80 ± 0.34	2.56 ± 0.14	ND	9.64 ± 5.68	5.83 ± 0.54	1.16 ± 0.01	0.43 ± 0.09	20.4
21J	ND	0.56 ± 0.09	0.22 ± 0.01	ND	ND	0.20 ± 0.04	0.31 ± 0.03	2.64 ± 0.69	3.93
22K	ND	1.45 ± 0.36	1.14 ± 0.11	ND	3.38 ± 4.20	4.52 ± 0.17	1.95 ± 0.19	0.54 ± 0.01	13.0
23K	ND	0.65 ± 0.11	0.98 ± 0.04	ND	0.10 ± 0.17	1.50 ± 0.19	1.03 ± 0.09	0.28 ± 0.17	4.54
24L	ND	0.47 ± 0.01	3.29 ± 0.18	0.21 ± 0.01	ND	ND	ND	ND	3.97
25L	ND	0.60 ± 0.02	9.32 ± 0.33	0.31 ± 0.03	1.05 ± 0.02	ND	ND	ND	11.4
26M	ND	0.41 ± 0.01	1.19 ± 0.53	ND	ND	ND	ND	ND	1.6
27M	ND	0.54 ± 0.02	2.79 ± 0.18	0.20 ± 0.01	ND	ND	1.29 ± 0.17	ND	4.82
28M	ND	4.13 ± 0.21	3.81 ± 0.34	0.60 ± 0.03	ND	ND	0.27 ± 0.05	ND	8.81
29M	ND	0.37 ± 0.05	13.06 ± 0.47	1.78 ± 0.08	ND	ND	ND	ND	15.2
30M	ND	0.54 ± 0.12	2.86 ± 0.23	0.25 ± 0.03	ND	ND	ND	ND	3.65
31M	ND	1.93 ± 0.23	0.39 ± 0.03	ND	7.81 ± 1.19	19.1 ± 2.58	1.54 ± 0.46	0.66 ± 0.10	31.5
Tianjin									
32N	ND	0.56 ± 0.01	0.50 ± 0.09	13.0 ± 0.71	0.75 ± 0.07	2.45 ± 0.27	3.82 ± 0.29	0.75 ± 0.09	21.8
33O	ND	0.36 ± 0.01	1.43 ± 0.06	ND	ND	ND	ND	ND	1.79
34P	ND	0.33 ± 0.03	4.79 ± 0.02	0.17 ± 0.01	1.15 ± 0.12	0.63 ± 0.07	ND	ND	7.07
35P	ND	ND	0.52 ± 0.03	ND	ND	ND	ND	ND	0.52
Xinjiang									
36R	ND	0.30 ± 0.11	6.49 ± 0.19	0.28 ± 0.09	0.64 ± 0.08	0.72 ± 0.04	ND	ND	8.43
37S	ND	ND	0.95 ± 0.05	ND	0.17 ± 0.08	ND	ND	ND	1.12
38T	ND	0.19 ± 0.01	5.08 ± 0.01	ND	ND	ND	ND	ND	5.27

[a] A–T: same letters signify same wineries of each region.
[b] ND, not detected.
[c] TAC, total amines content.

In all the red wine samples, the levels of aromatic and heterocyclic amines which have toxicological effects were not detectable for TRY, 0–4.58 mg/l for 2-PHE, 0–10.51 mg/l for HIM, and 0–19.13 mg/l for TYR. These values were generally low, especially for TRY and 2-PHE. About 94% of the wine samples contained less than 8 mg/l of HIM or TYR. However, the HIM content varied between 0 and 10.51 mg/l and TYR content between 0 and 19.13 mg/l. The amines associated with sanitary conditions (PUT and CAD) were also found to be present in very low ranges; 86.8% ranged between 0 and 1.0 mg/l, 10.5% ranged between 1.0 and 5.0 mg/l, with a maximum level at 13.0 mg/l for PUT and 39.5 % 0.19–1.0 mg/l, 36.8% 1.0–5.0 mg/l, with a maximum level at 19.0 mg/l for CAD. Ninety seven percent of the wine samples had SPM concentrations lower than 1 mg/l, with the maximum level at 2.64 mg/l. SPD never exceeded 4 mg/l and 97.4% of the wines presented concentrations lower 1 mg/l.

Overall, the average amount of the total biogenic amine contents was 8.92 mg/l. The samples from Shandong province had higher levels of total biogenic amines than the samples from other regions, with a maximum level at 31.5 mg/l. Thirty Portuguese wines (including fortified wines such as Port) have been analyzed with HPLC (OPA derivatization), the maximum content was found 1.7 mg/l for HIM. PUT and CAD were present in very low levels, varying between 0.2 and 0.6 mg/l (Mafra, Herbert, & Santos, 1999). The biogenic amine contents of 109 different commercial Rioja DOC wines were determined with HPLC (OPA derivatization) and the highest amine content was found in red wines. The maximum level was 33.1 mg/l for PUT, 1.74 mg/l for CAD, 5.98 mg/l for TYR and 8.72 mg/l for HIM in analyzed red wines (Vazquez-Lasa, Iniguez-Crespo, Gonzalez-Larraina, & Gonzalez- Guerrero, 1998). In France, 54 red, 15 rosé and 15 white commercial bottled wines from Vallée du Rhône have been analyzed with HPLC (FMOC derivatization) to determine their amine content such as HIM, TYR, 2-PHE, PUT and CAD. Only argmatine and PUT levels were found to be higher than 1 mg/l (8% of the samples contained more than 20 mg/l of PUT, 1.2% more than 10 mg/l of HIM and TYR) (Bauza, Blaise, & Daumas, 1995).

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 (Lu et al., 2007). The USFDA guideline value of HIM in food is 50 mg/kg, Silla Santos (1996) suggested that more than 1000 mg/kg (total amines in food) was dangerous for health. The total amine levels of red wines in this study were lower than values considered as dangerous for health. However, monoamine oxidase inhibitor and ethanol can increase the toxication of amines. Patients being treated with monoamine oxidase inhibitor should limit the consumption of red wines.

Although the recommended maximum limits for histamine in wine are 2 mg/l in Germany, 3 mg/l in Holland, 5–6 mg/l in Belgium, 8 mg/l in France, and 10 mg/l in Switzerland and Austria, (Busto, Guasch, & Borrull, 1996), the OIV (“Office International de la Vigne et du Vin”) has not set any maximal limits concerning levels of biogenic amines in wine, which could be used in future European legislation. In this work, most of red wines were found to have a low concentration of histamine. Over 86% ranged between 0 and 5.0 mg/l, 7.9% ranged between 5.0 and 8.0 mg/l, only 5.3% higher than 8.0%, with the maximum level at 10.5 mg/l for HIM.

4. Conclusions

In our work, eight important biogenic amines were determined in 38 samples of red wine in China. 2-PHE, PUT, CAD, HIM, TYR, SPD and SPM were found in the red wine samples. TRY was not found in any red wine samples analyzed. PUT was detected in all samples (100%), followed by 2-PHE (84.2%), SPD (60.5%), HIM (57.8%), TYR (57.8%), CAD (47.4%), and SPM (36.8%).

The concentrations of target biogenic amines in red wines tested were below the critical concentrations that may lead to direct adverse effects on consumers. The maximum level of total amines was 31.5 mg/l. 86.8% ranged between 0 and 5.0 mg/l, 7.9% ranged between 5.0 and 8.0 mg/l, only 5.3% higher than 8.0%, with the maximum level of 10.5 mg/l for HIM. However, patients being treated with monoamine oxidase inhibitors should be aware of the danger of amines in red wines and control their consumption.

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