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Modified QuEChERS combined with UPLC-MS/MS to determine eight biogenic amines in Xinjiang smoked horsemeat sausages

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Abstract

A modified QuEChERS method coupled with ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was established for the simultaneous determination of eight biogenic amines (BAs) in Xinjiang smoked horsemeat sausages. The conditions of UPLC-MS/MS, extraction solvent and QuEChERS purification were optimized. The internal standard method was used for quantitative analysis. Results showed that the eight BAs exhibited good linearity (1-200 μg/L, correlation coefficient > 0.99). The limits of detection and quantification were 5 μg/kg and 10 μg/kg, respectively. The accuracy was between 80.4% and 111.4%, and the precision was less than 10%. The established method was effectively used for the determination of eight BAs in forty Xinjiang smoked horsemeat sausages. Results showed that tyramine was the major amine, followed by cadaverine, putrescine and spermine. Total BAs content ranged from 26.5 mg/kg to 352.9 mg/kg, the contents of BAs in Xinjiang smoked horsemeat sausages may pose a potential health risk.

Keywords: biogenic amines; modified QuEChERS; ultra-high-performance liquid chromatography-tandem mass spectrometry; Xinjiang smoked horsemeat sausages.

Practical Application: Xinjiang smoked horsemeat sausages are one of the most famous Chinese traditional meat products. It has gained lots of acceptance and popularity over China for hundred years due to its unique qualities and excellent nutritional characteristics. However amino acids in horsemeat can also form biogenic amines by decarboxylation reaction during fermentation. The purpose of this research is to establish a modified QuEChERS purification method coupled with a UPLC-MS/MS method for the simultaneous determination of eight BAs in Xinjiang smoked horsemeat sausages by optimizing the extraction solvent and adsorbent of the QuEChERS procedure. This method is applied to evaluate and analyze the contents of biogenic amines in Xinjiang smoked horsemeat sausages.

1 Introduction

Biogenic amines (BA) is a kind of bioactive low molecular weight nitrogenous organic compounds which have been found in different kinds of foods such as aquatic products, meat products and fermented products (Hu et al., 2012; Ikonić et al., 2013; Ruiz-Capillas & Jimenez-Colmenero, 2004). BAs are mainly formed by amino acid decarboxylation via bacteria with specific decarboxylase activity (Alan & Yildiz, 2021). However, some other physiological effects (hypotension, nausea, palpitations, rashes, dizziness, headaches, tachycardia, hypertension, cardiac and emesis) may also occur if high concentrations of BAs are consumed (Gong et al., 2014). The determination of biogenic amines in food products has received considerable interests not only because of their toxicity but also their usage as indicators of the degree of freshness or spoilage of food (Vinci & Antonelli, 2002). In view of the degree of freshness food and the potential harmful effects of BAs, the development of sensitive and routine methods for their analysis in food is of great importance.

QuEChERS is a quick, easy, cheap, effective, rugged, and safe sample pretreatment technology based on dispersive solid phase extraction, which has been widely applied in the pretreatment of relatively simple samples, such as vegetable, fruit, beverage and wine (Xing et al., 2021). But for traditional fermented meat products, due to the high fat content and protein content, the direct application of QuEChERS pretreatment method was not very effective. Therefore, it was necessary to remove the fat and protein in the sample and combine with QuEChERS pretreatment method. In recent years, the determination of BAs mainly focused on the matrix of seafood (Kaufmann & Maden, 2018), meat (Motaghifar et al., 2021; Sun et al., 2016), dairy (Adımcılar et al., 2017), wine (Manetta et al., 2016; Redruello et al., 2017), beverages (Jain et al., 2015; Preti et al., 2016), sufu (Guan et al., 2013; Tang et al., 2011), soy (Shukla & Kim, 2016) and so on. Various methods have been developed for the analysis of BAs in foods mentioned above, including thin-layer chromatography (TLC) (Lapa-Guimarães & Pickova, 2004), gas chromatography-mass spectrometry (GC-MS) (Almeida et al., 2012; Papageorgiou et al., 2018), high performance liquid chromatography(HPLC) (Liu et al.,

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2018), high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Dong & Xiao, 2017; Mologni et al., 2018), liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-QTOF) (Jia et al., 2011). Among these methods, the sample pretreatment process of the TLC, GC-MS and HPLC methods was relatively complicated, time-consuming, requisite derivatization and low sensitivity, and the UPLC-MS/MS method was obviously better than other methods in analysis time, selectivity and anti-interference ability.

Xinjiang smoked horsemeat sausages are one of the most famous Chinese traditional meat products. It has gained lots of acceptance and popularity over China for hundred years due to its unique qualities and excellent nutritional characteristics (Lu et al., 2015). The making process of Xinjiang smoked horsemeat sausage is to mix the horse meat pieces with sugar, salt and spices in a certain proportion, and poured into the horse casings,after smoking, natural fermentation for 28 days (environmental temperature between 18 °C and 10 °C), one obtains the mature smoked horsemeat sausage. In the process of fermentation, horsemeat will form a unique flavor by lipid oxidation, protein oxidation and other processes improve the quality of smoked horsemeat sausage. However amino acids in horsemeat can also form biogenic amines by decarboxylation reaction during fermentation. Therefore, it is of great significance to evaluate and analyze the biogenic amine contents of Xinjiang smoked horsemeat sausages.

The purpose of this research is to establish a modified QuEChERS purification method coupled with a UPLC-MS/MS method for the simultaneous determination of eight BAs in Xinjiang smoked horsemeat sausages by optimizing the extraction solvent and adsorbent of the QuEChERS procedure. This method is applied to evaluate and analyze the contents of biogenic amines in Xinjiang smoked horsemeat sausages.

2 Materials and methods

2.1 Chemicals, reagents and standards

n-hexane and acetonitrile were purchased from Merck (HPLC grade, Darmstadt, Germany). Octadecylsilane (C18) adsorbent, primary secondary amine (PSA, 40-63 mm, 6 nm) adsorbent, graphitized carbon black (GCB) adsorbent, anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl) and formic acid (FA) were all purchased from CNW Technologies GmbH (Düsseldorf, Germany). Ultra-pure water (> 18.2 MΩ) was obtained from a Milli-Q water purification system (Millipore Corp., USA).

Standards of tyramine (TYR), phenethylamine (PEA), putrescine (PUT), cadaverine(CAD), histamine (HIS), tryptamine (TRP), spermidine (SPD), spermine (SPM) and 1,7-diaminoheptane (internal standard) were all acquired from Dr.Ehrenstorfer GmbH (Augsburg, Germany). All biogenic amines standards were of high purity grade (> 98%). Each standard substance was prepared 100 μg/mL individual standard stock solution with the acetonitrile, and all individual standard stock solutions were kept in a refrigerator at -20 °C.

2.2 Samples

Forty Xinjiang smoked horsemeat sausage samples were obtained from the local retail markets and department stores in Xinjiang Region. The origins of production were Urumchi city,Yili city, Changji city, Shihezi city and Tacheng city. All samples are vacuum packed. The samples were carried to the laboratory and stored at -20 °C until analysis.

2.3 Sample preparation

2.00 g smoked horsemeat sausage sample was accurately weighed, and transferred into a 50 mL plastic centrifuge tube. Then 100 μL of the 1.0 mg/L 1, 7-diaminoheptane and 5 mL of 10% ammonia water (*V*/*V*) were orderly added into the tube, and the mixture was vortexed for 30 s (A MS3 basic vortex mixer, IKA GmbH, Germany). After that, 10 mL of acetonitrile and 2 g of sodium chloride were further added, and the mixture was vortexed for 1 min and subsequently centrifuged for 3 min at 5000 g (3-330K high speed freezing centrifuge, Sigma Laborzentrifugen GmbH, Germany).

2.5 mL of the extraction solution was moved to a 10 mL high speed plastic centrifuge tube which contains 100 mg C18 and 100 mg of PSA. The above mixture was vortexed for 1 min and centrifuged for 5 min at 10000 g. After that,1.0 mL of supernatant was transferred into another 10 mL glass colorimetric tube, then it was subsequently blow-dried by nitrogen gas in a 40 °Cwaterbath(Nitrogen evaporator, Organomation Co., USA). Finally, 1.0 mL of 10% acetonitrile-water (*V*/*V*, containing 0.1% formic acid) was used to redissolve it. The redissolved solution was then filtered through a 0.22 μm filter membrane (Nylon, Shanghai ANPEL Laboratory Technologies Inc, China) and transferred into a sample bottle for UPLC-MS/MS analysis (Waters Xevo™ TQ-S tandem mass spectrometer, Waters Co., USA). With respect to the sample solutions contain target analytes beyond the linearity range, determinations were performed after appropriate dilution with 10% acetonitrile-water (*V*/*V*, containing 0.1% formic acid).

2.4 Method validation

The optimized analytical method was validated in terms of linearity, limits of detection (LODs), limits of quantitation (LOQs), accuracy and precision for eight BAs in Xinjiang smoked horsemeat sausages.

Fermented sausages generally contain a variety of biogenic amines according to previous reviews (Suzzi & Gardini, 2003). A series of mixed standard working solutions (1, 2, 5, 10, 20, 50, 100, 200 μg/L), which all contained 10 μg/L of the internal standard 1, 7-diaminoheptane, were all determined under the optimized conditions. The linear equations of the calibration curves were obtained by plotting the concentrations of the eight BAs and the corresponding peak areas, and the correlation coefficients (R^2 values) were calculated. The LODs and LOQs of the eight BAs were determined by serially diluting a mixed standard solution with 10% acetonitrile-water (*V*/*V*, containing 0.1% formic acid). The LODs were determined when the signal to noise ratio (SNR) was higher than or equal to 3, and the LOQs were taken when the SNR was higher than or equal to 10.

To evaluate the accuracy and precision of the method, mixed standard solution of eight BAs was added to Xinjiang smoked horsemeat sausages, including three different concentration levels, and the spiked samples were determined under the optimized pretreatment and instrument conditions. The spiked samples were determined continuously for 6 times and the RSDs of the eight BAs were calculated.

2.5 Analysis of the Xinjiang smoked horsemeat sausages

Xinjiang smoked horsemeat sausages (40 samples) were randomly collected from different region in Xinjiang, including Urumchi city (8 samples), Yili city (12 samples), Changji city (8 samples), Shihezi city (6 samples) and Tacheng city (6 samples). The collected smoked horsemeat sausage samples were homogenized at 10000 r/min for 1 min and stored at -20 °C. All prepared samples were pretreated according to section 2.3, which were determined under the optimal conditions of chromatography and mass spectrometry.

3 Results and discussion

3.1 Optimization of UPLC-MS/MS conditions

The MS condition of eight BAs and 1,7-heptyldiamine were optimized by flow injection analysis of standard at a concentration of 1 μg/mL. According to the chemical structure of BAs, eight BAs and 1,7-heptyldiamineare easy to form positive ions in the ion source. The cone voltage and collision energy were optimized to obtain the highest intensity of parent ions and daughter ions. The characteristic ion pairs, cone voltage and collision energy of eight BAs and 1,7-heptyldiamine were presented in Table 1.

The selection of chromatographic column and mobile phases has greatly influence on results of analysis. Firstly, the effects of BEH C18 chromatographic column (100 mm×2.1 mm, 1.7 μm) and BEH HILIC chromatographic column (100 mm × 2.1 mm, 1.7 μm) on resolution of eight BAs were evaluated. Secondly, in order to study the effect of ionization efficiency, the addition of formic acid in mobile phase were investigated, including water + acetonitrile, 0.1% FA water + acetonitrile, water + 0.1% FA acetonitrile and 0.1% FA water + 0.1% FA acetonitrile.

The results showed that C18 column presented much better selectivities for the BAs. The peak shapes of the BAs separated by the C18 column were much more symmetrical and had better separation than those separated by the HILIC column. The addition of formic acid to the mobile phase improves the retention and peak shape of the biogenic amine while making ionization more adequate. Therefore, the BEH C18 chromatographic column and acetonitrile+water mobile phase (containing 0.1% formic acid) is the best condition for the determination of BAs (Figure 1).

3.2 Optimization of the extraction solvent

BAs were organic bases widely found in fermented meat products. Since BAs mostly existed as ions at neutral and acidic conditions, the extraction solvent was adjusted to alkaline state (pH=12) by ammonia water, thus could keep these BAs in molecular state and make them easier to distribute in organic extractant. The selection of extraction solvents directly affects the extraction efficiency of biogenic amines in smoked horsemeat sausages. The paper used acetonitrile and ethyl acetate as extraction solvents to evaluate the extraction efficiency of the solvent by the experiment of adding BAs standard in smoked horse sausages. Recoveries were used to evaluate the extraction effect. Results were presented in Figure 2 and it could be clearly observed from Figure 2 that the overall extraction recoveries of acetonitrile were higher than those of ethyl acetate. Therefore, acetonitrile was finally selected as the extraction solvent, resulting in recoveries ranging from 82.7% for SPD to 103.4% for CAD.

Table 1. MS parameters of eight BAs and the internal standard.

Compounds	Parent (*m/z*)	Cone voltage (V)	Daughter(*m/z*)	Collision voltage(eV)
Tryptamine (TRP)	161.1	12	115 [q]	28
			144 [Q]	12
Phenethylamine (PEA)	122	15	77 [q]	24
			105 [Q]	11
Putrescine (PUT)	88.9	36	72 [Q]	12
Cadaverine (CAD)	103	14	69 [q]	15
			86 [Q]	10
Histamine (HIS)	112	20	68 [q]	18
			95 [Q]	13
Tyramine (TYR)	138	13	77 [q]	24
			121 [Q]	10
Spermidine (SPD)	146.1	18	72 [Q]	14
			112 [q]	14
Spermine (SPM)	203.1	18	112 [Q]	20
			129.1 [q]	13
1,7-diaminoheptane	131	20	55 [q]	16
			114.1 [Q]	11

[Q]Quantifier ion. [q]Qualifier ion.

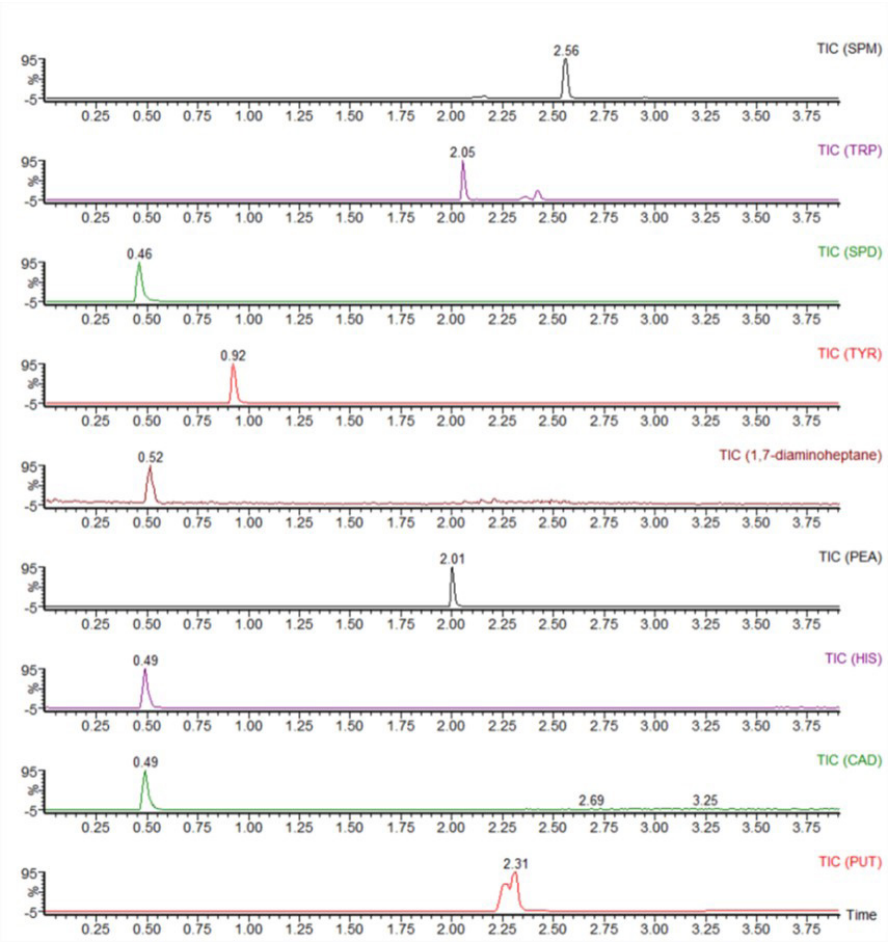

Figure 1. Total ion chromatograms of eight BAs and the internal standard (1, 7-heptyldiamine), the concentration is 20 ng/mL.

3.3 Optimization of the QuEChERS purification

QuEChERS technology is widely applied in the detection and analysis field. Chang et al. (2018) applied QuEChERS-based purification technology for the determination of heterocyclic amines in commercial meat products and favorable recoveries were obtained, indicating that QuEChERS technology is suitable for purifying target analytes in sausages. However GCB has noticeable adsorption to aromatic amine compounds (Dong & Xiao, 2017), the purification effects of C18 and PSA frequently-used adsorbents were compared with each other. Due to the high fat content in acetonitrile extract solution of smoked horse sausage samples, the fat in acetonitrile extract solution was firstly removed by hexane, and then the mixed standard solution of eight biological amines was added to the acetonitrile extract solution. 2 mL of the upper acetonitrile solution was accurately transferred into a 5-mL centrifuge tube pre-loaded with C18 and PSA. After that the extraction solutions were vortexed and centrifuged. Next, 1 mL of the supernatant was transferred to a glass centrifuge tube and dried under flowing nitrogen in a 40 °C water-bath. Subsequently, the residue was redissolved using 1.0 mL of 10% acetonitrile-water (with 0.1% FA, *V*/*V*) and filtered through a 0.22 μm organic filter membrane. Finally, the supernatant was determined by UPLC-MS/MS, and the recovery was calculated.

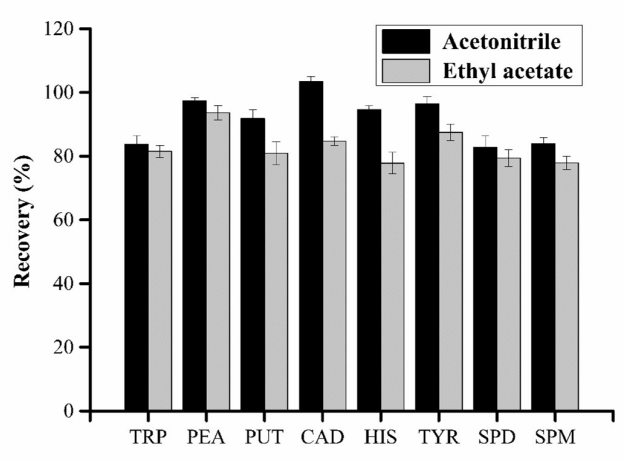

Figure 2. Comparison of the extraction efficiencies of different solvents (Spiked 100 μg/kg, n=3).

The QuEChERS method is to add the right amount of adsorbent directly to the extracting solution, adsorbs as many impurities as possible while retaining the target. Compared with the ordinary SPE method, this is fewer steps such as washing and elution, which is faster and simpler. PSA adsorbent can remove polar interferences

such as organic acids, pigments and sugars. C18 adsorbent removed non-polar interferences such as fat and ester from the sample matrix. Therefore, in this study, modified QuEChERS method was adopted in purification, and anhydrous magnesium sulfate was used to remove water, while PSA and C18 mixed adsorbents were used to effectively remove interferences in the extract. The results were shown in Figure 3. When the dosage of C18 and PSA was 100 mg respectively, the recovery of biogenic amine was the best and the purification effect was the best. Because the amount of adsorbent was too small, impurities can't be completely adsorbed, if the amount of adsorbent was too large, it will adsorb certain biological amines. The results are consistent with those reported in the literature.

3.4 Method validation

Linearity, detectability of the method

In the linearity studies, all the standard working solutions were determined under the optimal conditions of chromatography and mass spectrometry. Taking the concentration as the X-axis, the peak area as the Y-axis, linear regression analysis was performed. The results shown in Table 2 indicate that favourable linearities were obtained in the corresponding concentration range of each BA, and the coefficients of determination (R^2) were higher than 0.99.

The LODs and LOQs of the method were calculated according to the validated experimental results. The results showed that the LODs were 5 μg/kg, and the LOQs were 10 μg/kg (Table 2). The LODs and LOQs of this method are higher than those of the HPLC methods reported (Herrero et al., 2016; Piasta et al., 2014), and are basically consistent with those of the LC-MS/MS methods reported (Sagratini et al., 2012; Sirocchi et al., 2014; Dong & Xiao, 2017; Nalazek-Rudnicka & Wasik, 2017).

Accuracyand precision

The accuracy and precision were evaluated though which determined for each BA recoveries and RSDs at three different spiked concentrations in Xinjiang smoked horsemeat sausages.

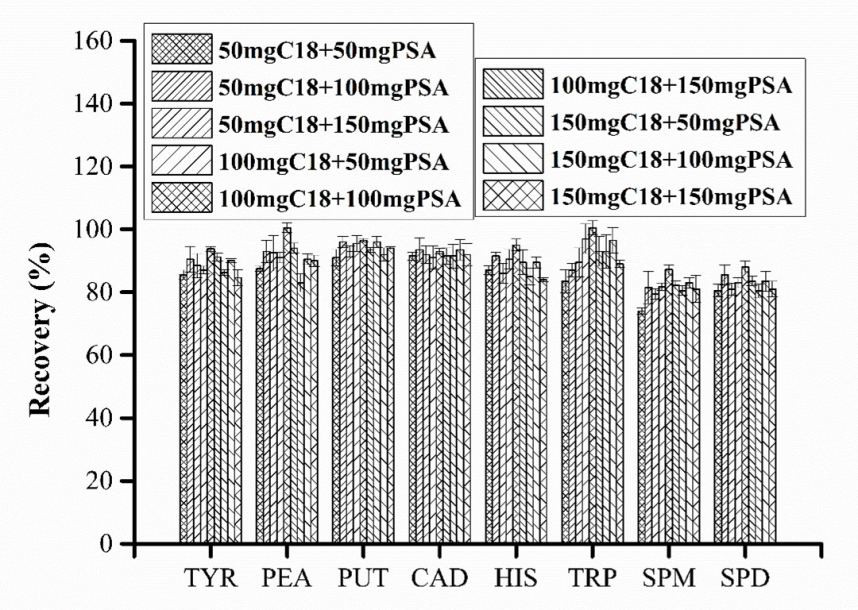

Figure 3. Recoveries of eight BAs purified with different absorbent types and dosages (Spiked 100 μg/kg, n=3).

Table 2. Liner range, linear equation, R^2, LODs and LOQs of the eight BAs.

Component	Liner range (μg/L)	Linear equation	R^2	LODs[b] (μg/kg)	LOQs[b] (μg/kg)
TRP[a]	1~200	y=555.41 x-33310	0.9995	5	10
PEA[a]	1~200	y=2405.83x+2085.30	0.9996	5	10
PUT[a]	1~200	y=1509.07x+569.27	0.9998	5	10
CAD[a]	1~200	y=244.24x+149.51	0.9994	5	10
HIS[a]	1~200	y=1054.34x+1868.98	0.9987	5	10
TYR[a]	1~200	y=669.32x+1002.12	0.9992	5	10
SPD[a]	1~200	y=236.29x-47.25	0.9997	5	10
SPM[a]	1~200	y=275.99x+45.11	0.9996	5	10

[a]TRP, tryptamine; PEA, phenethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPD, spermidine; SPM, spermine; [b]LODs, limits of detection; LOQs, the limits of quantification.

The results were shown in Table 3. The average recoveries ranged from 80.4% to 111.4% and RSDs were less than 10.0%. Indicating the accuracy and precision of the determination were satisfactory for eight BAs in Xinjiang smoked horsemeat sausages. The results are acceptable, which also comply with the AOAC criteria.

3.5 Analysis of the Xinjiang smoked horsemeat sausages

The concentration of BAs in smoked horsemeat sausages in different regions of Xinjiang is shown in Table 4, and the average content of each region is shown in Figure 4. It can be seen from Table 4 that the BAs content of most samples is considerable difference, and the content of various BAs in the same sample is also significantly different. At the same time, the table also shows that YL1, YL12, WS5, WS6, CJ7 and TC4 six samples have only PUT, SPD, SPM three kinds of biological amine detected, which is completely different from other samples. After investigation and analysis, the reason for this result is mainly because these six samples are vacuum-packed products after high temperature sterilization.

Tyramine is the dominant biogenic amine in Xinjiang smoked horsemeat sausages, the tyramine content ranged from ND to 151.5 mg/kg, which had a mean value of 57.8 mg/kg. Figure 4 shows that the average content of tyramine in Changji region is higher than that in other regions. Tyramine has also been found in fermented sausages in Turkey, Spain, France, Italy and Belgium, with a content range of 0 ~ 510 mg/kg (Bozkurt & Erkmen, 2004; Papavergou et al., 2012; Suzzi & Gardini, 2003; Ansorena et al., 2002). The acceptable levels of tyramine should be ranged from 100 mg/kg to 800 mg/kg. Based on the present results, 25% of the fermented sausages were higher than 100 mg/kg. Similar result was reported by Lu et al. (2010), who found that the mean value of tyramine was 151.56 mg/kg in traditional Chinese sausages, and the concentration of tyramine in 57.1% samples was higher than 100 mg/kg.

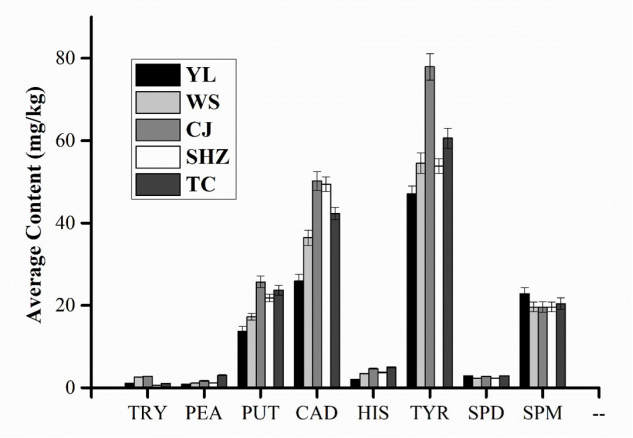

Figure 4. Average contents of eight BAs in five different regions.

Table 3. Average recovery and RSD of the eight BAs in Xinjiang smoked horsemeat sausages (n=6).

Matrix	Contents (mg/kg)	Spiked	Component	Recovery						Average recovery	RSD[c]
		(μg/kg)		(%)						(%)	(%)
Xinjiang smoked horsemeat sausages	ND[a]	1.0	TRP[b]	86.7	85.3	84.2	84.7	82.7	83.5	84.5	1.7
	ND	1.0	PEA[b]	94.6	91.9	94.9	92.2	93.7	95.5	93.8	1.6
	1.1	1.0	PUT[b]	86.2	88.4	84.1	87.4	83.7	86.9	86.1	2.2
	ND	1.0	CAD[b]	86.2	83.1	82.5	83.2	80.5	81.8	82.9	2.3
	ND	1.0	HIS[b]	93.9	84.9	90.9	83.6	89.0	84.9	87.9	4.6
	ND	1.0	TYR[b]	88.1	86.2	88.4	94.8	87.4	89.5	89.1	3.4
	1.0	1.0	SPD[b]	83.6	80.4	85.4	83.0	87.6	83.4	83.9	2.9
	9.6	10.0	SPM[b]	82.6	81.5	89.4	86.9	83.8	83.8	84.7	3.5
	ND	10.0	TRP	93.2	88.9	90.7	89.1	92.9	91.8	91.1	2.0
	ND	10.0	PEA	98.4	103.1	102.5	96.6	101.3	95.9	99.6	3.1
	1.1	10.0	PUT	93.8	92.9	91.9	94.3	89.9	96.7	93.3	2.5
	ND	10.0	CAD	92.6	95.1	99.2	89.3	94.8	93.4	94.1	3.5
	ND	10.0	HIS	86.4	87.7	87.3	92.6	88.2	88.7	88.5	2.4
	ND	10.0	TYR	85.6	90.1	85.4	87.3	91.9	84.2	87.4	3.4
	1.0	10.0	SPD	87.9	90.9	85.3	86.7	93.9	84.5	88.2	4.1
	9.6	50.0	SPM	101.3	93.2	103.7	104.2	99.1	101.4	100.5	4.0
	ND	100	TRP	96.8	90.8	94.7	93.9	91.3	99.3	94.5	3.4
	ND	100	PEA	104.2	109.1	108.2	103.7	111.4	101.2	106.3	3.6
	1.1	100	PUT	93.7	95.3	94.5	89.6	97.2	88.7	93.2	3.6
	ND	100	CAD	92.3	89.8	93.8	93.9	92.1	94.5	92.7	1.9
	ND	100	HIS	92.6	97.5	97.2	94.8	88.5	92.8	93.9	3.6
	ND	100	TYR	95.4	94.7	85.3	101.2	86.6	97.9	93.5	6.7
	1.0	100	SPD	93.2	92.4	91.8	99.5	93.8	94.7	94.2	2.9
	9.6	100	SPM	92.4	91.7	96.3	94.5	96.9	91.8	93.9	2.5

[a]"ND" means not detected; [b]TRP tryptamine; PEA, phenethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPD, spermidine; SPM, spermine; [c]RSD, relative standard deviation.

Table 4. Detection results of the Xinjiang smoked horsemeat sausage samples.

Samples	TRY[a]	PEA[a]	PUT[a]	CAD[a]	HIS[a]	TYR[a]	SPD[a]	SPM[a]
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
YL1[b]	ND[c]	ND	8.5	ND	ND	ND	4.2	39.1
YL2	7.5	ND	17.8	60.2	2.3	132.9	1.9	19.0
YL3	ND	ND	9.9	28.2	6.4	35.6	3.4	27.2
YL4	ND	ND	5.9	21.9	4.6	16.7	2.7	19.3
YL5	ND	2.9	5.3	24.9	ND	111.6	3.0	25.5
YL6	ND	ND	7.7	24.2	ND	32.5	2.6	18.5
YL7	ND	4.2	59.8	114.1	ND	90.9	1.9	11.7
YL8	ND	ND	2.5	3.2	1.4	13.1	0.6	5.6
YL9	ND	3.1	19.6	18.5	3.3	18.9	3.7	27.8
YL10	ND	ND	13.0	3.1	1.3	11.2	4.5	33.4
YL11	6.1	ND	8.8	12.5	5.8	102.2	3.0	21.1
YL12	ND	ND	5.7	ND	ND	ND	3.5	24.9
WS1 [b]	ND	2.6	3.1	2.8	4.0	47.1	2.1	14.5
WS2	5.4	ND	44.6	70.5	ND	103.3	1.2	10.1
WS3	ND	3.5	5.3	4.4	3.3	35.4	3.0	28.1
WS4	ND	3.1	3.1	5.0	4.4	63.6	2.2	18.1
WS5	ND	ND	3.7	ND	ND	ND	4.8	35.7
WS6	ND	ND	1.1	ND	ND	ND	1.0	9.6
WS7	ND	ND	43.5	72.5	9.9	67.1	1.8	13.1
WS8	15.6	ND	32.9	136.1	6.2	119.7	2.3	27.3
CJ1 [b]	8.6	4.1	29.1	84.9	1.9	98.4	2.1	21.9
CJ2	14.0	ND	27.9	34.7	10.1	108.0	1.3	9.4
CJ3	ND	3.1	4.5	30.2	7.8	48.4	2.9	19.3
CJ4	ND	3.7	41.5	98.2	5.4	90.9	2.6	21.9
CJ5	ND	ND	29.8	62.8	3.4	151.5	2.0	15.4
CJ6	ND	ND	57.3	83.6	6.8	78.4	5.3	26.7
CJ7	ND	ND	3.6	ND	ND	ND	2.9	22.8
CJ8	ND	2.8	11.8	7.5	1.9	47.6	3.2	19.3
SHZ1 [b]	3.8	3.5	17.2	68.9	3.6	107.9	2.1	23.3
SHZ2	ND	3.7	21.7	43.1	1.8	41.9	3.2	26.3
SHZ3	ND	ND	62.6	133.3	6.7	63.8	1.4	16.3
SHZ4	ND	ND	15.7	31.2	ND	32.5	2.4	15.9
SHZ5	ND	ND	7.0	11.7	3.3	40.9	2.3	16.7
SHZ6	ND	ND	6.4	8.1	7.1	36.1	2.5	18.8
TC1 [b]	ND	ND	7.0	9.4	6.9	31.8	3.3	18.1
TC2	2.6	5.4	70.2	117.6	4.9	137.9	1.6	12.8
TC3	ND	3.6	10.1	11.7	7.6	26.5	3.1	28.2
TC4	ND	ND	2.8	ND	ND	ND	2.9	25.5
TC5	3.9	2.9	40.5	98.1	3.7	114.4	2.5	11.4
TC6	ND	6.4	11.5	17.2	6.7	53.1	3.9	26.5

[a] TRP tryptamine; PEA, phenethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPD, spermidine; SPM, spermine; [b]YL, Yili city; WS, Urumchi city; CJ, Changji city; SHZ, Shihezi city; TC, Tacheng city; [c]"ND" means not detected.

Cadaverine is the second most abundant biogenic amine followed by tyramine in Xinjiang smoked horsemeat sausages. Due to the complex interaction between processing parameters, fresh meat quality, and microbial flora, cadaverine content varies greatly in different regions of Xinjiang. In this study, the content of cadaverine in Xinjiang smoked horsemeat sausages ranged from ND to 136.1 mg/kg, with an average content of 38.9 mg/kg.

The only amines present at significant levels in fresh meat used for fermented sausage production are spermidine and spermine, and, to a lesser extent, putrescine (Hernandez-Jover et al., 1997). High concentrations of putrescine and the presence of other amines have been attributed to microbial growth and depend on meat freshness. Putrescine and spermine are important BAs in the natural fermentation of smoked horsemeat sausages. The content of putrescine ranges from 1.1 mg/kg to 70.2 mg/kg,

with an average of 19.5 mg/kg, and the content of spermine ranges from 5.6 mg/kg to 39.1 mg/kg, with an average of 20.7 mg/kg. The average content of putrescine and spermine (<30 mg/kg) was less than that of tyramine and cadaverine.

From a toxicological point of view, histamine is the most important biogenic amine, which can cause urticaria, hypotension, headache, flushing, abdominal pain, chemical poisoning and other human health problems (Kaufmann & Maden, 2018). In this study, the content of histamine was low and ranged from ND to 10.1 mg/kg, with an average of 3.6 mg/kg. The low content histamine could be explained that low temperature conditions do not favor histamine formation. Also, indigenous microflora apparently prevented the development of specific strains of some *Enterobacteriace* species capable to decarboxylate histidine (Bover-Cid et al., 2001).

Tryptamine, phenylethylamine and spermine were relatively low in Xinjiang smoked horsemeat sausages, and could be viewed as minor amines happening in fermented sausage. Tryptamine content ranged from ND to 15.6 mg/kg, with an average of 1.7 mg/kg. The content of phenylethylamine ranged from ND to 6.4 mg/kg, with an average of 1.5 mg/kg. The content of spermidine ranged from 0.6 mg/kg to 5.3 mg/kg, with an average of 2.7 mg/kg. This is similar to the results reported by Lu et al. (2010) that tryptamine content in traditional Chinese sausage samples ranged from 0 to 28.2 mg/kg with an average of 7.24 mg/kg, and spermine content ranged from 0.1 mg/kg to 85.32 mg/kg with an average of 14.1 mg/kg. Gençcelep et al. (2008) also found similar results, with spermidine concentrations ranging from 0 to 10.7 mg/kg.

No standards or guidelines exist for the allowable concentrations of biogenic amines in fermented sausages. Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual. Moreover, it must be noted that smaller amounts of biogenic amines may cause poisoning. Handling of raw materials and production technology for Xinjiang smoked horsemeat sausages are relatively primitive. These results indicate that the natural fermentation process used for Xinjiang smoked horsemeat sausages can result in the accumulation of high biogenic amines levels. Different environmental conditions and regions have some effect on biogenic amines content.

4 Conclusions

Xinjiang smoked horsemeat sausage is a kind of self-fermentation sausage, easy to produce various BAs. Up to now, the determination of BAs in fermented sausages mainly focused on HPLC method, because the determination of BAs by HPLC method is complicated and requires derivatization derivative process. So an easy, fast and effective method that can simultaneously measure eight BAs in Xinjiang smoked horsemeat sausage samples using a modified QuEChERS method and UPLC-MS/MS was established. Under optimized conditions of chromatography and mass spectrometry, Xinjiang smoked horsemeat sausage samples were extracted by acetonitrile and purified with 100 mg C18 adsorbent and 100 mg PSA adsorbent. This method had good selectivity, accuracy and precision using internal standard for quantification. The LODs and LOQs of the method were 5 μg/kg and 10 μg/kg, respectively. This method was successfully applied for the determination of eight BAs in forty Xinjiang smoked horsemeat sausage samples. The results showed that the tyramine was the major biogenic amines, followed by cadaverine, putrescine and spermine, which have a mean value of 57.8 mg/kg (ranging from ND to 151.5 mg/kg), 38.9 mg/kg (ranging from ND to 136.1 mg/kg), 19.5 mg/kg (ranging from 1.1 mg/kg to 70.2 mg/kg) and 20.7 mg/kg (ranging from 5.6 mg/kg to 35.7 mg/kg), respectively. TRY, PEA, HIS and SPD content were less than 20 mg/kg. Total biogenic amines content ranged from 26.5 mg/kg to 352.9 mg/kg in Xinjiang smoked horsemeat sausages. On the one hand, the presence of these amines has been related to the low quality of raw materials in which high proliferation of microorganisms occurs. On the other hand, the same raw material can lead to very different amine levels in final products depending on the presence of decarboxylating microorganisms, either derived from environmental contamination or from starter cultures, and the conditions supporting the growth and activity of amine-producing bacteria. Xinjiang smoked horsemeat sausages may pose a potential health risk. Therefore, it must be developed methods for preventing formation of biogenic amines that aim at eliminating the decarboxylating microbes in Xinjiang smoked horsemeat sausages, the use of high-quality raw materials, amine negative starter cultures and processing conditions which favour growth of the starter strains.

Conflict of interest

The authors declare no conflicts of interest.

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Author contributions

Conceptualization, Yuan Wang and Shiling Lu; sample collection, Ruifeng Luo and Xianyi Li; sample analysis, Fei Zhang and Yuan Wang; statistical analysis, Shiling Lu and Ruifeng Luo; writing-original draft preparation, Shiling Lu and Yuan Wang; writing-review and editing, Yuan Wang. All authors have read and agreed to the published version of the manuscript.

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