Bioactive amines in soy sauce: Validation of method, occurrence and potential health effects

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A B S T R A C T

The objective of this study was to investigate the levels of bioactive amines in soy sauce. A method for the extraction of amines was optimized and an ion pair-HPLC method was validated. Overall, tyramine was the prevalent amine, followed by putrescine, histamine, phenylethylamine and cadaverine. The concentrations of amines varied widely among samples. The brands could be divided into two groups. The first one contained three amines; there was prevalence of cadaverine followed by tyramine and putrescine; and total amine levels were low. The second group contained four amines; there was prevalence of tyramine followed by histamine, phenylethylamine and putrescine; and total amine levels were high. A brand with lower NaCl levels contained significantly higher amine levels. Based on the levels of amines detected, a high percentage of samples could cause adverse effects to human health.

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1. Introduction

Soy sauce is a traditional seasoning in China and many other Asian countries. It has been used, for more than 2500 years, to improve the flavour and taste of foods, imparting a salty taste and sharp flavour. Today it is widely used worldwide, mainly due to the increased consumption of oriental foods both at restaurants and at home, where it is used in cooking and as a table condiment. Besides the use as a seasoning, soy sauce has also been used as a salt substitute and also due to its recently recognized health promoting properties (Stute, Petridis, Steinhart, & Biernoth, 2002; Yang, Yang, Li, & Jiang, 2011; Zhu et al., 2010).

Soy sauce is traditionally prepared by months of enzymatic brewing of a mixture of soybean and roasted wheat. During the manufacturing process, a mixture of steam cooked soybean or defatted soybean and roasted wheat flour is allowed to ferment in the presence of koji mould (Aspergillus oryzae or Aspergillus sojae), which produce enzymes that hydrolyze proteins and polysaccharides. After incubation at 25–30 °C for 2–3 days, the koji is mixed with 1.2–1.5 volumes of 22–23% saline to make a soy sauce mash with a final NaCl concentration of 16–18%. In the following step, yeasts and lactic acid bacteria are responsible for the formation of alcohol, flavour compounds and for the lowering of the pH. After ageing at room temperature for about a year, the mash is pressed and the soy sauce is pasteurized (Matsudo et al., 1993; Su, Wang, Kwok, & Lee, 2005; Yongmei et al., 2009). Soy sauce can also be made artificially through HCl hydrolysis, which speeds up the production process (acid-hydrolyzed vegetable protein, HVP). Some soy sauces are economically prepared as a blend of traditionally brewed soy sauce and acid-hydrolyzed vegetable or soy protein (Luh, 1995; Sano et al., 2007; Zhu et al., 2010).

Due to the presence of microorganisms and protein hydrolysis, soy sauce can be a potential source of biogenic amines. However, information on the presence and levels of amines in soy sauce is scarce. Baek et al. (1998) found high levels of tyramine and histamine in Japanese soy sauces. Stute et al. (2002) detected high tyramine levels (up to 5250 mg/kg) in soy sauce available in the German market. They also observed the presence of histamine, phenylethylamine, putrescine and cadaverine. Yongmei et al. (2009) detected high levels of tyramine and histamine in Chinese soy sauce. No information was found regarding the types and levels of amines in soy sauce available in the Brazilian market.

The knowledge of the levels of amines in soy sauce is relevant as it can be used as indices of both quality and safety. The presence of certain amines in soy sauce can indicate poor hygienic-sanitary conditions during processing or the use of low quality ingredients. Moreover, the presence of high levels of histamine, tyramine, tryptamine and phenylethylamine in soy sauce can cause adverse effects to human health: histamine can cause histamine poisoning whereas the other amines are implicated in migraines (Gloria, 2005; Rauscher-Gabernig, Grossgut, Bauer, & Paulsen, 2009). Chinese restaurant syndrome is a combination of symptoms experienced after eating a Chinese meal that include feelings of...
burning, flushing, tingling, tightness and headache – symptoms that are also typical of high levels of biogenic amines. Therefore, it is possible that high levels of biogenic amines in soy sauce may hasten Chinese restaurant syndrome (Yongmei et al., 2009).

The analysis of amines in soy sauce was performed recently by HPLC after extraction with perchloric acid, derivatization with dansyl chloride and UV detection (Yongmei et al., 2009). However, perchloric acid is explosive and dangerous to deal with. Furthermore, the derivatization with dansyl chloride is laborious and time consuming. Therefore, the use of a safer acid and a faster and more selective post-column derivatization followed by fluorimetric detection would be more appropriate for the analysis of these compounds. Such a method was validated and information regarding the profile and the levels of biogenic amines in Brazilian soy sauce was provided.

2. Materials and methods

2.1. Samples

Samples (n = 42) of soy sauce were purchased at supermarkets in Belo Horizonte, MG, Brazil, from July 2009 until February 2010. Seven different brands were available in the market (A–G) and six different lots of each brand were included in this study.

According to the manufacturers, samples from brands C, D, E, F and G were naturally fermented. However, no information was provided regarding fermentation for samples from brands A and B.

According to the labels of the products, they contained water, refined salt, soybean, corn, sugar and glucose syrup and some additives (sodium glutamate, caramel, potassium sorbate, and sodium benzoate). Brand C also listed hydrolyzed soy protein as ingredient on the label. Products from brand E were described as having lower levels of NaCl (32% less). Interesting to observe that corn is used as the adjunct for soy sauce production in Brazil whereas wheat and rice are usually used in Asian countries (Baek et al., 1998; Matsudo et al., 1993; Su et al., 2005; Yongmei et al., 2009).

2.2. Reagents and solvents

The reagents used were of analytical grade, except HPLC solvents (acetone and methanol) which were chromatographic grade. The organic solvents were filtered through HVLP membranes with 0.45 μm pore size (Millipore Corp., Milford, MA, USA). The water used was ultrapure, obtained from Milli-Q Plus System (Millipore Corp., Milford, MA, USA).

Standards of putrescine (PUT, dihydrochloride), cadaverine (CAD, dihydrochloride), histamine (HIM, dihydrochloride), tyramine (TYM, hydrochloride), and 2-phenylethylamine (PHM, hydrochloride), as well as the derivatization reagent o-phthalaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Methods

2.3.1. Optimization of the method for the extraction of amines from soy sauce

In order to obtain the best conditions for the extraction of five amines (putrescine, cadaverine, histamine, tyramine and phenylethylamine) from soy sauce, a sequence of factorial designs was used. The first was a Plackett–Burman design with 12 tests and four repetitions at the central point (Rodrigues &lemma, 2009). The variables studied were sample volume (1, 2 and 3 ml), trichloroacetic acid (TCA) volume (3, 6 and 9 ml) and TCA concentration (1%, 5% and 9%), agitation time at 250 rpm (2, 4 and 6 min) and centrifugation time at 11,250 × g and 0 °C (0, 5 and 10 min).

A second Plackett–Burman design was used with 12 tests and four repetitions at the central point. The variables were sample volume (2, 4 and 6 ml), TCA volume (5, 10 and 15 ml), agitation time (2, 4 and 5 min) and centrifugation time (0, 5 and 10 min). The concentration of TCA was set at 5% because it provided the best results in the first design.

A third experiment was undertaken, fixing the volumes of the sample (6 ml) and of TCA (15 ml). Three tests were performed and the variables investigated were agitation time (2 and 4 min) and centrifugation time (0 and 5 min).

2.3.2. Validation of the optimized method

The fitness of the method for the determination of amines in soy sauce was investigated by means of linearity, selectivity, matrix effect, accuracy, precision, detection limit and quantification limit (Eurachem, 1996; Inmetro, 2010). The standard solutions were prepared by adding the five amines to a solvent (0.1 mol/l HCl) and to a soy sauce matrix at concentrations of 0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/l. The calibration curves were prepared with three independent replicates at each level and analyzed randomly.

2.3.3. Determination of the profile and levels of amines in soy sauce

Aliquots of 6 ml of soy sauce were added to 15 ml of 5% TCA and agitated during 4 min at 250 rpm. The samples were filtered through Whatman # 1 paper and cellulose ester HAWP membrane (0.45 μm pore size, Millipore Corp., Milford, MA, USA). The samples were analyzed by ion-pair HPLC using a reversed phase column, post-column derivatization with o-phthalaldehyde and fluorescence detection at 340 and 450 nm of excitation and emission, respectively, as described by Manfroi, Silva, Rizzon, Sabaini, and Gloria (2009).

The amines were identified by comparison of retention times in samples with those of standard solutions and by adding a known amount of the suspect amines to the sample. Amines levels were calculated by interpolation in the matrix calibration curve.

2.3.4. Determination of some physico-chemical characteristics in soy sauce

The samples of soy sauce were also analyzed for some physico-chemical characteristics according to AOAC (1995), among them, total titratable acidity, total soluble solids and pH. The pH was determined by means of a pH meter (Digimed DM20, São Paulo, SP, Brasil). Total acidity was determined by titration of 10 ml samples with 0.1 mol/l NaOH, up to pH 8.2 and the results were reported as meq/l of soy sauce. The total soluble solids were determined at 25 °C as Brix using a refractometer (RL1-PZO, Warsaw, Poland).

2.4. Statistical analysis

The Plackett–Burman experiments were performed using Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA) at 10% significance. The percent recoveries of amines during extraction as well as the levels of amines and the physico-chemical characteristics of the soy sauces were submitted to analysis of variance and the means were compared by the Tukey test (p < 0.05). Pearson's correlation (p < 0.001) was used to investigate significant correlations between the levels of amines and the physico-chemical characteristics of the soy sauces.
3. Results and discussion

3.1. Optimization of the extraction of amines from soy sauce

The first Plackett–Burman design indicated recoveries which were not acceptable (EC, 2002): 46.1–85.6% for putrescine, 36.9–75.6% for cadaverine, 52.1–85.9% for histamine, 53.1–78.9% for tyramine and 54.7–88.8% for phenylethylamine (Guidi, 2010). It also indicated that the volumes of the samples and of the extracting acid had positive effects on the recoveries (p < 0.1).

In the second Plackett–Burman design, improved recoveries were obtained compared to the first design, with values ranging from 50.7% to 122.0% for putrescine, 75.8–103.1% for cadaverine, 60.0–80.2% for histamine, 76.4–90.3% for tyramine and 68.8–103.4% for phenylethylamine (Guidi, 2010). However, the best extraction condition still provided histamine and tyramine recoveries which were near the lower limit established by EC (2002). According to Stute et al. (2002) and Yongmei et al. (2009), histamine and tyramine are the prevalent amines in soy sauce; therefore further studies were undertaken to improve their recoveries.

In the third Plackett–Burman design, the volumes of the sample and of TCA were set at 6 and 15 ml, respectively, whereas agitation and centrifugation times varied as indicated in Table 1. Treatment 2, which consisted of 6 ml sample, 15 ml TCA, 4 min agitation and no centrifugation, provided the best recoveries of the five amines.

The elimination of the centrifugation step is advantageous as it decreases analysis time as well as its costs. The optimized extraction procedure was reliable, simple and fast. The use of solid-phase extraction recommended by Stute et al., 2002 was not necessary. Furthermore, it was simpler than the method proposed by Yongmei et al. (2009) and did not require the use of perchloric acid, which is explosive.

3.2. Validation of the method for the determination of amines in soy sauce

3.2.1. Linearity

The assumptions that the regression residues followed normal distribution and were homoscedastic and independent were confirmed: the Ryan–Joiner coefficient of correlation indicated that the normality deviations were not significant (p > 0.10); the error variance over the concentrations estimated by the modified Levene test was not significant (p > 0.05), suggesting homoscedasticity; and Durbin–Watson statistics showed independence of the residues (p > 0.10).

The data adjusted well to a linear model, showing correlation coefficients in the range 0.9959–0.9987. Significant regression (p < 0.001) and lack of significant linearity deviation (p > 0.05) indicated that the range from 2.0 mg/l to 10.0 mg/l was linear for the amines using both solvent and soy sauce matrix.

3.2.2. Selectivity and matrix effect

The selectivity of the method was confirmed. There was good resolution among peaks and the presence of the matrix did not affect retention times. Furthermore, there was no interference from other amines which can be present simultaneously in some foods, among them, serotonin, agmatine, spermidine, spermine and tryptamine.

In order to investigate if there was matrix effect, the slopes and intercepts of the linear equations for the calibration curves in solvent and in the matrix were compared by the t-test. Significant difference (p < 0.05) was observed between intercepts of the curves for putrescine, histamine, tyramine and phenylethylamine and also between the inclinations of the curves for cadaverine (Table 2). These results confirmed the matrix effect and, therefore, calibration curves in a soy sauce matrix were used.

3.2.3. Precision and accuracy

Grubbs test indicated the absence of outliers at every concentration investigated. The residues obtained for the apparent recovery for each day and for each concentration, showed normal distribution (p > 0.10) and homoscedasticity (p > 0.05), allowing estimates, by variance analysis, of the relative standard deviation of repeatability (RSDr) and of reproducibility (RSDa).

The mean apparent recoveries for the soy sauce spiked with 2.0–10.0 mg/l of standards varied from 101.3% to 108.2% for putrescine, 92.0–97.1% for cadaverine, 88.8–93.8% for histamine, 86.8–89.9% for tyramine and 93.7–97.7% for phenylethylamine, which is within the acceptable range (80.0–110.0%) established by EC (2002). The RSDr ranged from 0.65% to 6.40% and the RSDa values from 0.97% to 9.20%. These results confirm the applicability of the validated method in the range of 2.0–10.0 mg/l for putrescine, cadaverine, histamine, tyramine and phenylethylamine in soy sauce.

3.2.4. Quantification and detection limits

The detection limits for the amines in soy sauce were 0.18 mg/l for putrescine, 0.13 mg/l for cadaverine, 0.19 mg/l for histamine, 0.16 mg/l for tyramine and 0.20 mg/l for phenylethylamine. The quantification limit of the method was 2.0 mg/l for the five amines. These limits of detection and quantification are adequate for the analysis of amine in soy sauce. No information was found in the literature regarding the limits of quantification for these amines in the soy sauces.

3.3. Occurrence of bioactive amines in soy sauce

The five amines investigated were detected in the soy sauce samples. According to Fig. 1, tyramine was the prevalent amine (100% occurrence), followed by putrescine (97.6%) and histamine (78.6%). Phenylethylamine was detected in 57.1% of the samples whereas cadaverine was present in only 28.6% of the samples.

Similar occurrence of tyramine, histamine in soy sauce has been reported (Baek et al., 1998; Kirschbaum, Rebscher, & Bruckner, 2000; Stute et al., 2002; Yongmei et al., 2009), as well as putrescine (Baek et al., 1998; Kirschbaum et al., 2000; Stute et al., 2002), cadaverine and phenylethylamine (Kirschbaum et al., 2000; Stute et al., 2002).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (min)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agitation</td>
<td>Centrifugation</td>
<td>PUT</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

*The volume of the sample was 6 ml and the volume of TCA was 15 ml. Mean values (n = 3) with different superscripts in the same column are significantly different (Tukey test, p < 0.05). PUT–putrescine; CAD–cadaverine; HIM–histamine; TYM–tyramine; PHM–phenylethylamine.
among samples, from 3.0 mg/l up to 1133 mg/l (Table 3). Wide variability on the levels of amines in soy sauces has also been reported. It has been attributed to the different types and quality of ingredients, applied manufacturing process, soaking period, type of fermentative microorganisms, boiling, storage temperature and time, and, therefore, minimizes amine formation (Su et al., 2005; Yongmei et al., 2009). The lower concentrations of salt in samples from brand E, could have allowed higher protease activity and, therefore, the formation of amines.

When considering the contribution of each amine to total levels (Fig. 2), the brands could be divided into two different groups. In the first one, including brands A and B, the prevalent amine was cadaverine, reaching 39.0–57.0% of total levels, followed by putrescine and tyramine. These were the only three types of amines present. In the second group that included brands C, D, E, F and G, the prevalent amine was tyramine (41.0–51.9%) followed by histamine (33.8–39.6%). Putrescine and phenylethylamine were also present, contributing with less than 13.0% and 12.7% of total levels, respectively. With regard to the levels of amines found in each brand (Table 4), significantly higher putrescine and phenylethylamine from 290.6 mg/l to 1313 mg/l, and total solids varying from 22.0 °Brix to 39.0 °Brix. When grouping samples according to the brands, mean values for the physico-chemical characteristics varied significantly among brands. Higher pH values were observed for brands B, D, F and G (4.75–4.95). Higher acidity was observed for brand C (1185 meq/l) followed by brand E (1014 meq/l), whereas lower acidity was observed for brand D (316.8 mg/l). Total soluble solids were higher for brands F and G (38.8 and 37.8 °Brix, respectively) and lower for brands A, B and E (25.4–27.0 °Brix).

Total levels of amines also varied widely among brands (Table 3). Significantly lower mean total levels were found in brands A and B (12.6 mg/l and 35.4 mg/l, respectively), whereas higher levels were found in brand E (775.9 mg/l). Lower amines levels in brands A and B could be associated with differences in the fermentation process. According to Kirschbaum et al. (2000), the use of acid hydrolysis compared to natural fermentation can affect the formation of amines.

Higher amines levels in brand E could result from lower concentrations of NaCl in the product. In traditionally manufactured soy sauce, the added salt limits protease activity, prolonging fermentation time, and, therefore, minimizes amine formation (Su et al., 2005; Yongmei et al., 2009). The lower concentrations of salt in samples from brand E, could have allowed higher protease activity and, therefore, the formation of amines.

### 3.4. Levels of amines in soy sauce

Overall, mean total levels of amines in soy sauce varied widely among samples, from 2.0 mg/l up to 1133 mg/l (Table 3). Wide variation among levels of individual amines was also observed (nd-33.8–39.6%). Putrescine and phenylethylamine were also present, prevalently contributing with less than 13.0% and 12.7% of total levels, respectively. Putrescine and phenylethylamine (PHM) in soy sauce from the market of Belo Horizonte, MG, Brazil (n = 42).

### 3.5. Physico-chemical characteristics and amines in different brands of soy sauce

The physico-chemical characteristics of the samples (Table 3) varied widely, with pH values ranging from 4.00 to 5.27, acidity values are significant at 5% probability.

### Table 3

<table>
<thead>
<tr>
<th>Brand</th>
<th>Mean values a (± range)</th>
<th>pH</th>
<th>Acidity (meq/l)</th>
<th>Total soluble solids (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.6 b (3.00–32.5)</td>
<td>4.53 c (4.47–4.65)</td>
<td>410.2 b (371.1–462.1)</td>
<td>25.4 b (22.0–28.5)</td>
</tr>
<tr>
<td>B</td>
<td>35.4 a (4.43–110)</td>
<td>4.75 b (4.24–5.27)</td>
<td>407.3 b (301.1–616.2)</td>
<td>27.7 b (25.0–30.0)</td>
</tr>
<tr>
<td>C</td>
<td>460.2 a (252.9–641.5)</td>
<td>4.28 a (4.00–4.37)</td>
<td>1185 a (1110–1313)</td>
<td>34.9 a (33.0–34.5)</td>
</tr>
<tr>
<td>D</td>
<td>231.4 a (130.9–266.7)</td>
<td>4.95 b (4.93–5.06)</td>
<td>3168 a (290.6–346.6)</td>
<td>34.9 ab (28.3–37.0)</td>
</tr>
<tr>
<td>E</td>
<td>772.9 a (363.2–1133)</td>
<td>4.52 a (4.45–4.60)</td>
<td>1014 a (934.8–1092)</td>
<td>27.9 b (27.0)</td>
</tr>
<tr>
<td>F</td>
<td>476.9 a (293.5–573.3)</td>
<td>4.79 ab (4.77–4.82)</td>
<td>4851 a (406.1–521.6)</td>
<td>38.8 b (37.5–39.0)</td>
</tr>
<tr>
<td>G</td>
<td>366.4 a (208.6–435)</td>
<td>4.92 a (4.82–4.96)</td>
<td>5001 a (438.0–525.2)</td>
<td>37.8 ab (37.5–39.0)</td>
</tr>
</tbody>
</table>

**Table 2**

Comparison between slopes and intercepts of the calibration curves of five amines from 2.0 to 10.0 mg/l in soy sauce and in solvent.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Amines</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUT</td>
<td>CAD</td>
</tr>
<tr>
<td><strong>Comparison between intercepts</strong></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>4.876</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Comparison between slopes</strong></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>1.349</td>
</tr>
<tr>
<td>p</td>
<td>0.189</td>
</tr>
</tbody>
</table>

**Footnotes**

a t = t-Statistic for contrasts between intercepts or slopes, p = significance. Bold values are significant at 5% probability.

b PUT–putrescine; CAD–cadaverine; HIM–histamine; TYM–tyramine; PHM–phenylethylamine.

**Fig. 1.** Occurrence (%) of putrescine (PUT), cadaverine (CAD), histamine (HIM), tyramine (TYM) and phenylethylamine (PHM) in soy sauce from the market of Belo Horizonte, MG, Brazil (n = 42).

**Fig. 2.** Contribution of individual amines to total levels in seven brands of soy sauce available in the market of Belo Horizonte, MG, Brazil. (PUT–putrescine; CAD–cadaverine; HIM–histamine; TYM–tyramine; PHM–phenethylamine).
levels of histamine and other biogenic amines (Gloria, 2005; Yongmei et al., 2009).

Therefore, it is necessary to take into account the levels of bioactive amines as a quality control tool for soy sauce in order to warrant the quality and safety of the product which has become very popular in the Brazilian diet. Furthermore, studies are needed to investigate the sources and the conditions allowing amine formation in order to better understand the mechanisms involved in the formation and accumulation of amines in soy sauce. Such information would be valuable in establishing critical control points during soy sauce processing to prevent or limit the formation and build up of undesirable amines and to improve quality.

4. Conclusions

The extraction of amines from soy sauce was optimized and the ion-pair HPLC method with post-column derivatization and fluorimetric detection was validated for the determination of five amines in soy sauce. Samples of Brazilian soy sauce were analyzed. Tyramine was the prevalent amine, present in 100% of the samples. It was followed by putrescine (97.6%), histamine (78.6%), phenylethylamine (57.1%) and cadaverine (28.6%). There was significant difference on the profile and levels of amines among samples. Samples could be grouped into two different types: (i) cadaverine was the prevalence amine, followed by tyramine and putrescine; lower amines levels were present and they would not cause adverse effects to human health; and (ii) tyramine was the prevalent amine, followed by histamine, phenylethylamine and putrescine; higher amine levels were detected and, in some samples, they were high enough to cause adverse effects to human health. Significantly higher levels were detected in samples with lower NaCl content. High levels of some biogenic amines can indicate poor hygienic conditions during soy sauce production.

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References


Table 4

Levels of bioactive amines on different brands of soy sauce available in the market of Belo Horizonte, MG, Brazil.

<table>
<thead>
<tr>
<th>Brands</th>
<th>PUT Mean levels (range) in mg/l</th>
<th>CAD</th>
<th>HIM</th>
<th>TYM Mean levels (range) in mg/l</th>
<th>PHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.50 (7.84-12.7)</td>
<td>4.91 (10.6-16.2)</td>
<td>nd</td>
<td>5.18 (3.09-8.06)</td>
<td>nd</td>
</tr>
<tr>
<td>B</td>
<td>6.85 (14.1-19.7)</td>
<td>20.0 (20.0-26.8)</td>
<td>nd</td>
<td>8.55 (3.30-21.8)</td>
<td>nd</td>
</tr>
<tr>
<td>C</td>
<td>57.7 (13.3-96.7)</td>
<td>nd</td>
<td>155.5 (94.2-196.4)</td>
<td>188.5 (101.8-298.2)</td>
<td>58.5 (19.4-99.3)</td>
</tr>
<tr>
<td>D</td>
<td>13.8 (21.3-38.5)</td>
<td>nd</td>
<td>78.2 (21.8-99.4)</td>
<td>116.3 (37.7-155.6)</td>
<td>23.0 (6.7-41.4)</td>
</tr>
<tr>
<td>E</td>
<td>14.4 (13.3-96.7)</td>
<td>nd</td>
<td>307.0 (102.3-395.0)</td>
<td>402.5 (110.7-659.9)</td>
<td>52.1 (11.1-121.9)</td>
</tr>
<tr>
<td>F</td>
<td>24.6 (11.3-96.7)</td>
<td>nd</td>
<td>184.4 (81.1-315.2)</td>
<td>225.0 (63.0-313.0)</td>
<td>42.9 (11.1-90.9)</td>
</tr>
<tr>
<td>G</td>
<td>33.2 (18.0-180)</td>
<td>nd</td>
<td>135.1 (63.4-201.7)</td>
<td>164.8 (52.3-232.1)</td>
<td>34.3 (6.6-82.0)</td>
</tr>
</tbody>
</table>

a Mean values (n = 6) with different superscripts in the same column are significantly different (Tukey test, p < 0.05). PUT–putrescine; CAD–cadaverine; HIM–histamine; TYM–tyramine; PHM–phenylethylamine.

levels were found in brand C; whereas higher tyramine and histamine levels were found in brand E.

There was no significant correlation between pH or total soluble solids and the concentrations of amines in the samples. However, significant positive correlation (p < 0.001) was found between acidity and levels of histamine, tyramine and total amines. Based on these results, the higher the acidity of the soy sauce, the higher the levels of histamine and tyramine. These could be associated with the response of the microorganisms to the high acidity, which could be detrimental to their survival (Gloria, 2005).

Significant positive correlation (p < 0.001) was found between putrescine and phenylethylamine and also between histamine and tyramine. This suggests that the formation of putrescine and phenylethylamine as well as histamine and tyramine are affected by similar factors.

Further studies are needed to elucidate the role of fermentation and acid hydrolysis on the formation and build up of amines in soy sauce.

3.6. Health effects of soy sauce associated with bioactive amines

It is well established that high levels of biogenic amines in foods constitute a potential public health concern due to toxicological effects. Histamine at levels higher than 100 mg/kg, tyramine levels higher than 100 mg/kg, and phenylethylamine levels above 30 mg/kg can cause adverse effects to human health (Gloria, 2005; Rauscher-Gabernig et al., 2009; Yongmei et al., 2009). Based on this criterion, 48% of the samples could cause histamine poisoning; 61% could induce migraine headache due to tyramine; and 31% could cause headache due to phenylethylamine (Table 5).

The number of samples capable of causing adverse effects to human health could increase when considering the potentiating effect of some amines on histamine poisoning. In fact, putrescine and cadaverine are concomitantly present in some soy sauce samples (Kirschbaum et al., 2000). Another concern would be the consumption of soy sauce with raw fish, typical of some oriental dishes. This combination could increase the chances of having histamine poisoning, because both ingredients may contain high

<table>
<thead>
<tr>
<th>Amines levels (mg/l)</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Tyramine</td>
</tr>
<tr>
<td>&lt;29</td>
<td>13</td>
</tr>
<tr>
<td>30–99</td>
<td>9</td>
</tr>
<tr>
<td>100–499</td>
<td>9</td>
</tr>
<tr>
<td>500–799</td>
<td>0</td>
</tr>
<tr>
<td>≥800</td>
<td>0</td>
</tr>
</tbody>
</table>

Eurachem (1998). The fitness for purpose of analytical methods, a laboratory guide to method validation and related topics (p. 61). Teddington: LGC.


