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

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ABSTRACT

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.

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

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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 (acetonitrile 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 & Iemma, 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, 1998; Inmetro, 2007). 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.