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Tyramine Content of Asian and Pacific Foods Determined by High Performance Liquid Chromatography

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ABSTRACT

Tyramine was analyzed in selected Asian and Pacific foods utilizing high performance liquid chromatography. The method is rapid, sensitive and specific. It gave comparable results when food extracts were analyzed by the fluorescence procedure. Fermented salted black beans and shrimp sauce prepared in Hong Kong contained high levels of tyramine, and 18 other Asian and Pacific foods contained low to moderate amounts. Tyramine levels of some food items increased during refrigerator storage.

INTRODUCTION

Tyramine is formed in foods by decarboxylation of tyrosine by the microorganisms that are present (Rice *et al.*, 1976). The microorganisms may be present as a result of their role in the production of the food, as in certain cheeses, shoyu products and alcoholic beverages (Sen, 1969). Microorganisms may also be present due to contamination during food handling, preparation and storage (Lovenberg, 1973).

Tyramine is harmful to human health as a result of acute ingestion and

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also possibly as a result of long term consumption of low levels of the substance. A sudden increase in blood pressure can occur as a result of ingestion of 10–25 mg of tyramine, particularly in patients receiving monoamine oxidase inhibitor drug regimens (McCabe, 1986). Tyramine is metabolized by monoamine oxidase, an enzyme which also metabolizes catecholamines. Tyramine causes elevation of catecholamines by competing with these substances for monoamine oxidase combining sites, and by stimulation of the release of catecholamines from nerve tissue (Yamamoto *et al.*, 1980).

Chronic effects of tyramine exposure can be postulated to result from frequent reaction of nitrite with small amounts of tyramine under the weakly acidic conditions of the stomach to form a stable diazo product (Ochiai *et al.*, 1984) which has recently been shown to be a potent direct acting carcinogen in rats (Fugita *et al.*, 1987).

Analysis of tyramine is usually carried out by derivatization with 1-nitroso-2-naphthol to form a fluorescent compound whose fluorescence is measured at 565 nm (Spector *et al.*, 1963). This procedure is very sensitive, but is subject to interference by other substances present in food extracts. The method also requires multiple extraction of the sample and 30 min derivatization at 50°C.

We report here a rapid, sensitive, and specific procedure for determination of tyramine using high performance liquid chromatography. The method requires only three solvent-partition steps and determines tyramine directly by optical absorption at 278 nm. A similar but somewhat more complicated procedure has been reported (Wakabayashi *et al.*, 1984*a*).

METHODS

Tyramine . HCl was obtained from Aldrich Chemical Co., Milwaukee, WS. Other chemicals were obtained from common commercial sources. Foods were obtained from local supermarkets as ordinary articles of commerce. Homemade foods were obtained from local residents at community markets.

Tyramine was extracted from foods by first cutting a 3·0 g sample into ~0·5 cm cubes. The diced foods were then placed in a 150 ml Corex glass centrifuge tube (Fisher Scientific Co., Springfield, NJ) and 25 ml of NaCl-saturated borax buffer, adjusted to pH 10·5, were added. The suspensions were homogenized using a Polytron homogenizer (Brinkman Instruments, Westbury, NY). Thirty millilitres of ethyl acetate were then added and the two-phase mixtures were again homogenized. The homogenates were then centrifuged in a GSA rotor of an RC-2 centrifuge (DuPont Co. Biomedical

Products Div., Wilmington, DE) at 5000 rpm ($4000 \times g$) for 10 min. The upper ethyl acetate phases were removed and placed in a second set of 150 ml glass Corex centrifuge tubes each containing 10 ml borate buffer. The tubes were capped and shaken to thoroughly mix the two phases. The resulting mixtures were centrifuged and the upper phases were removed and transferred to a third set of 150 ml glass centrifuge tubes, each containing 6·0 ml 0·2N HCl. After mixing the two phases, they were again separated by centrifugation, and each acidic aqueous phase was transferred to an appropriate container for subsequent analysis by HPLC.

The analysis of tyramine was done using an isocratic HPLC system consisting of a model 110A pump, a 421 Controller unit, and a Hitachi Model 100-10 spectrophotometer variable wave length detector (Beckman Instruments Co., Berkeley, CA), and a No. 4270 electronic integrator (Spectra-Physics Co., San Jose, CA) and a 250 × 4·6 mm column containing 10 micron diameter strong cation exchange resin (SCX, Whatman Co., Clifton, NJ). The mobile phase was 20% water and 80% 0·1M sodium phosphate buffer, pH 7·0. The flow rate was typically 1·5 ml/min. The column eluate was monitored at 278 nm. The tyramine extract was injected onto the column by means of a 100 μl loop injector. (Model 210 sample injection valve, Beckman Co., Berkeley, CA.)

RESULTS

When tyramine . HCl was injected in amounts ranging from 2·0 to 100 μg on the SCX column, the upper chromatogram shown in Fig. 1 was typically obtained. The average tyramine retention time was $10{\cdot}4 \pm 0{\cdot}5$ min ($n = 15$). The retention time was found to be sensitive to the composition of the mobile phase, decreasing with increasing amount of phosphate buffer. The lower chromatogram of Fig. 1 is typical of a food extract, in this case shrimp sauce (Hong Kong), which contains tyramine. These chromatograms have a large complex early eluting peak containing unknown substances. When the food is mixed, i.e. spiked, with tyramine prior to extraction the tyramine peak is greatly enlarged as shown in the middle chromatogram of Fig. 1.

Figure 2 shows a plot of the area of the tyramine elution peak versus the amount of tyramine injected on the column. The area units were obtained by electronic integration of the tyramine optical absorption curve of each solution. The plot extrapolates to zero when 1·0 to 100 μg tyramine are injected on the SCX column.

The recovery of tyramine was determined with known amounts ranging from 2·0 to 100 μg added to 3·0 ml volumes of water, or to a food material containing no tyramine (red bean from Taiwan), mixed with homogenizing

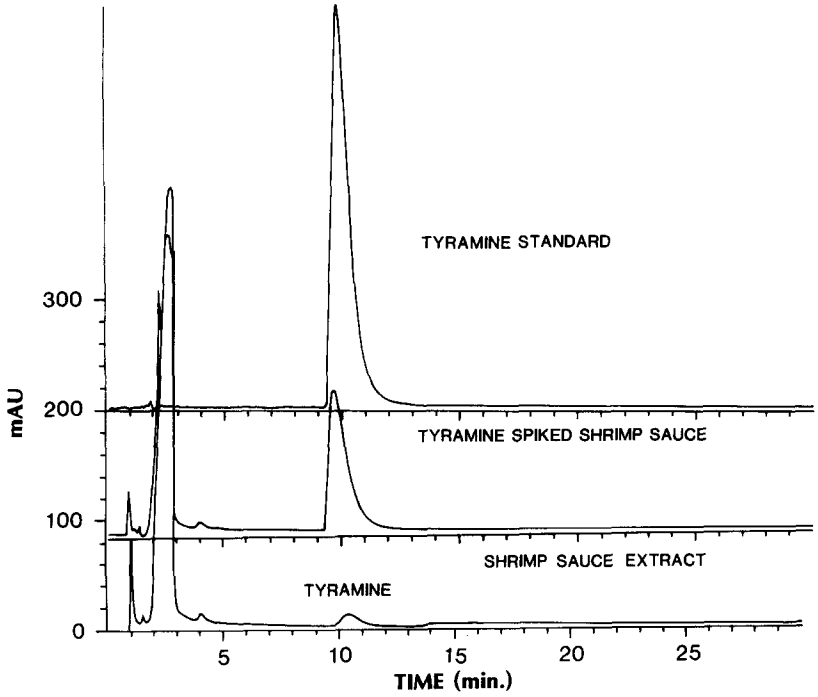

Fig. 1. High performance liquid chromatogram of tyramine standard solution and of food extract containing tyramine.

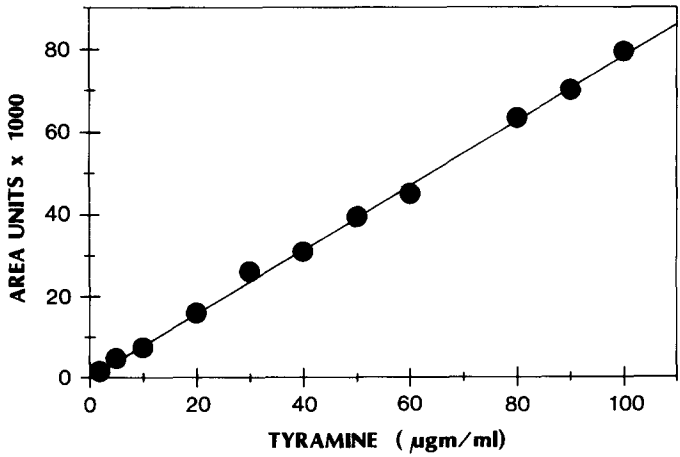

Fig. 2. Tyramine concentration versus HPLC area units.

TABLE 1
Recovery of Tyramine

Tyramine (μg) added to 3·0 g water or red bean	*Tyramine found (μg)*	
	Water	*Red bean*
2·0	1·6	1·4
5·0	4·1	3·9
20·0	17·0	17·1
50·0	43·5	43·0
75·0	64·5	63·0
100·0	90·0	87·4

buffer, and extracted with ethyl acetate. The recovery data are shown in Table 1. The minimum detectable level of tyramine by the procedure is 0·5 μg/g.

Tyramine content of ethnic foods of Hawaii

Table 2 shows the tyramine content of some common ethnic foods eaten in Hawaii. The foods were analyzed on the same day purchased, and analyzed a

TABLE 2
Tyramine Content of Ethnic Foods of Hawaii (μg/g)

Salted Black Beans (Hong Kong)	450·0[a]	563·0[b]	$p < 0·02$[c]
Shrimp Sauce (Hong Kong)	245·5	307·2	$p < 0·02$
Fish Sauce (Thailand)	23·8	19·7	$p < 0·1$
Ziganid Fish (Philippines)	5·4	2·7	$p < 0·1$
Red Bean (Taiwan)	0·8	0·0	NS
Urume-Zuke (Hawaii)	2·1	3·5	NS
Urume-Zuke (Homemade)	8·4	9·7	NS
Kim Chee (Hawaii)	6·9	22·4	$p < 0·02$
Kim Chee (Homemade)	25·7	49·2	$p < 0·05$
Takuan (Honolulu)	0·7	0·5	NS
Pickled Eggplant (Honolulu)	0·0	2·3	NS
Pickled Mango (Hawaii)	1·1	0·6	NS
Pickled Plum (Hawaii)	0·0	0·9	NS
Dried Shrimp (Honolulu)	13·6	11·2	NS
Somen Soup Base (Japan)	35·5	32·3	NS

[a] Analysis on the day acquired.
[b] Analysis after 3 weeks at 5°C.
[c] Significance of difference in tyramine content of food sample measured on day acquired and after 3 weeks' storage at 5°C.
NS = not significant.

second time after 3 weeks' storage in a common household refrigerator. The food with the highest amount of tyramine was a black bean preparation manufactured in Hong Kong. Shrimp sauce (Hong Kong) and fish sauce (Thailand) contained moderate amounts of tyramine. However, a commercial sample of bagoon padas (salted Ziganid Fish), which is a traditional fermented fish product of the Philippines, contained little tyramine.

We found only low levels of tyramine in a series of commercial samples of Japanese pickled vegetables and in kim chee, a traditional Korean fermented cabbage. However, samples of homemade kim chee and urume-zuke had higher levels of tyramine than did the same commercial products. Upon storage at $+5°C$, the levels of tyramine in some foods increased substantially. This was particularly true for the kim chee samples. Food items, such as natto, tofu, yogurt, miso and soya based cheeses, did not contain detectable amounts of tyramine even after storage.

When the same food samples were measured for tyramine by different persons, the results were in agreement within $\pm 15\%$. The same food samples analyzed by the HPLC procedure and by the fluorescence technique contained the same amount of tyramine within $\pm 20\%$.

DISCUSSION

The HPLC procedure for the analysis of tyramine has certain advantages over other methods (Spector *et al.*, 1963; Wakabayashi *et al.*, 1984*a*). The fluorometric procedure of Spector *et al.* (1963) is about ten times more sensitive, but has the disadvantage that the usable linear concentration range is only from 0·05 to 0·4 μg tyramine/ml. Furthermore, the high sensitivity of the fluorescence method is of little advantage since only foods with $\sim 100\,\mu$g/tyramine/g food are a potential health hazard. The use of solvent extraction as the means of purifying the food extract prior to HPLC seems to make our method somewhat more sensitive (0·5 vs 2·0 μg/g) than that of Wakabayashi *et al.* (1984*a*). The latter procedure employs gravity ion exchange chromatography, which causes dilution of the sample during elution from the column.

Levels of tyramine between 1000 and 2000 μg/g have been reported in some cheeses (Sen, 1969) and some soya preparations (Wakabayashi *et al.*, 1984*b*). The highest value found in this study is about 1/4 as high, and would require the ingestion of about 20 g of such foods to give a dose of 10 mg tyramine. The latter amount is considered a minimum value necessary to cause hypertensive crisis in a sensitive individual (McCabe, 1986).

Our results are in general agreement with those of Wakabayashi *et al.*

(1983, 1984*b*). These authors measured mutagens in oriental foods that are formed by the reaction of tyramine with nitrite.

Tyramine levels can change on storage even at refrigerator temperatures, and it is possible that foods thought to be tyramine free might cause concern if an unusually long time elapses between purchase and consumption.

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