ISOLATION AND IDENTIFICATION OF BIOLOGICALLY ACTIVE AMINES IN FERMENTED FISH PASTE

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

DEDI FARDIAZ

1977

ABSTRACT

ISOLATION AND IDENTIFICATION OF
BIOLOGICALLY ACTIVE AMINES
IN FERMENTED FISH PASTE

By

Dedi Fardiaz

This investigation was carried out to determine biologically active amines qualitatively and quantitatively in fermented fish paste by using gas liquid and thin layer chromatographies. Amines were extracted with peroxide-free ether under alkaline condition. Amine hydrochlorides obtained after extraction were treated with trifluoroacetic anhydride in ether (1:1) to form N-trifluoroacetyl derivatives.

Separation of the compounds was achieved using a 6 ft. x 0.125 in. o.d. 3% SP-2100 on 100/120 Supelcoport with FID detector. The column was programmed from 60 to 240°C at 8°C per minute with nitrogen as carrier gas at a flow rate of 18.5 ml per minute. Precoated silica gel and cellulose plates were used in TLC with four solvent systems, n-butanol:pyridine:water (1:1:1); n-butanol: pyridine:glacial acetic acid:water (60:8:12:20); n-butanol:

glacial acetic acid:water (4:1:5); and chloroform:methanol: ammonium hydroxide (12:7:1).

Chromatograms obtained from six different commercial fermented fish pastes showed from 7 to 17 major peaks, several of which were positively identified as ethanolamine, 2-methylbutylamine, β-phenylethylamine, tyramine, dopamine, octopamine, cadaverine, tryptamine, and β-mercaptoethylamine. Depending on the origin of the product the concentration of these amines ranged as follows: ethanolamine (15.3-106.5 μg/g), 2-methylbutylamine (5.0-12.6 μg/g), β-phenylethylamine (18.8-600.0 μg/g), tyramine (34.2-376.2 μg/g), dopamine (17.6-300.6 μg/g), octopamine (7.6-53.8 μg/g), cadaverine (35.0 μg/g), tryptamine (22.8-162.8 μg/g), and β-mercaptoethylamine (12.5-35.0 μg/g).

ISOLATION AND IDENTIFICATION OF BIOLOGICALLY ACTIVE AMINES IN FERMENTED FISH PASTE

By

Dedi Fardiaz

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1977

ACKNOWLEDGMENTS

The author would like to thank his major professor, Dr. P. Markakis, for his encouragement and guidance throughout the course of this study and assistance in the preparation of this manuscript.

Appreciations are also expressed to Dr. E. S. Beneke of the Department of Botany and Plant Pathology, Dr. R. F. McFeeters, and Dr. J. N. Cash of the Department of Food Science and Human Nutrition for their helpful suggestions as members of the graduate committee.

Finally, the author is deeply grateful to his parents, his wife, Andi, his daughter, Miri, and his close friends for their support, encouragement, and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Biologically active amines can be synthesized endogenously in certain plants or formed by microbial activity. Many bacteria produce decarboxylases which catalyze the conversion of several amino acids to amines. Consequently, food substances prepared by a fermentative process are likely to contain amines.

Fermented fish paste is an oriental food eaten almost everywhere in Southeast Asia. It is made of small fish or shrimp which are fermented for at least one month to form a paste. As a result of microbial activity the paste may contain amines generally and physiologically active ones particularly.

It has been known that ingestion of foods containing biologically active amines may be deleterious to health. The toxic effects are often fatal for people taking monoamine oxidase inhibitory drugs. It is important, therefore, to isolate, identify, and determine the level of amines which may be present in fermented fish paste, so that we can measure the degree of safety of this food.

Amines are substances which have widely differing chemical and physical properties. It is difficult to

analyze free amines by gas chromatography since they are generally polar, basic in nature, nonvolatile, and unstable at high temperature. Therefore, suitable derivatives are used in the analysis of these amines by gas chromatography. The purpose of this research was to determine qualitatively and quantitatively biologically active amines present in fermented fish paste.

LITERATURE REVIEW

Biologically Active Amines and Their Effects

Biologically active amines or biogenic amines are naturally occurring organic amino compounds which play a biochemical role in the life processes. They can be found as aliphatic or aromatic amines in plants, animals, and decomposed products of living organisms (Doby, 1965). It has been known that derivatives of phenylalkylamine may have a physiological effect in man (Udenfriend *et al.*, 1959; Lovenberg, 1973).

There are two major reasons why attention should be given to the presence of biologically active amines in foods. First, ingestion of amine-containing foods may cause a health hazard to a person treated with drugs which contain monoamine oxidase inhibitor. Second, amines by themselves may be toxic.

Several reports published during 1962-1963 showed that ingestion of cheese in patients treated with the drug tranylcypromine resulted in toxicity. The toxic effects reported were a dangerous rise in blood pressure, severe hypertension, intracerebral hemorrhage or even death (Asatoor *et al.*, 1963; Blackwell, 1963; Lovenberg, 1973).

It was found that cheese contained a high level of tyramine. And monoamine oxidase inhibitor present in drugs was responsible for the toxic effect (Asatoor et al., 1963; Horwitz et al., 1964). Under normal conditions, tyramine is oxidized by monoamine oxidase in the body to the harmless phenolic acid, p-hydroxyphenylacetic acid, as shown in Figure 1. Likewise, other amines are inactivated by monoamine oxidase to harmless compounds (Davison, 1958).

If the pathway for catabolism and inactivation of the amines is blocked due to either monoamine oxidase inhibitors or disfunction of the enzyme, amines will be circulated in the blood. And this abnormally high level of circulating amines is considered as the major cause of toxemias in human. Horwitz et al. (1964) reported that as little as 6 mg of tyramine hydrochloride was sufficient to produce hypertension in persons treated with monoamine oxidase inhibitory drugs. Generally drugs containing hydrazine derivatives such as iproniazid and isoniazid act as monoamine oxidase inhibitors.

Following these findings, several foods were reported to contain biologically active amines which produce the same toxic effects as caused by cheese. Hodge et al. (1964) reported that hypertensive crises occurred in persons treated with monoamine oxidase inhibitors after eating cooked broad beans (Vicia faba L.). He suggested

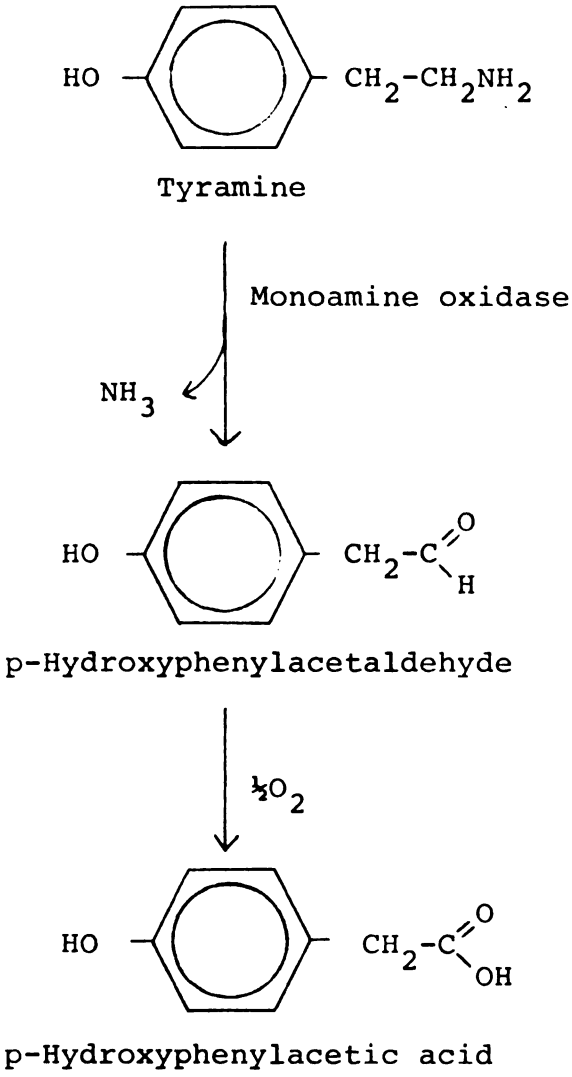


Fig. 1.--Inactivation of tyramine by monoamine oxidase in the body.

that 3,4-dihydroxyphenylalanine or its amine derivative, dopamine, was responsible for the toxicity. Blackwell et al. (1965) reported that eating yeast extract, marmite, produced hypertension and severe headache in persons receiving monoamine oxidase inhibitors. It was found that the high level of tyramine and histamine in that product were the cause of the disease.

Since 1962 it has been reported that biologically active amines may cause direct toxic effects to human. Davies (1960) found that there was a high incidence of heart disease (endomyocardial fibrosis) in a group of Africans who eat plantain as a major food source. This disease has not been found among Europeans or Indians of East or West Africa who do not eat plantain. Based on this fact, it was suggested that the high level of 5-hydroxytryptamine present in plantain might play a role in causing the disease (Crawford, 1962; Foy and Parratt, 1962).

Another possible direct toxic effect of biologically active amines in foods is headaches. Hanington (1967) reported that oral tyramine could induce migraine attacks in susceptible subjects. According to his experiment, an attack of migraine can be precipitated by a dose of 100-125 mg of tyramine. Sandler et al. (1970) suggested that persons who respond to tyramine with headache absorb an abnormally high load of tyramine from the foods.

Besides tyramine, β-phenylethylamine is also considered as a powerful potentiator of migraine headaches. Clinical trials have shown that 3 mg of β-phenylethylamine is sufficient to initiate a migraine attack; indeed, it is a more potent migraine precipitant than tyramine (Sandler et al., 1974; Chaytor et al., 1975). Many suggestions have been published to explain why biologically active amines produce migraine attacks; however, the exact mechanism involved is not known.

Formation of Biologically Active Amines in Decomposed Products

Food substances which have been exposed to microbial contamination undergo biodeterioration. Eskin et al. (1971) defined biodeterioration as any undesirable change in the properties, chemical composition, or structure of a material or substance caused by the activities of organisms. In the microbial spoilage of foods, various complex organic substances are broken down to low molecular weight compounds by the action of microorganisms. The type of spoilage is influenced by the chemical composition of the foods and the type of microorganisms.

Studies of the microbial spoilage of fish showed that fish juice which contains low molecular weight nitrogenous substances such as free amino acids, simple peptides, trimethylamine oxide, etc. is the most important fraction in which the biochemical changes take place.

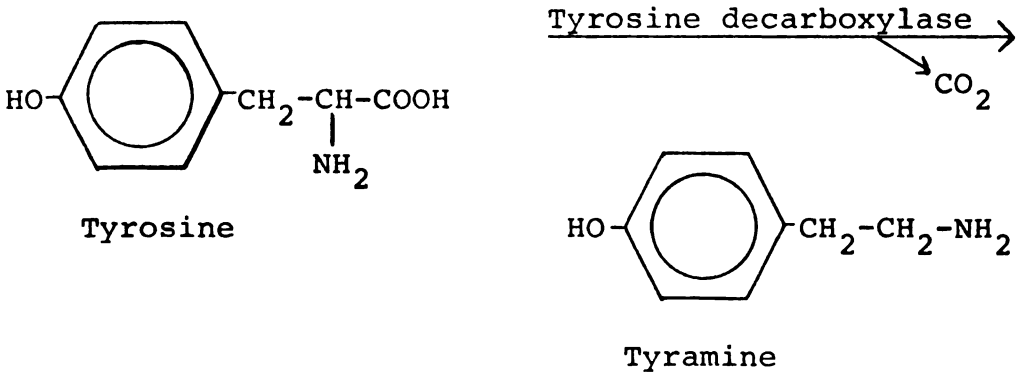
Proteolytic breakdown of the proteins occurs only during the advanced stages of putrefaction (Lerke et al., 1967; Eskin et al., 1971).

In spoiled foods amines arise directly from amino acids by the decarboxylation reaction. It has been known that α-decarboxylases produced by microorganisms catalyze the α-decarboxylation reaction of amino acids during the spoilage, according to the following equation (Boeker and Snell, 1972):

$$R - \underset{\underset{NH_3^+}{|}}{CH} - C\begin{matrix}=O\\ -O^-\end{matrix} + H \xrightarrow{\text{α-amino acid decarboxylases}} R - CH_2 - NH_2 + CO_2$$

Several examples of microbial α-decarboxylase catalyzing reaction during the spoilage of protein foods are given below.

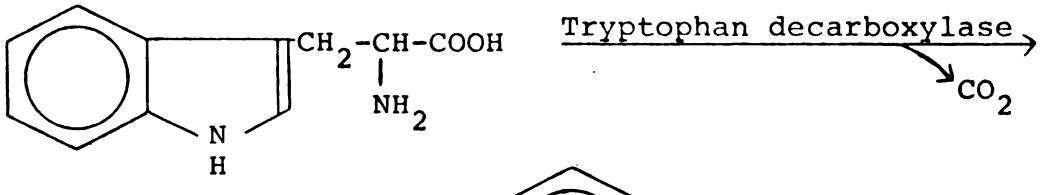
1. Formation of tyramine from tyrosine by Streptococcus faecalis (Gale, 1940).



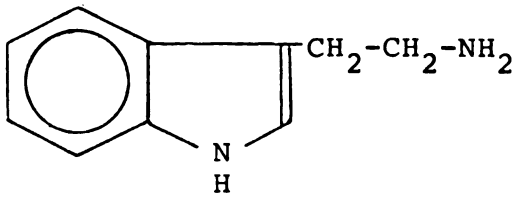
Tyrosine

Tyramine

2. Formation of tryptamine from tryptophan by Streptococcus faecalis and Clostridium welchii (Eskin et al., 1971).

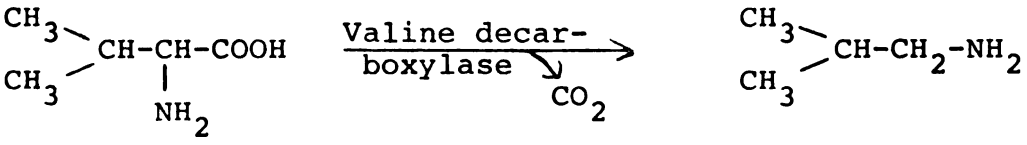


Tryptophan



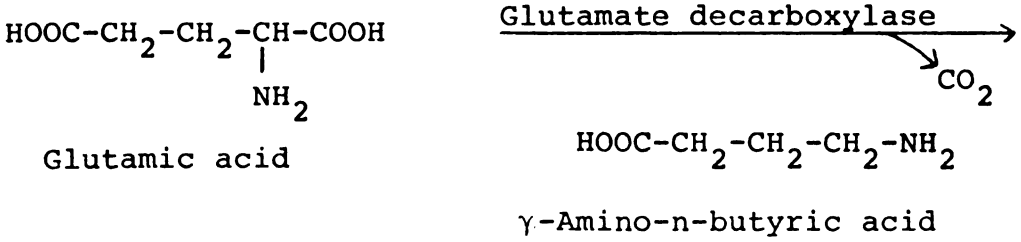
Tryptamine

3. Formation of isobutylamine from valine by Proteus vulgaris (King, 1953; Ekladius, 1957) and Pseudomonas cocovenans (Eskin et al., 1971).

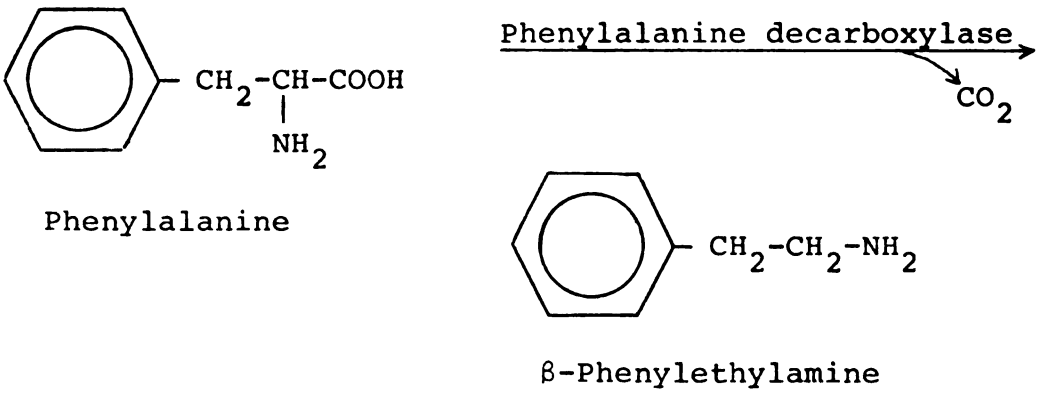


Valine Isobutylamine

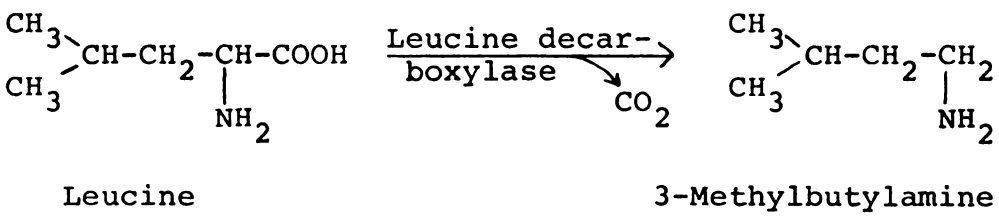
4. Formation of γ-amino-n-butyric acid from glutamic acid by Escherichia coli (Homola, 1967; Strausbauch and Fischer, 1970), Streptococcus faecalis (Eskin et al., 1971), and Clostridium perfringens (Cozzani et al., 1970).



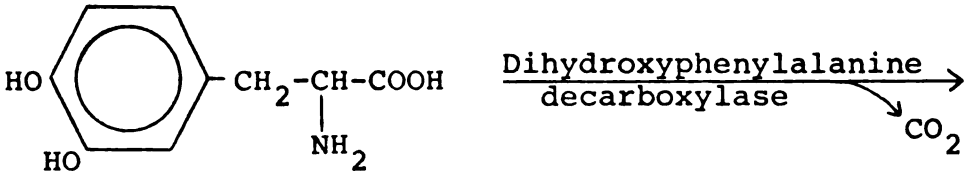
5. Formation of β-phenylethylamine from phenylalanine by Streptococcus faecalis (McGilvery and Cohen, 1948).



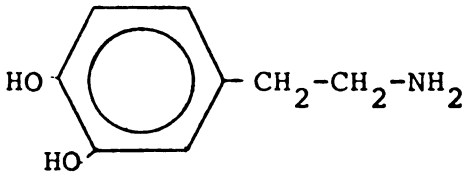
6. Formation of 3-methylbutylamine from leucine by Proteus vulgaris (King, 1953).



7. Formation of dopamine from dihydroxyphenylalanine by Streptococcus faecalis (Epps, 1944).

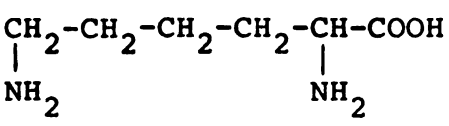


Dihydroxyphenylalanine

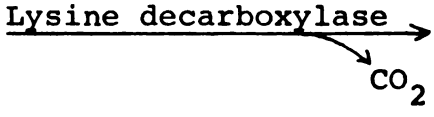


Dopamine

8. Formation of cadaverine from lysine by Bacterium cadaveris (Soda and Moriguchi, 1969) and Escherichia coli (Meretzki and Mallette, 1962).



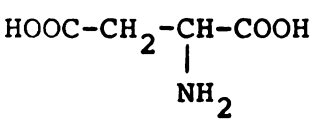
Lysine



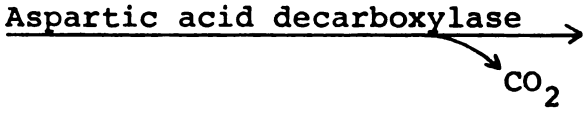
$NH_2-CH_2-CH_2-CH_2-CH_2-CH_2-NH_2$

Cadaverine

9. Formation of β-amino-n-propionic acid from aspartic acid by Achromobacter sp. (Wilson, 1963) and Alcaligenes faecalis (Tate and Meister, 1971).



Aspartic acid



$HOOC-CH_2-CH_2-NH_2$

β-amino-n-propionic acid

Fermented Fish Paste Manufacture

Fermented fish paste called "Bagoong" in the Philippines, "Kapi" in Thailand, and "Blachan" in Malaysia, is a condiment for rice dishes eaten almost everywhere in Southeast Asia. It is prepared by a fermentative process. The method for preparing fermented fish paste in one place or country may be different from another; however, the basic principle is almost the same.

Traditionally fermented fish paste is made of small fish such as Stolephorus sp. or small shrimp such as Atya sp. Sometimes various forms of carbohydrates and dyestuffs are added. The first step in the preparation of fermented fish paste is the mixing of fish or shrimp with about 10 to 15% salt. After a few days the mixture is spread out on large floors and sun dried for 1 to 3 days. The mixture is then kneaded carefully to form a paste. Sometimes during kneading red synthetic dyestuffs are added. The mass is fermented for about 1 month at ambient temperature, about 30°C. The finished product is a red-colored, sticky mass which has distinct aroma and flavor (Van Veen, 1953).

So far microorganisms responsible for the fermentation have not been clearly identified. Although there is no certain microorganism used as inoculum, spontaneous fermentation can occur due to bacteria which originate from the fish, the salt used in the process or from contamination. High concentration of salt used may act as

culture selector. Consequently, halophilic or halotolerant bacteria predominate in the fermentation process.

Since the fish tissue is exposed to bacterial activity during the fermentation process, the fermented fish paste is likely to contain biologically active amines. These amines are produced from decarboxylase-catalyzed reactions of free amino acids liberated by proteolytic breakdown of the proteins.

Analysis of Amines

Several solvents can be used to extract biologically active amines from food samples. Solvents which may react with the amines must be avoided. Chloroform may react with amines under alkaline conditions to form isocyanides. Carbon tetrachloride often reacts with amines especially in the presence of light to form hydrochlorides and other compounds. Alcohols may extract many other polar materials along with amines. Ether may contain hydrogen peroxide which can oxidize amines (Fales and Pisano, 1964). However, hydrogen peroxide in ether is easily removed by washing it with aqueous 5% ferrous sulfate to form peroxide-free ether suitable for extraction of amines (Stecher et al., 1968).

Previous work on the analysis of amines in foods was done by paper or thin layer chromatographic methods. Paper and thin layer chromatographies can produce specific separations; however, these are time-consuming, and cannot

be used for quantitative analysis at low level. Amines in foods can also be measured by fluorometric methods either directly (Udenfriend et al., 1959) or indirectly after separation by thin layer chromatography (Voigt and Eitenmiller, 1974). The direct fluorometric method is rapid and sensitive, but interference by other compounds is common. Combination of ion-exchange chromatography and measurement of UV absorbance was also used to analyze amines (Wheaton and Stewart, 1965); this method is only limited to phenolic amines.

Amines are difficult to analyze by gas chronatography, since they have widely differing chemical and physical properties. Fales and Pisano (1962) attempted to separate aromatic amines by gas chromatography on SE-30 column. It was found that the direct method of analysis of amines by gas chromatography produced poor peaks and was not suitable for the analysis of the more complicated amines. Failure to get a good separation is due to the fact that biologically active amines are generally polar, basic in nature, nonvolatile, and unstable at high temperature.

O'Donnell and Mann (1964) suggested several ways to avoid the problem of tailing of amine peaks: (1) using a support with an inert surface, (2) treatment of the surface with a basic organic compound, (3) using a basic liquid phase, and (4) treatment of the support or liquid

phase with an alkali hydroxide to decrease the tendency of the column to absorb amines. Although these methods are good, the conversion of amines to suitable volatile derivatives turns out to be the best solution to peak tailing problem.

Sen and McGeer (1963) found that the trimethylsilylation was a suitable method to convert catecholamines to volatile trimethylsilyl ethers. However, several derivatives such as the trimethylsilyl derivatives of epinephrine and norepinephrine could not be separated from each other on the SE-30 column.

Brydia and Persinger (1967) described a method for the quantitative gas chromatographic analysis of ethanolamines as N-trifluoroacetyl derivatives on the neopentylglycol succinate column. The formation of derivatives was based on the reaction of the amino and hydroxyl groups of ethanolamines with trifluoroacetic anhydride, as shown in Figure 2.

There are two advantages in the conversion of amines to N-trifluoroacetyl derivatives: (1) increase in volatility, and (2) removal of the basicity of the corresponding amines. This method appeared to be suitable for analysis of biologically active amines by gas liquid chromatography; therefore, it was used in this investigation. Because of its reactivity with moist air and its dangerous properties, the reactant must be handled with

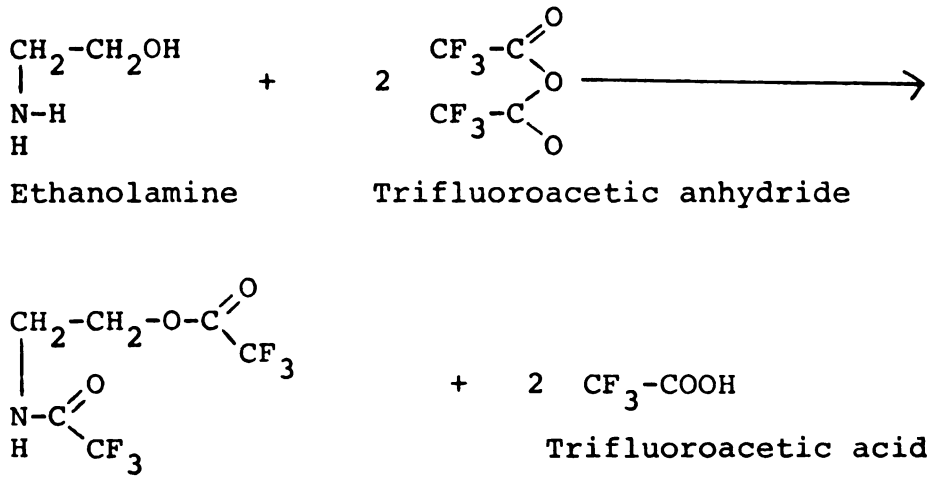


Fig. 2.--Formation of N-trifluoroacetyl derivative of ethanolamine (Brydia and Persinger, 1967).

special precaution. Likewise, the N-trifluoroacetyl derivatives are not stable for a long time and they should be analyzed immediately.

MATERIALS AND METHODS

Fermented fish paste samples were purchased from local stores. Those samples were products from Malaysia, Thailand and the Philippines. Five cans or bottles of each sample were mixed and homogenized in order to get a uniform paste, placed in a new bottle, labeled, and refrigerated at about 35°F.

The samples used were:

A. Philippine Bagoong "Anchovy Sauce." It was made of anchovy extract, manufactured by Besana Enterprises, Manila, the Philippines.

B. "Shrimp Paste." It was made from shrimp and fish, packed by Mae Tu Co., Bangkok, Thailand.

C. "Salted Anchovy." It was made of anchovies, processed and packed by Rapenco Food Products, Quezon City, the Philippines.

D. "Malaysian's Prawn Cake" (Blachan). It was made of prawn, packed for Lucky Foods, Co., San Francisco.

E. "Lorenzana Bagoong." It was made of oyster, manufactured by Lorenzana Fish Products, Rizal, the Philippines.

F. Salted Shrimp Fry "Bagoong Alamang." It was made of shrimp, processed and packed by Rapenco Food Products, Quezon City, the Philippines.

Amine standards were purchased from the following commercial sources: tyramine hydrochloride, tryptamine hydrochloride, histamine dihydrochloride, and cadaverine hydrochloride from Calbiochem; dopamine from Nutritional Biochemicals Corporation; octopamine hydrochloride, ethanolamine, putrescine, γ-amino-n-butyric acid, 2-methylbutylamine, isoamylamine, phenylmethylamine, β-mercaptoethylamine hydrochloride, synephrine, and β-phenylethylamine from Sigma Chemicals.

Extraction and Isolation

Diethyl ether was used to extract all amines from the sample filtrate. Since it frequently contains hydrogen peroxide capable of oxidizing amines (Fales and Pisano, 1964) it must be washed by using 5% ferrous sulfate (Stecher et al., 1968). Every 100 ml diethyl ether was washed four times with 100 ml aqueous 5% ferrous sulfate in a 500 ml separatory funnel. Peroxide-free diethyl ether obtained was suitable for the extraction of the amines.

A method of extraction by Silverman and Kosikowski (1956) was used with several modifications. Ten grams of homogenized sample was blended with 40 ml of distilled water. The slurry was filtered under suction on a Whatman

No. 1 filter paper through a Buchner funnel. The blender was washed with another 10 ml of distilled water. The filtrate was heated in a boiling water bath for 10 minutes, and cooled to room temperature.

Twenty ml of the filtrate was transferred into a 125 ml Erlenmeyer, and 2 ml of 50% trichloroacetic acid was added. The mixture was well agitated in a mechanical shaker for 10 minutes and then filtered through Whatman No. 1 filter paper. Fifteen ml of this clear acid filtrate was pipetted into another 125 ml Erlenmeyer, and 30 ml of a tri-potassium phosphate-sodium sulfate buffer (pH 12) was added slowly. The buffer was made by mixing 45.6 grams anhydrous K_3PO_4 and 272.8 grams anhydrous Na_2SO_4 in 1 litre distilled water. By adding of 10% sodium hydroxide solution, the pH of the mixture was adjusted to pH 12 using Coleman pH Meter. While adjusting the pH, the mixture was stirred magnetically.

The mixture was then filtered through Whatman No. 1 filter paper. Forty ml of the filtrate was extracted with 80 ml peroxide-free ether for 3 minutes in a 250 ml separatory funnel. After extraction the ether layer was transferred through Whatman No. 1 filter paper containing several grams of anhydrous Na_2SO_4 into a 500 ml beaker containing 2 ml of 0.02 N hydrochloric acid. The extraction was repeated on the same 40 ml filtrate three times with fresh peroxide-free ether.

The ether collected in the beaker was evaporated in a steam bath. The acid solution of amines was transferred into a 5 ml glass stoppered tube. The beaker was washed with 1 ml of 0.02 N hydrochloric acid, and the acid was transferred into the same tube. The solution was evaporated at 50-60°C to dryness in a vacuum oven. The solid residue consisting largely of amine hydrochlorides was washed with anhydrous diethyl ether and the ether was discarded. The amine hydrochlorides obtained were used for analysis in either gas or thin layer chromatography.

Preparation of N-trifluoroacetyl Derivatives

One ml of trifluoroacetic anhydride and 1 ml of anhydrous diethyl ether were transferred into the 5 ml glass stoppered tube containing the amine hydrochlorides. The mixture was agitated carefully using a small magnetic stirrer for 2 hours until all amine hydrochlorides dissolved. The solution was evaporated to dryness by passing a stream of nitrogen gas through the solution. The residue obtained was N-trifluoroacetyl derivatives. Since these derivatives are not stable, they were analyzed immediately. For the preparation of the N-trifluoroacetyl derivative standards, standards of amine hydrochloride were taken through the whole procedure including the extraction steps with peroxide-free ether from buffer.

Gas Chromatograph and Separation Conditions

All gas chromatographic separations were carried out using a Perkin-Elmer 900 Gas Chromatograph equipped with a Servo/Riter II Flushmount Recorder and a Flame Ionization Detector.

A 6 ft. x 0.125 in. o.d. aluminum column was packed with 3% SP-2100 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA.). The packed column was conditioned at 275°C for 4 hours, and then from 60 to 275°C at 1°C per minute with a nitrogen flow rate of 37 ml per minute.

The standard chromatographic conditions for N-trifluoroacetyl derivative separations were accomplished with nitrogen as the carrier gas at an inlet pressure of 50 psi and the flow rate of 18.5 ml per minute. The flame ionization detector was operated at 250°C with hydrogen pressure of 25 psi and air pressure of 40 psi. The injection port temperature was 235°C, attenuation was x 32 with attenuation range x 100, and chart speed was 15 in. per hour. Temperature of the column was programmed from 60 to 240°C at 8°C per minute.

Injection of the Samples Into the Gas Chromatograph

A 5 ml glass stoppered tube containing the N-trifluoroacetyl derivatives was placed in an ice bath, and 50 µl to 2 ml anhydrous diethyl ether was added into the tube. Proper dilution was carried out in order to get the

right peak response according to the standard curves previously made. One to 5 µl of aliquots were immediately injected into the gas chromatograph by using a 10 ul Hamilton syringe #701 (Hamilton Co., Reno, Nev.). The syringe was cleaned between injections with anhydrous diethyl ether and used again after it had been dried.

Thin Layer Chromatography

Thin layer plates (20 x 20 cm) of precoated silica gel 60 F-254 (Merck, Darmstadt) and precoated cellulose (Eastman Kodak Co., Rochester, N.Y.) were used with layer thickness of 250 µ and 100 µ respectively. The adsorbents were reactivated at 100°C for 30 minutes immediately before used. Chamber saturation (CS) system was used in this experiment. Chamber saturation system is a chamber which has paper lining inside to equilibrate the solvent vapor in the chamber and improve the separation (Stahl, 1969).

The dried amine hydrochlorides were dissolved in 0.3 ml 70% methanol. Three µl aliquots were spotted with a capillary pipette on thin layer plates for qualitative analysis. Three µl of amine hydrochloride standards (2 mg/ml) were spotted as above. Spots were dried immediately using a Sears hair dryer, and chromatographed in a single dimension with 4 solvent systems: n-butanol: pyridine:water (1:1:1); n-butanol:pyridine:glacial acetic acid:water (60:8:12:20); n-butanol:glacial acetic acid: water (4:1:5); and chloroform:methanol:ammonium hydroxide

(12:7:1). Thin layer plates were developed simultaneously by ascending chromatography. The spray reagent used was a mixture of 0.3 gram ninhydrin, 3 ml acetic acid, and 100 ml n-butanol. After spraying, the plates were heated at 110°C until the color showed up.

RESULTS AND DISCUSSION

Analysis by Gas Liquid Chromatography

The formation of N-trifluoroacetyl derivatives is of great importance in the analysis of amines by gas liquid chromatography. Heuvel et al. (1964) found that gas chromatographic analysis of free amines showed peak tailing and partial irreversible adsorption, even with relatively inert column packings. It was suggested that the strong hydrogen bonding character of the amines caused the excessive peak tailing (Brydia and Persinger, 1967). By comparing the peak height response of the free amines with the N-trifluoroacetyl derivatives, it was proven that the formation of these derivatives resulted in a large increase in sensitivity (McCurdy and Reiser, 1966).

The result of gas chromatographic separation of N-trifluoroacetyl derivatives obtained from a standard mixture of 13 amines is shown in Figure 3. The chromatogram exhibits symmetric peaks of all amine standards without appreciable peak tailing. This indicated no irreversible adsorption of N-trifluoroacetyl derivatives on the column packing materials. Consequently, identification of amines in the samples based on retention time of the standards

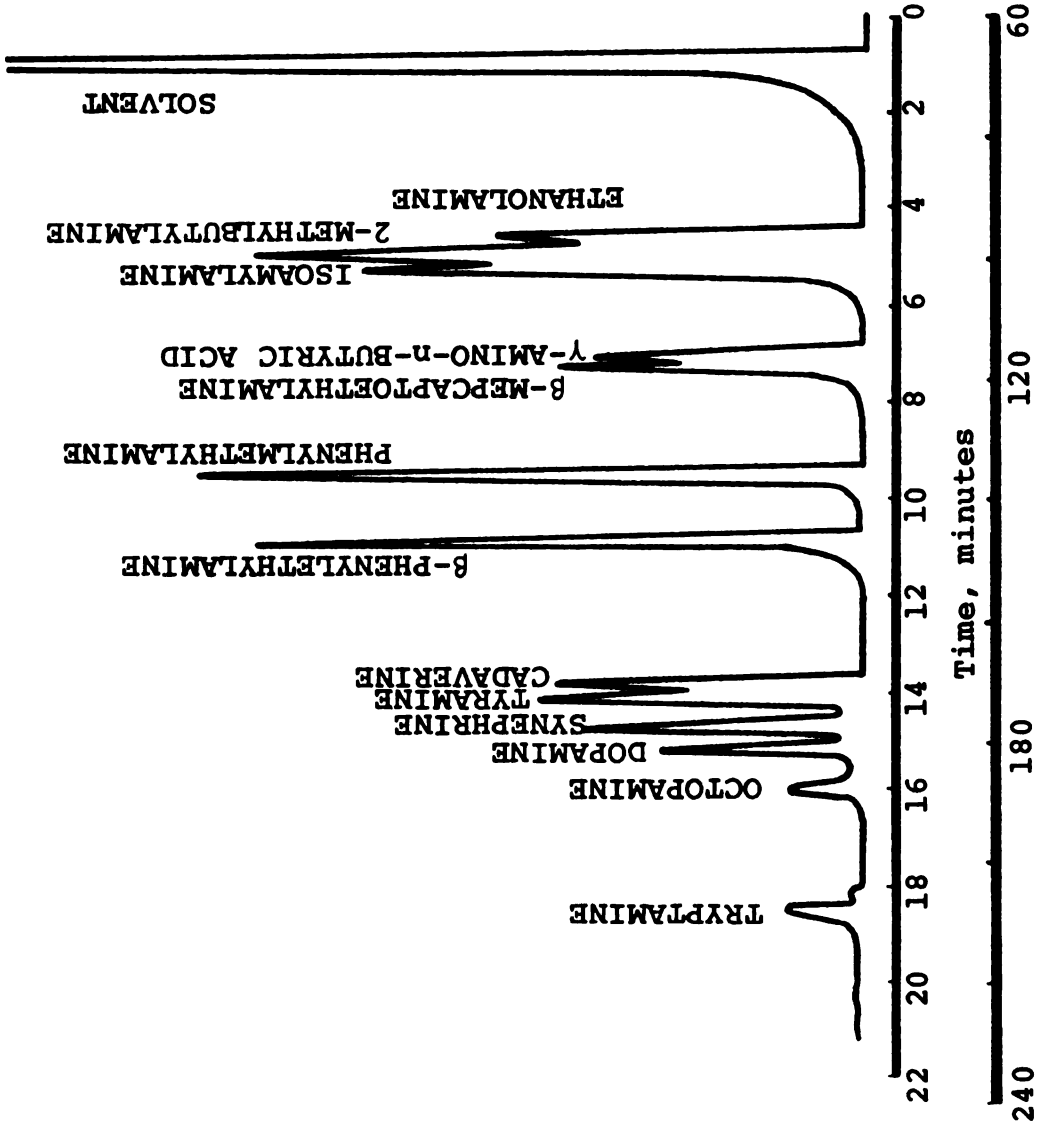


Fig. 3.--Chromatogram of a standard mixture of amines analyzed as N-trifluoroacetyl derivatives.

could be done in this system. The system used in this analysis allows the complete separation of N-trifluoroacetyl derivatives in less than 22 minutes. The retention times (Rt) of the amines analyzed as N-trifluoroacetyl derivatives are shown in Table 1.

Table 1.--Retention times (Rt) of amines analyzed as N-trifluoroacetyl derivatives.

Amines	Rt* (minutes)
Ethanolamine	4.63
2-Methylbutylamine	5.05
Isoamylamine	5.38
γ-Amino-n-butyric acid	7.25
β-Mercaptoethylamine	7.38
Phenylmethylamine	9.70
β-Phenylethylamine	11.05
Cadaverine	14.00
Tyramine	14.38
Synephrine	15.00
Dopamine	15.38
Octopamine	16.25
Tryptamine	18.75

*Values are the average of 4 replications.

Pretrials indicated that holding the column temperature at 275°C for 10 minutes between injections of the samples greatly improved the base line characteristic. Likewise, washing the amine hydrochlorides isolated from the samples with anhydrous diethyl ether was important to remove fatty trace materials. Attempts to separate putrescine and histamine by this procedure were unsuccessful. Perhaps, the boiling points of the N-trifluoroacetyl

derivatives of these amines were out of the range between 60 and 240°C.

For quantitative analysis, standard curves were prepared by taking pure amine hydrochlorides through the whole procedure including the extraction steps. Within a certain range of concentrations the peak width remained constant, while the peak height was directly proportional to the quantity of N-trifluoroacetyl derivatives. Above this range increasing quantities of sample resulted in a nonlinear curve, concave downward. This condition might be due to an overload of detector.

Based on these facts the relationship between the peak height and the quantity of N-trifluoroacetyl derivatives was chosen to prepare the standard curves. The sample then was taken in sufficiently small volume that the quantity of component injected into the chromatograph did not exceed the limit mentioned above.

In order to make calculations easier, a linear regression equation $Y = aX + b$ was computed for each standard of amine, where Y represents peak height in mm while X represents quantity of amine in μg. Table 2 shows that every amine has its own concentration limit up to which a linear relationship between peak height and amine quantity exists. Figures 4, 5, and 6 show typical standard curves obtained from all amine standards.

Table 2.--Linear regression equations of the amine standards analyzed gas chromatographically as N-trifluoroacetyl derivatives.

Amines	Conc. Range (ug)	Linear Regression Equations*
Ethanolamine	2- 6	Y = 6.8500 X - 8.7400
2-Methylbutylamine	1- 6	Y = 5.8257 X - 0.3067
Isoamylamine	2- 6	Y = 9.3900 X - 9.5400
γ-Amino-n-butyric acid	2- 6	Y = 4.4100 X - 3.7800
β-Mercaptoethylamine	2-10	Y = 3.4850 X - 2.3500
Phenylmethylamine	2-12	Y = 2.5186 X - 0.8133
β-Phenylethylamine	1- 5	Y = 7.0200 X - 0.0600
Cadaverine	2-12	Y = 3.7243 X - 6.4867
Tyramine	1- 6	Y = 7.8514 X - 0.7800
Synephrine	1- 4	Y =12.6700 X - 8.7500
Dopamine	1- 6	Y = 5.2743 X - 2.9267
Octopamine	1- 6	Y = 6.4286 X - 1.5333
Tryptamine	2-12	Y = 1.8614 X - 0.0133

*Data obtained from the average of 4 replications.

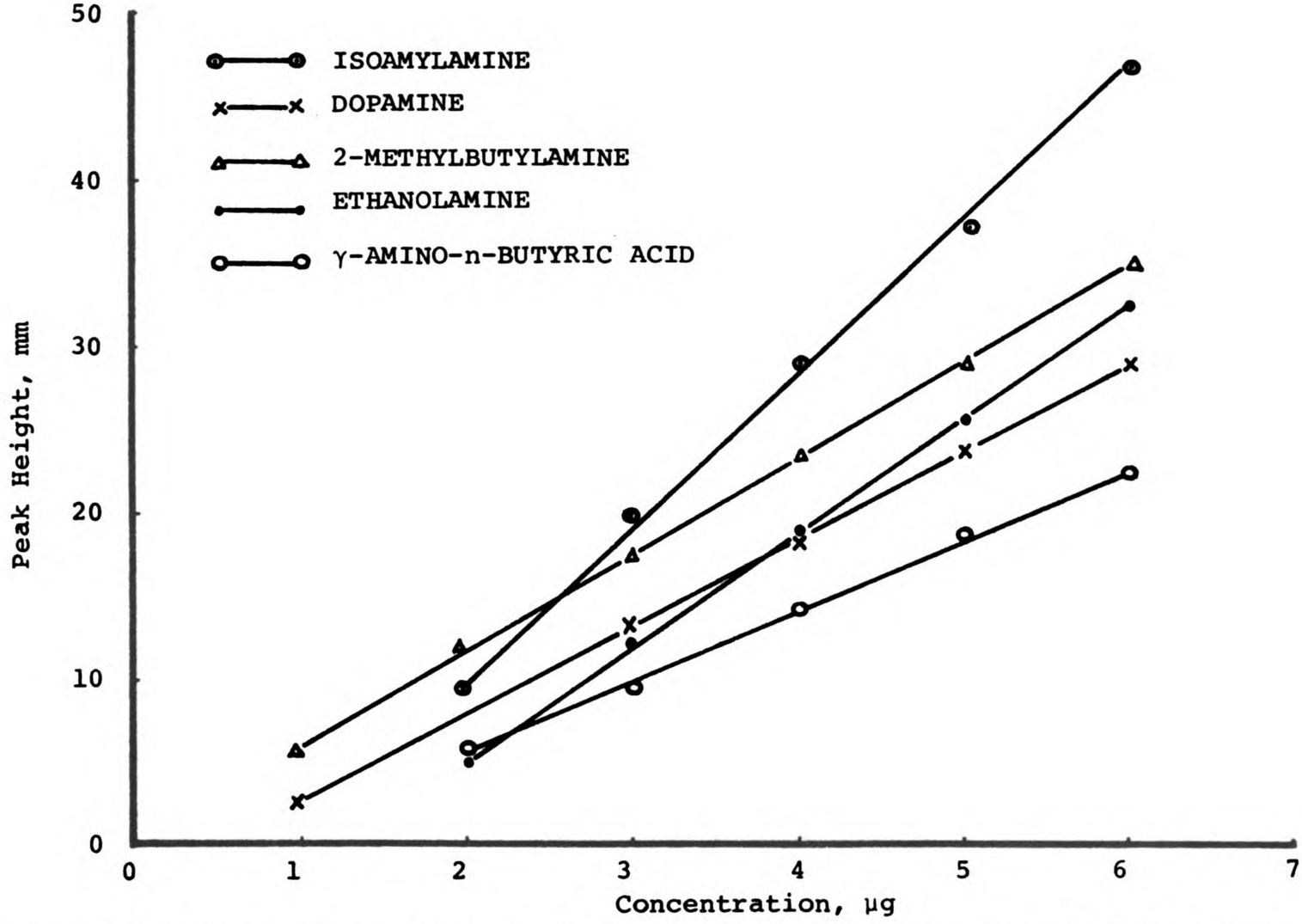


Fig. 4.--Standard curves showing the relationship of µg of isoamylamine, dopamine, 2-methylbutylamine, ethanolamine, and γ-amino-n-butyric acid to peak height response. Compounds were analyzed gas chromatographically as the N-trifluoroacetyl derivatives.

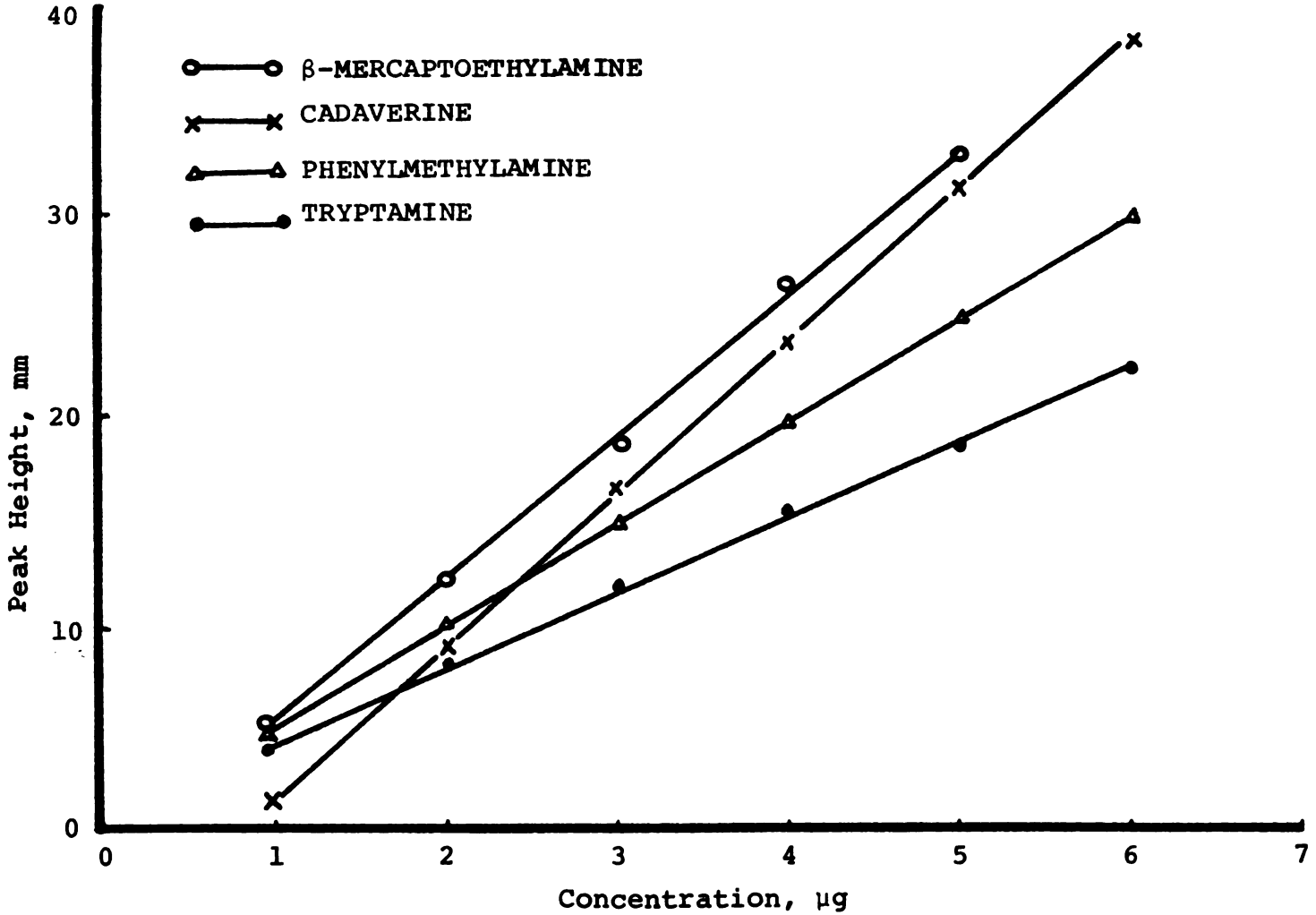


Fig. 5.--Standard curves showing the relationship of µg of β-mercaptoethylamine, cadaverine, phenylmethylamine, and tryptamine to peak height response. Compounds were analyzed gas chromatographically as the N-trifluoroacetyl derivatives.

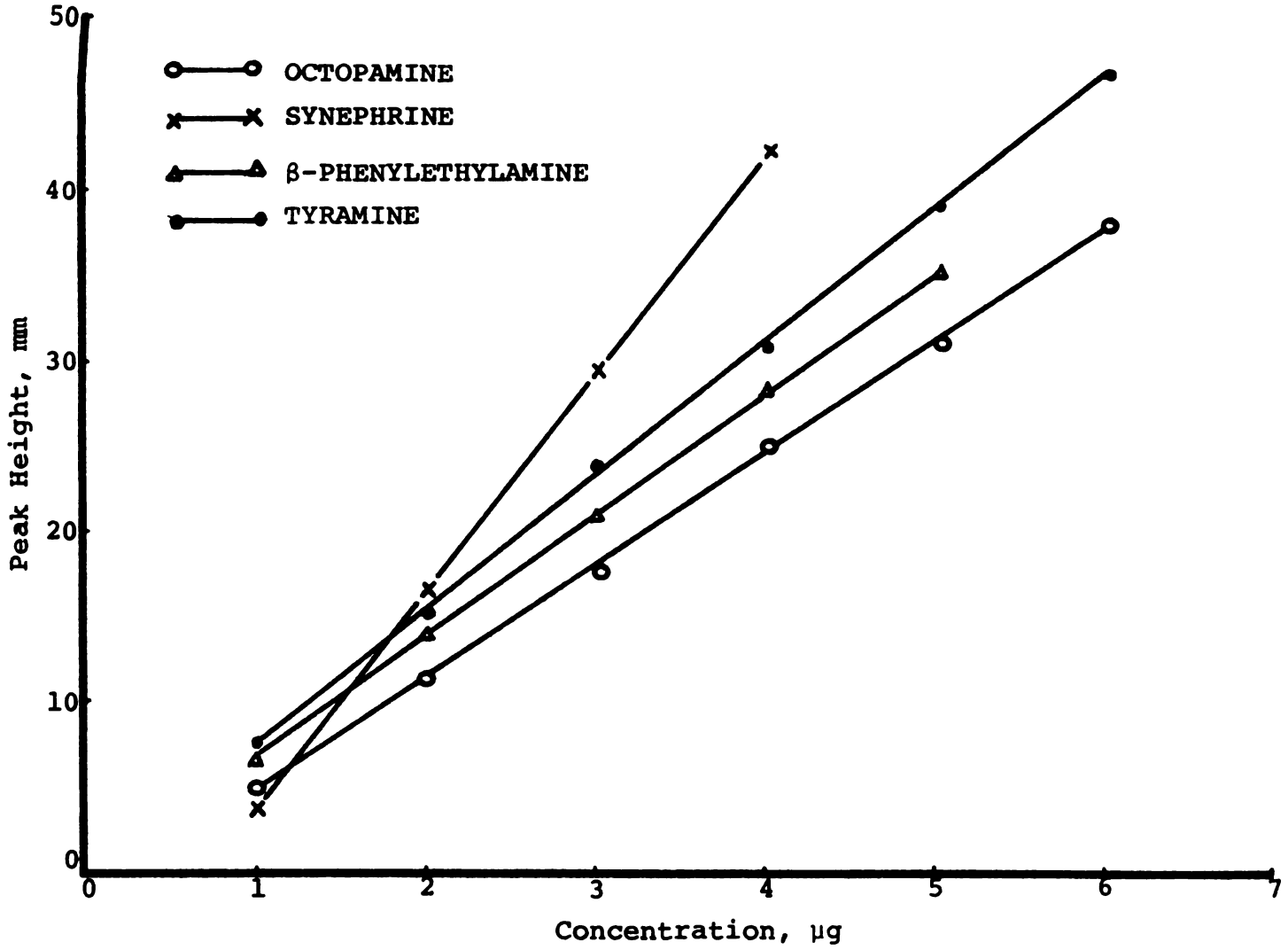


Fig. 6.--Standard curves showing the relationship of µg of octopamine, synephrine, β-phenylethylamine, and tyramine to peak height response. Compounds were analyzed gas chromatographically as the N-trifluoroacetyl derivatives.

Gas chromatographic analysis of six commercial fermented fish paste exhibited different chromatograms, as shown in Figures 7 to 12. Depending on the origin of the product, 7 to 17 major chromatographic peaks appeared, several of which had identical retention times with authentic ethanolamine, 2-methylbutylamine, β-phenylethylamine, tyramine, dopamine, octopamine, cadaverine, tryptamine, and β-mercaptoethylamine. Tables 3 to 8 show the retention times and concentration of biologically active amines found in fermented fish pastes.

The wide differences in the concentration of amines between samples are probably due to differences in the amino acid composition of the raw material, microorganisms involved in the fermentation process, and environmental conditions. According to Gale (1946) the following conditions, are required for the formation of active amino acid decarboxylases in bacteria: (1) the organism concerned must possess such enzymes in its potential enzymic constitution, (2) growth must take place in the presence of the specific substrate, (3) the organism must be capable of synthesizing codecarboxylase or, if the organism cannot accomplish this synthesis, the growth medium must contain certain factors involved in the formation of codecarboxylase, (4) the growth medium must be acid, (5) with some organisms, amino acid decarboxylases are formed to a significant extent only if growth occurs

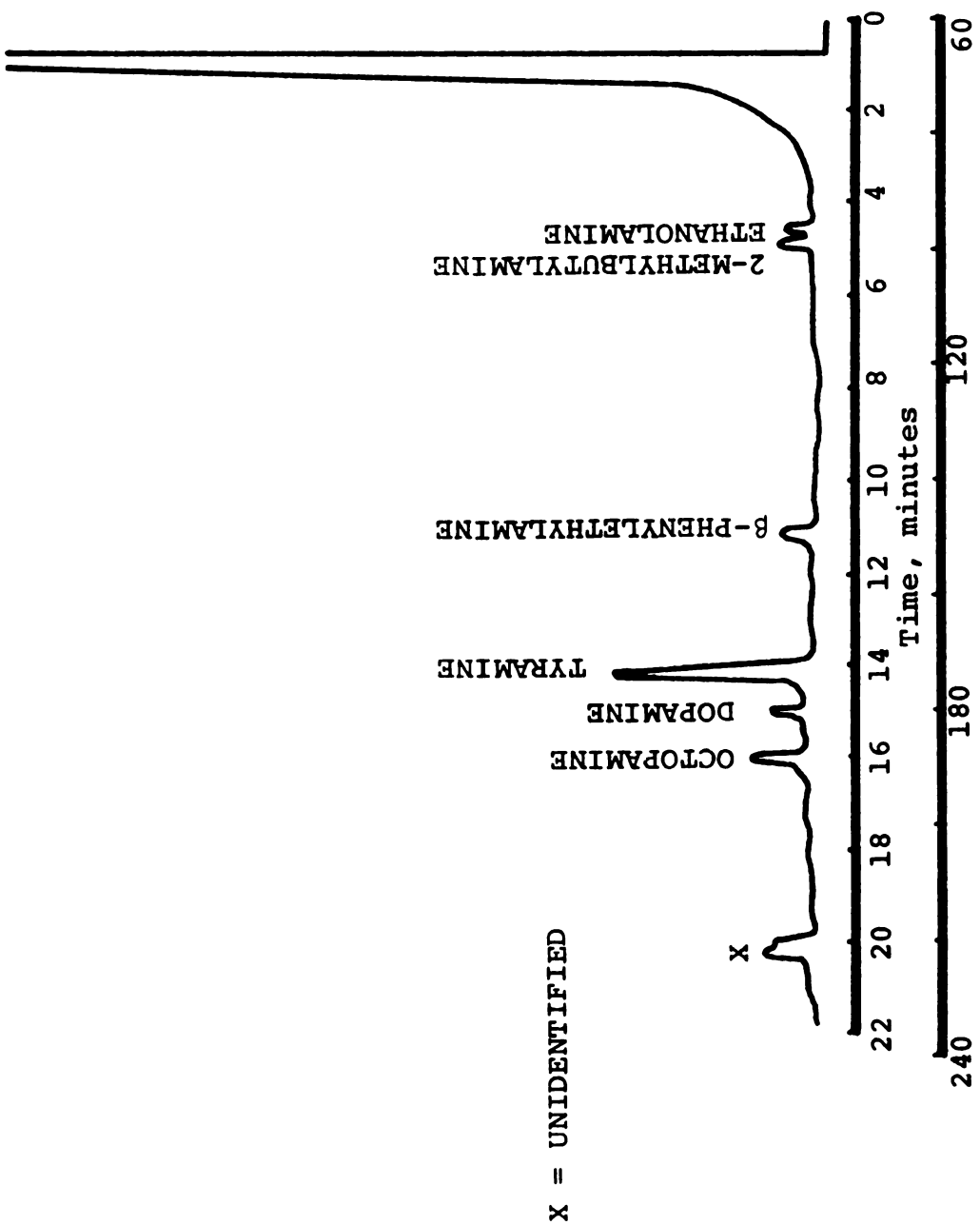


Fig. 7.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample A.

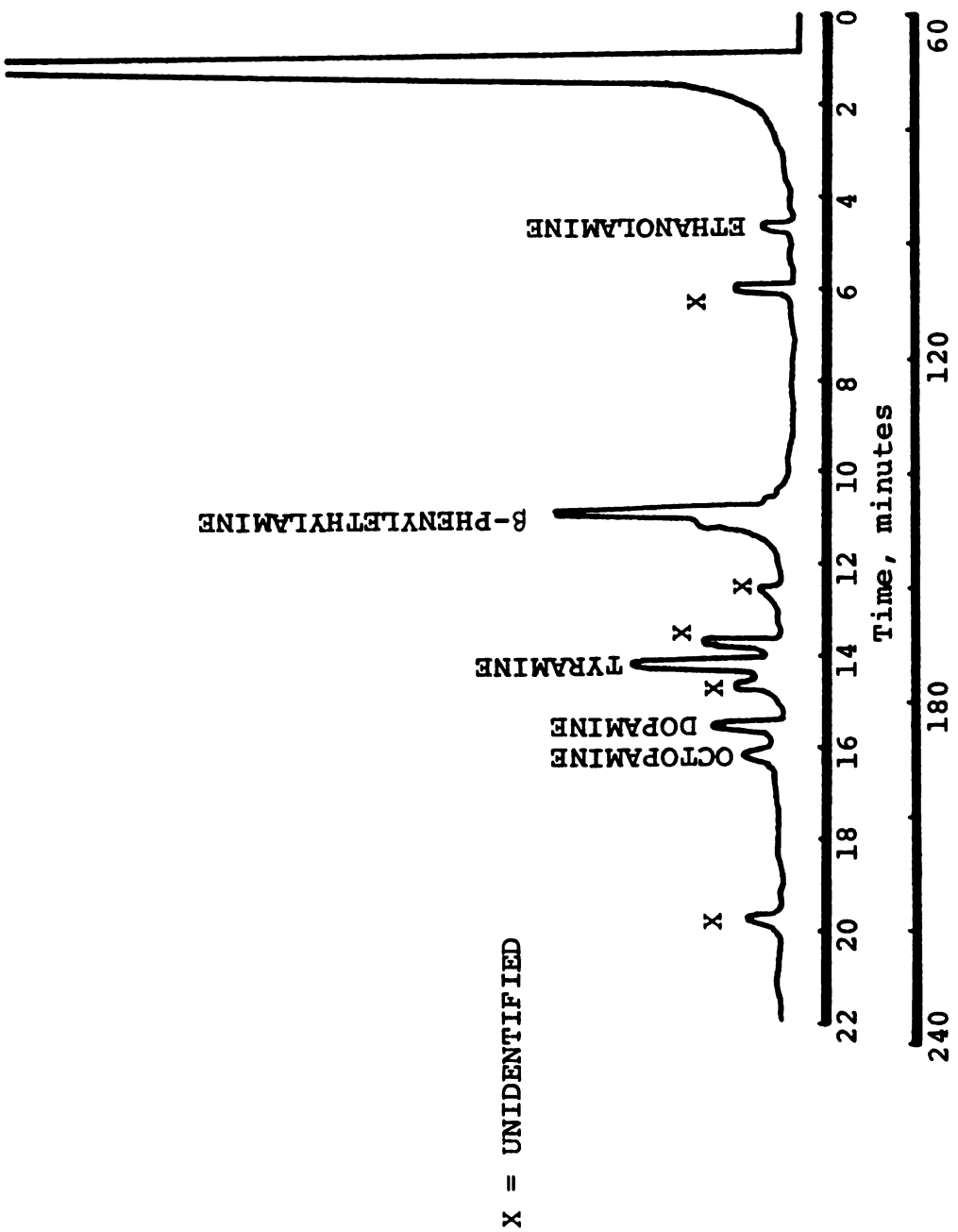


Fig. 8.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample B.

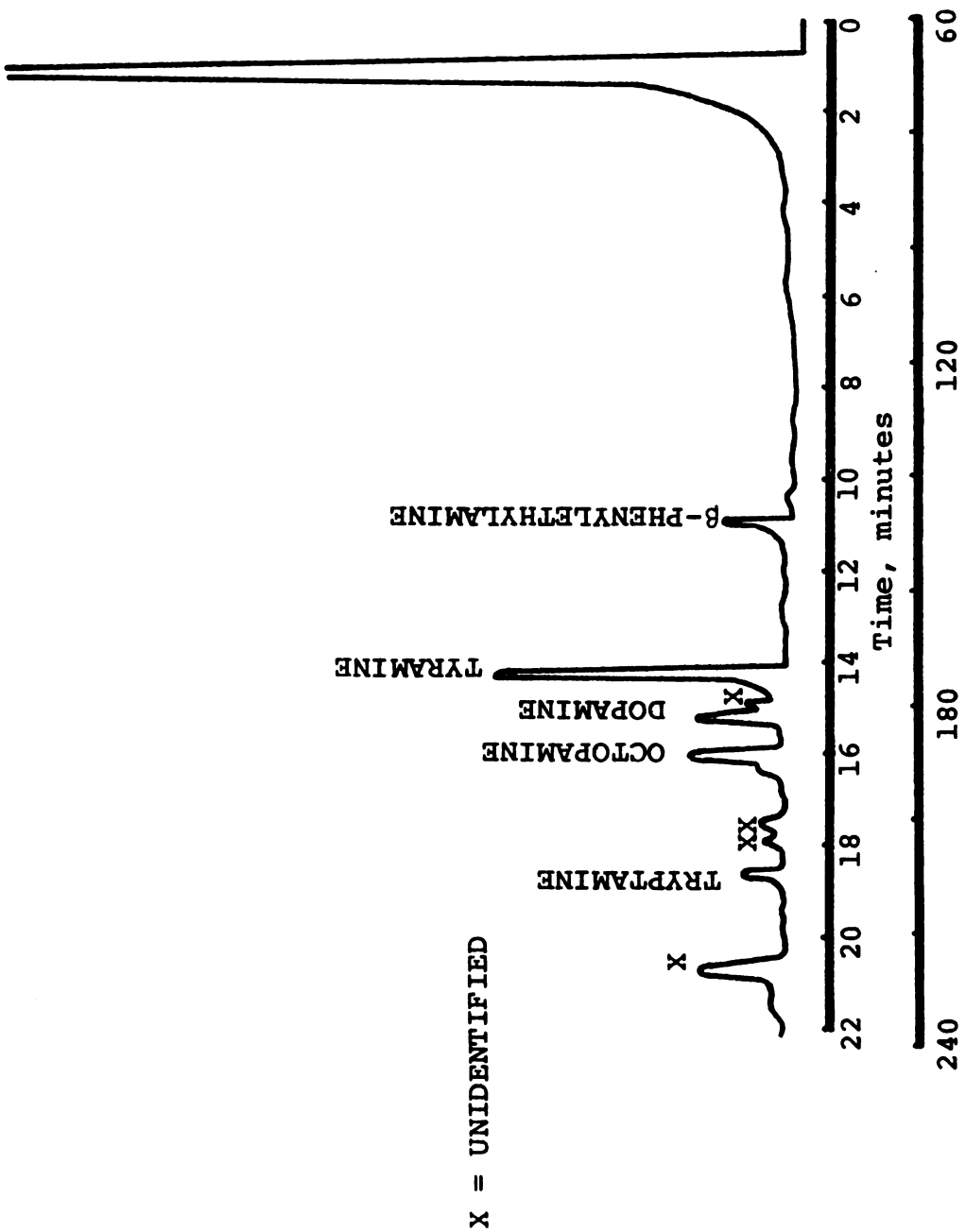


Fig. 9.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample C.

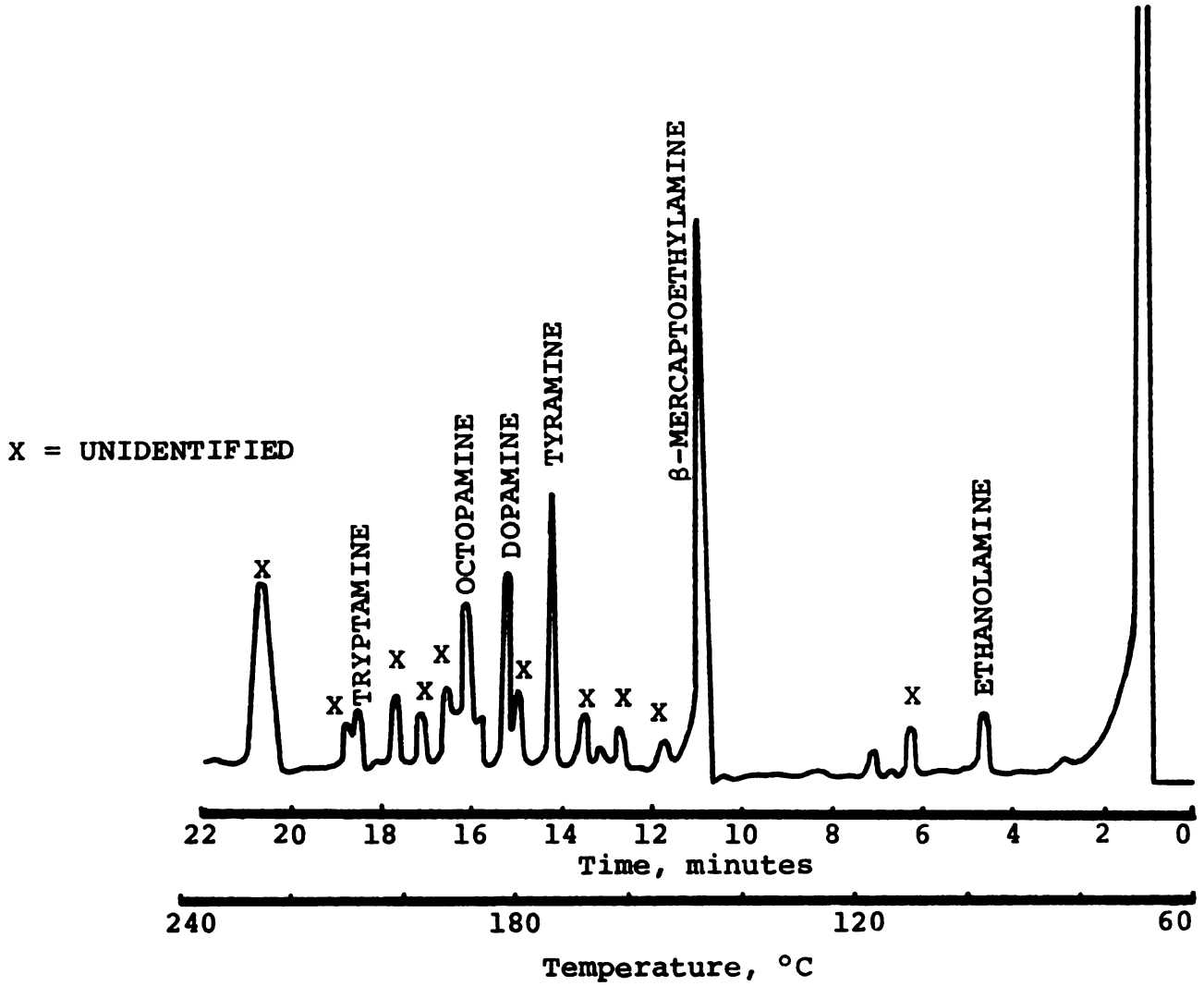


Fig. 10.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample D.

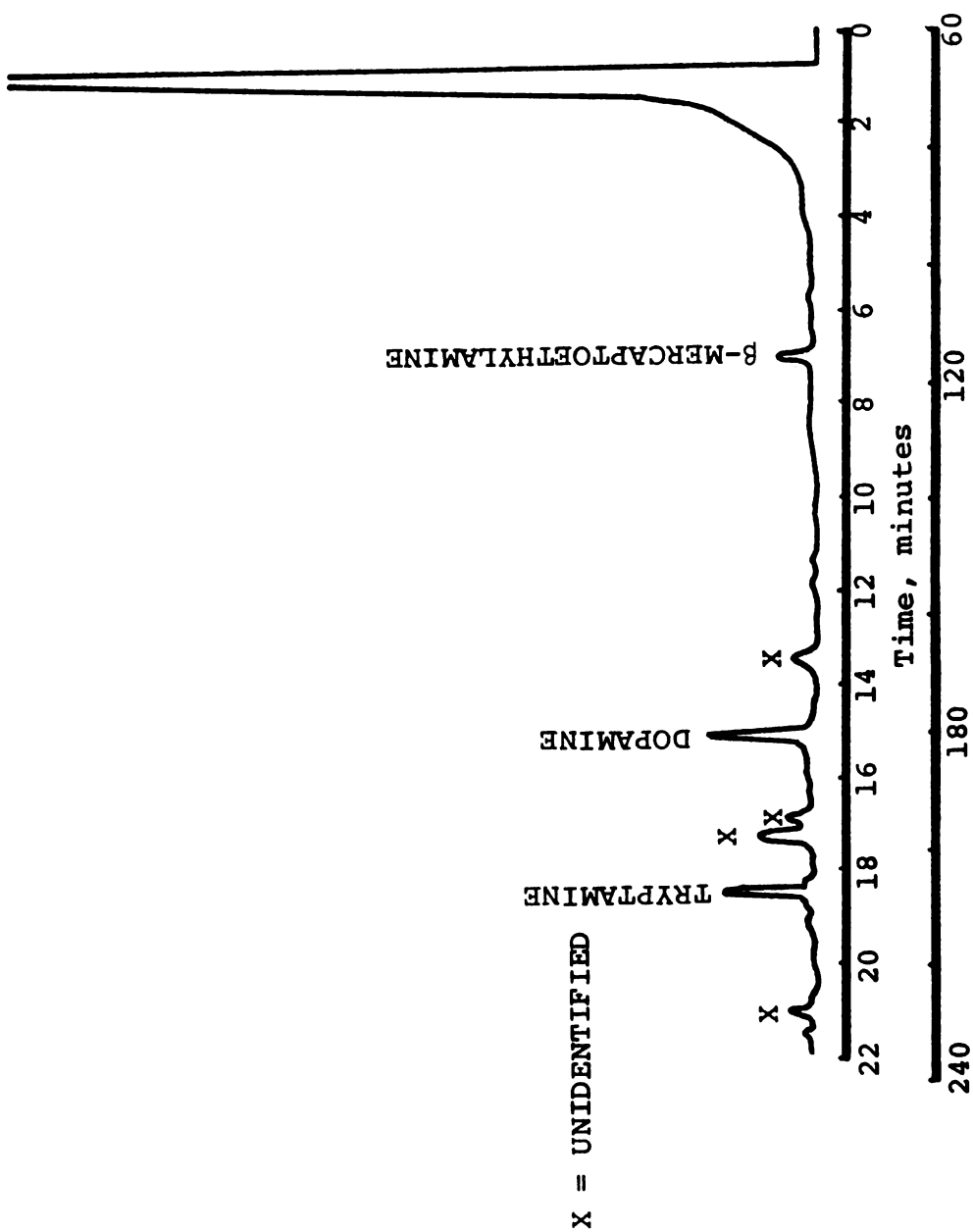


Fig. 11.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample E.

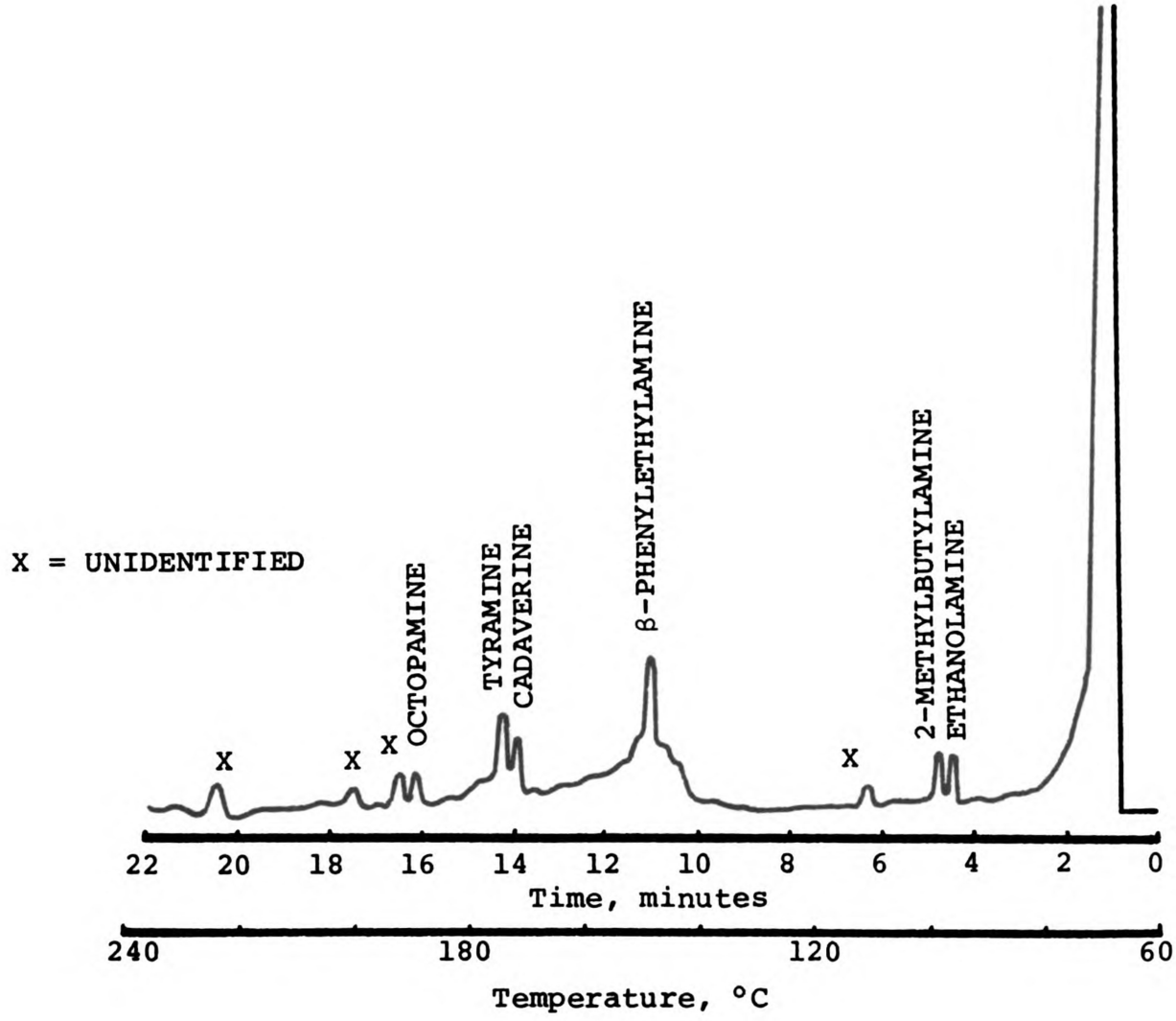


Fig. 12.--Chromatogram of N-trifluoroacetyl derivatives isolated from Sample F.

Table 3.--Retention time (Rt) and concentration of amines isolated from Sample A.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	4.63	Ethanolamine	15.3
2	5.05	2-Methylbutylamine	5.0
3	11.05	β-Phenylethylamine	18.8
4	14.38	Tyramine	34.2
5	15.38	Dopamine	17.6
6	16.25	Octopamine	16.5
7	20.38	Unidentified	-

*A = Philippine Bagoong "Anchovy Sauce."

†Average of 3 replications.

Table 4.--Retention time (Rt) and concentration of amines isolated from Sample B.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	4.63	Ethanolamine	41.0
2	6.00	Unidentified	-
3	11.05	β-Phenylethylamine	115.8
4	12.55	Unidentified	-
5	13.80	Unidentified	-
6	14.38	Tyramine	160.2
7	14.75	Unidentified	-
8	15.38	Dopamine	66.0
9	16.25	Octopamine	21.5
10	18.30	Unidentified	-

*B = "Shrimp Paste" (Product of Thailand).

†Average of 3 replications.

Table 5.--Retention time (Rt) and concentration of amines isolated from Sample C.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	11.05	β-Phenylethylamine	130.2
2	14.38	Tyramine	54.5
3	15.00	Unidentified	-
4	15.38	Dopamine	28.2
5	16.25	Octopamine	27.0
6	17.70	Unidentified	-
7	18.00	Unidentified	-
8	18.75	Tryptamine	22.8
9	20.80	Unidentified	-

*C = "Salted Anchovy" (Product of the Philippines).

†Average of 3 replications.

Table 6.--Retention time (Rt) and concentration of amines isolated from Sample D.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	4.63	Ethanolamine	106.5
2	6.30	Unidentified	-
3	7.38	β-Mercaptoethylamine	35.0
4	11.05	β-Phenylethylamine	600.0
5	11.75	Unidentified	-
6	12.75	Unidentified	-
7	13.50	Unidentified	-
8	14.38	Tyramine	376.2
9	15.00	Unidentified	-
10	15.38	Dopamine	300.6
11	16.25	Octopamine	53.8
12	16.70	Unidentified	-
13	17.25	Unidentified	-
14	17.75	Unidentified	-
15	18.75	Tryptamine	162.8
16	18.95	Unidentified	-
17	20.80	Unidentified	-

*D = "Malaysian's Prawn Cake."

†Average of 3 replications.

Table 7.--Retention time (Rt) and concentration of amines isolated from Sample E.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	7.38	β-Mercaptoethylamine	12.5
2	13.70	Unidentified	-
3	15.38	Dopamine	26.0
4	17.00	Unidentified	-
5	17.50	Unidentified	-
6	18.75	Tryptamine	46.0
7	21.25	Unidentified	-

*E = "Lorenzana Bagoong" (Product of the Philippines).

†Average of 3 replications.

Table 8.--Retention time (Rt) and concentration of amines isolated from Sample F.*

Peak No.	Rt (minutes)	Amines	Conc.† (µg/g)
1	4.63	Ethanolamine	19.2
2	5.05	2-Methylbutylamine	12.6
3	5.63	Unidentified	-
4	11.05	β-Phenylethylamine	40.5
5	14.00	Cadaverine	35.0
6	14.38	Tyramine	87.8
7	16.25	Octopamine	7.6
8	16.50	Unidentified	-
9	17.63	Unidentified	-
10	20.80	Unidentified	-

*F = "Bagoong Alamang" (Product of the Philippines).

†Average of 3 replications.

at temperatures lower than 30°C, and (6) the enzymes are fully developed within the organism only at the end of active cell division.

Several suggestions have been reported why microorganisms produce amino acid decarboxylases. Gale (1946) suggested that the formation of decarboxylases in acid media might be due to inability of microorganisms to utilize carbohydrates and other substances at this pH. Other workers speculated that amines might act as reaction buffers to protect microorganisms from the accumulation of H ions in their protoplasm (Tabor, 1954). However, their exact metabolic function remains unknown.

The pH is an important factor in the formation of decarboxylases in bacteria, since generally bacteria grown in alkaline medium attack amino acids by deamination, while in acid medium by decarboxylation. The optimum pH for decarboxylation reaction ranges from 2.5 to 6.0 (Gale, 1946). The pH measurements of samples A, B, C, D, E, and F showed the pH values of 4.65, 5.10, 6.10, 6.00, 6.10, and 5.70 respectively. Indeed, these are the pH optima for the production of amino acid decarboxylase in bacteria.

Analysis by Thin Layer Chromatography

Thin layer chromatography was carried out by using two different adsorbents and four different developing solvent systems. From the four solvent systems used, only the following three gave good separations: n-butanol:

pyridine:water (1:1:1), n-butanol:pyridine:glacial acetic acid:water (60:8:12:20), n-butanol:glacial acetic acid: water (4:1:5). Failure to get good separations with the mixture chloroform:methanol:ammonium hydroxide (12:7:1) might be due to insufficient polarity of this solvent mixture. Increasing polarity of the developing solvent system with water as in the three solvent systems mentioned above greatly improved the separations. Rf values of amines by thin layer chromatography are shown in Table 9. Values represent the average of three different experiments.

The wide distribution of Rf values shown in Table 9 indicated that some solvent systems are more suitable than others for the separation of certain amines. This might be due to the wide variability in polarity between amines, as well as between developing solvent systems used.

According to the Rf values obtained (Table 9) the amines may be divided into two groups; group S, amines which can be separated, and group US, amines which cannot be separated by the solvent systems used in this work. Table 10 shows this grouping; amines which have Rf values differing less than 0.01 are considered unseparated, since we will not be able to distinguish the colored spots on TLC if the spots are too close to each other. As an example by using the BPW solvent on silica gel (Table 10)

Table 9.--Rf values of several amines on silica gel and cellulose plates.*

Amines	BPW		BPAW		BAW	
	Silica gel	Cellu-lose	Silica gel	Cellu-lose	Silica gel	Cellu-lose
Ethanolamine	-	0.57	0.24	0.41	0.02	0.48
2-Methylbutylamine	0.35	0.79	0.53	0.84	0.46	0.91
Isoamylamine	0.37	0.78	0.54	0.84	0.46	0.95
γ-Amino-n-butyric acid	0.18	0.32	0.23	0.38	0.18	0.49
β-Mercaptoethyl-amine	-	0.52	0.15	0.56	0.05	0.62
Phenylmethylamine	0.35	0.70	0.53	0.77	0.47	0.85
β-Phenylethylamine	0.41	0.71	0.53	0.82	0.46	0.88
Cadaverine	-	0.04	0.09	0.25	0.03	0.41
Tyramine	0.39	0.69	0.49	0.69	0.46	0.76
Synephrine	0.34	0.71	0.41	0.64	0.33	0.73
Dopamine	0.28	0.68	0.40	0.52	0.36	0.59
Octopamine	0.34	0.69	0.46	0.56	0.43	0.64
Tryptamine	0.43	0.72	0.51	0.74	0.48	0.83
Histamine	-	0.52	0.10	0.27	0.02	0.40
Putrescine	-	0.68	0.40	0.26	0.04	0.38

*Values are the average of 3 replications.

BPW = n-butanol:pyridine:water (1:1:1).

BPAW = n-butanol:pyridine:glacial acetic acid: water (60:8:12:20).

BAW = n-butanol:glacial acetic acid:water (4:1:5).

Table 10.--Grouping of amines based on Rf values.

Solvent/ Adsorbent	Group S (can be separated)	Group US* (cannot be separated)
BPW		
Silica gel	Isoamylamine, γ-amino-n-butyric acid, β-phenylethylamine, tyramine, dopamine, tryptamine	(1) Ethanolamine, β-mercaptoethylamine, cadaverine, histamine, putrescine
		(2) 2-Methylbutylamine, phenylmethylamine, synephrine, octopamine
Cellulose	Ethanolamine, γ-amino-n-butyric acid, β-mercaptoethylamine, cadaverine, tryptamine, histamine	(1) 2-Methylbutylamine, Isoamylamine
		(2) Phenylmethylamine, β-phenylethylamine, synephrine
		(3) Tyramine, dopamine, octopamine, putrescine
BPAW		
Silica gel	β-Mercaptoethylamine, tyramine, octopamine, tryptamine	(1) Ethanolamine, γ-amino-n-butyric acid
		(2) 2-Methylbutylamine, Isoamylamine, Phenylmethylamine, β-phenylethylamine
		(3) Cadaverine, histamine
		(4) Synephrine, dopamine, putrescine
Cellulose	Ethanolamine, γ-amino-n-butyric acid, β-mercaptoethylamine, phenylmethylamine, β-phenylethylamine, tyramine, synephrine, dopamine, octopamine, tryptamine	(1) 2-Methylbutylamine, isoamylamine
		(2) Cadaverine, histamine, putrescine

Table 10.--Continued.

Solvent/ Adsorbent	Group S (can be separated)	Group US* (cannot be separated)
BAW		
Silica gel	γ-amino-n-butyric acid, synephrine, dopamine, octopamine	(1) 2-Methylbutylamine, isoamylamine, phenylmethylamine, β-phenylethylamine, tyramine, tryptamine
		(2) Ethanolamine, β-mercaptoethylamine, cadaverine, histamine, putrescine
Cellulose	2-Methylbutylamine, isoamylamine, β-mercaptoethylamine, phenylmethylamine, β-phenylethylamine, tyramine, synephrine, dopamine, octopamine, tryptamine, putrescine	(1) Ethanolamine, γ-amino-n-butyric acid
		(2) Cadaverine, histamine

*Amines which have different Rf of 0.01 on TLC with solvents developed for 15 cm.

we will not be able to separate ethanolamine (Rf = 0) from β-mercaptoethylamine (Rf = 0); also, 2-methylbutylamine (Rf = 0.35) from phenylmethylamine (Rf = 0.35), etc. However, the amines of one subgroup can be separated from the amines of another subgroup, e.g., we can separate ethanolamine (subgroup US1) from 2-methylbutylamine (subgroup US2).

This grouping allows anyone to select the right solvent and adsorbent in order to get the best thin layer chromatographic separation for the amines studied here. According to our results, using at least two different solvent systems and two adsorbents allows complete separation of all the amines studied here. However, it may be necessary to try other adsorbent solvent systems for the separation of amines which have not been included in this study.

Biologically Active Amines Found in Fermented Fish Paste

β-Phenylethylamine

Five of the six different fermented fish pastes contained β-phenylethylamine. The level of this amine varied from 18.8 to 600.0 μg/g. Its presence in fermented fish paste should get attention since β-phenylethylamine is a powerful migraine precipitant. Clinical trials have shown that 3 mg of β-phenylethylamine is sufficient to initiate a migraine attack (Chaytor et al., 1975).

β-phenylethylamine in fermented fish paste may be formed by the action of phenylalanine decarboxylase produced by bacteria during fermentation. Among the bacteria commonly associated with the production of amino acid decarboxylase, Streptococcus faecalis is known to produce phenylalanine decarboxylase. However the question as to whether this organism is involved in the fermentation of fish paste may be answered by further microbiological investigation.

Tyramine

Tyramine is the second major biologically active amine found in fermented fish paste. Its concentration varied from 34.2 to 376.2 μg/g. Among biologically active amines found in nature, tyramine is the most common amine present in fermented products such as cheese. So far, tyrosine decarboxylase has been found primarily in Streptococcus faecalis. Although tyramine is considered as migraine precipitant, it is less potent than β-phenylethylamine in triggering off migraine attacks.

Dopamine

Dopamine was found in fermented fish paste in quantities ranging from 17.6 to 300.6 μg/g. We do not know yet what the effect of ingestion of dopamine-containing foods to health is, since the presence of dopamine in foods is rare. Only one report published (Hodge et al., 1964)

suggesting that 3,4-dihydroxyphenylalanine (dopa) after its conversion to dopamine by decarboxylation might cause hypertensive crises in persons receiving monoamine oxidase inhibitors. Unfortunately the authors did not mention the quantities of dopamine which caused the toxicity. However, Goldberg (1972) found that intravenous injection of 2 mg of dopamine hydrochloride markedly elevated the blood pressure in dogs.

There are two main sources known from which dopamine can be formed. First, dopamine can be formed by decarboxylation of 3,4-dihydroxyphenylalanine. Second, it can be formed from tyramine by the action of catechol-forming enzymes (Axelrod, 1963). The formation of dopamine from tyramine occurs only in animals and has not been found in microorganisms, whereas, the formation from 3,4-dihydroxyphenylalanine occurs both in animals and in microorganisms. Epps (1944) showed that in addition to tyrosine and phenylalanine, Streptococcus faecalis enzymes also decarboxylated 3,4-dihydroxyphenylalanine at one-sixth the rate of tyrosine decarboxylation.

Octopamine

Octopamine was found in low quantities ranging from 7.6 to 53.8 µg/g. The presence of this amine in fermented fish paste is rather surprising since octopamine is only found in animal and certain plant sources. In animals and citrus fruit octopamine is formed from tyramine by

dopamine β-oxidase and appears to be an intermediate precursor in the formation of synephrine, norepinephrine, and epinephrine (Axelrod, 1963).

Recently Devi et al. (1975) found that certain species of Arthrobacter are capable of forming octopamine by breaking down synephrine. However, it is rather unlikely this finding can apply to the formation of octopamine in fermented fish paste since synephrine was not found in these samples even in small quantities.

There are two things that could happen; octopamine may be already present in raw materials, or there is a certain group of bacteria which can produce enzymes capable of converting tyramine to octopamine during fermentation. However, the exact mechanism can only be answered by further investigation.

Tryptamine

Half of the six samples analyzed contained tryptamine, the concentration of which ranged from 22.8 to 162.8 μg/g. Tryptamine has been known to be present in several plant foods such as pineapple, tomato, and plum. Recently, it was found that tryptamine can be formed from tryptophan by the action of certain bacteria. Streptococcus faecalis and Clostridium welchii are two bacteria capable of decarboxylating tryptophan (Eskin et al., 1971).

Cadaverine

Only one sample of fermented fish was found to contain cadaverine; its concentration was 35.0 µg/g. Cadaverine is responsible for the common unpleasant odor present in putrefied protein foods. It can be produced by the bacterial decarboxylation of lysine. Bacterium cadaveris, Escherichia coli, and some lactobacilli are bacteria capable of producing lysine decarboxylase. Cadaverine is considered poisonous as it may cause skin irritation (Stecher et al., 1968).

2-Methylbutylamine

Two commercial fermented fish pastes appeared to contain 2-methylbutylamine at the concentration of 5.0 to 12.6 µg/g. Unlike other amines which originated from acid or basic amino acids, 2-methylbutylamine is formed from a neutral amino acid, isoleucine. Ekladius et al. (1957) showed that Proteus vulgaris produced enzymes which could decarboxylate neutral amino acids such as leucine, isoleucine, valine, and α-amino-n-butyric acid.

Ethanolamine and β-Mercaptoethylamine

Ethanolamine was found in four fermented fish pastes; its concentration varied in the range of 15.3 to 106.5 µg/g. Only two samples were found to contain β-mercaptoethylamine; they contained 12.5 and 35.0 µg/g. It is a little difficult to explain the presence of these

two amines in fermented fish paste since there is no publication available demonstrating the ability of bacteria in producing decarboxylase to form ethanolamine and β-mercaptoethylamine. However, ethanolamine is often found in putrefied foods along with other amines. Since the structure of ethanolamine and β-mercaptoethylamine are similar to that of amino acids serine and cystein respectively, they might be formed from those amino acids by decarboxylation. Further studies on bacterial serine and cystein decarboxylases are desirable.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to identify and quantitatively determine biologically active amines which might be present in fermented fish paste. Six different commercial fish pastes were purchased from local stores. They had been imported from the Philippines, Thailand, and Malaysia.

After preliminary treatments to remove insoluble materials and soluble proteins from the sample, biologically active amines were extracted with peroxide-free ether under alkaline conditions. The amines were transferred from the ether solution to an aqueous 0.02 N hydrochloric acid. The acid solution was evaporated to dryness; the residue consisted largely of amine hydrochlorides. Further separation of amines was done using TLC and GLC.

Thin layer chromatography was carried out on silica gel and cellulose adsorbents, with four different developing solvent systems; the following three gave satisfactory separations; n-butanol:pyridine:water (1:1:1), n-butanol:pyridine:glacial acetic acid:water (60:8:12:20), and n-butanol:glacial acetic acid:water (4:1:5), all ratios by volume.

Before amines were analyzed by GLC, they were converted to N-trifluoroacetyl derivatives. Analysis of amines as their derivatives was accomplished in a Perkin-Elmer 900 gas chromatograph. Separation of the derivatives was achieved using a 6 ft. x 0.125 in. o.d. 3% SP-2100 on 100/120 mesh Supelcoport with FID detector. Column was programmed from 60 to 240°C at 8°C per minute with nitrogen as a carrier gas at a flow rate of 18.5 ml per minute.

Qualitative identification of the amines was accomplished by comparing the retention times of unknowns with standards similarly treated. For quantitative analysis standard curves were prepared by taking pure amine hydrochlorides through the whole procedure including the extraction steps. Within a certain range of concentrations the peak height is directly proportional to the quantity of amine. Each amine has its own concentration limit up to which a linear relationship between peak height and amine quantity exists.

Nine amines were identified as ethanolamine, 2-methylbutylamine, β-phenylethylamine, tyramine, dopamine, octopamine, cadaverine, tryptamine, and β-mercaptoethylamine. Depending on the origin of the product the concentration of these amines ranged as follows, ethanolamine (15.3-106.5 μg/g), 2-methylbutylamine (5.0-12.6 μg/g), β-phenylethylamine (18.8-600.0 μg/g), tyramine (34.2-376.2 μg/g), dopamine (17.6-300.6 μg/g), octopamine (7.6-53.8

μg/g), cadaverine (35.0 μg/g), tryptamine (22.8-162.8 μg/g), and β-mercaptoethylamine (12.5-35.0 μg/g).

Five from the nine amines identified, β-phenylethylamine, tyramine, dopamine, octopamine, and tryptamine are classified as physiologically active amines. These amines may be deleterious to health if ingested at relatively large quantities, especially in persons taking monoamine oxidase inhibitory drugs.

It was found that β-phenylethylamine and tyramine appeared to be the major amines present in fermented fish paste. People, such as those living in Southeast Asia, who regularly include fermented fish paste in their diet should be aware of the possibility of amine toxicity. However, further investigation is needed to determine the degree of safety of this food.

LITERATURE CITED

LITERATURE CITED

Asatoor, A. M.; A. J. Levi; and M. D. Milne. 1963. Tranylcypromine and cheese. Lancet 2:733-734.

Axelrod, J. 1963. Enzymatic formation of adrenaline and other catechols from monophenols. Science 140: 499-500.

Blackwell, B. 1963. Tranylcypromine. Lancet 2:414.

Blackwell, B.; E. Marley; and L. A. Mabbitt. 1965. Effects of yeast extract after monoamine-oxidase inhibition. Lancet 1:940-943.

Boeker, E. A., and E. E. Snell. 1972. Amino acid decarboxylase. In P. D. Boyer, ed., The Enzymes 6. Academic Press, New York.

Brydia, L. E., and H. E. Persinger. 1967. Quantitative gas chromatographic determination of ethanolamines as trifluoroacetyl derivatives. Anal. Chem. 39: 1318-1320.

Chaytor, J. P.; B. Crathorne; and M. J. Saxby. 1975. The identification and significance of 2-phenylethylamine in foods. J. Sci. Fd. Agric. 26:593-598.

Cozzani, I.; A. Misuri; and C. Santoni. 1970. Purification and general properties of glutamate decarboxylase from Clostridium perfringens. Biochem. J. 118:135-141.

Crawford, M. A. 1962. Excretion of 5-hydroxyindolylacetic acid in East Africans. Lancet 1:352-353.

Cruickshank, P. A., and J. C. Sheehan. 1964. Gas chromatographic analysis of amino acids as N-trifluoroacetyl amino acid methyl esters. Anal. Chem. 36:1191-1197.

Davies, J. N. P. 1960. Some considerations regarding obscure diseases affecting the mural endocardium. Am. Heart J. 59:600-631.

Davison, A. N. 1958. Physiological role of monoamine oxidase. Physiol. Rev. 38:729-746.

Devi, N. A.; R. K. Kutty; V. N. Vasantharajan; and P. V. S. Rao. 1975. Microbial metabolism of phenolic amines: Degradation of dl-synephrine by an unidentified Arthrobacter. J. Bacteriol. 122:866-873.

Doby, G. 1965. Plant Biochemistry. Interscience Publisher, London.

Ekladius, L.; H. K. King; and C. R. Sutton. 1957. Decarboxylation of neutral amino acids in Proteus vulgaris. J. Gen. Microbiol. 17:602-619.

Epps, H. M. R. 1944. Studies on bacterial amino acid decarboxylases: 2. l(-) Tyrosine decarboxylase from Streptococcus faecalis. Biochem. J. 38:242-249.

Eskin, N. A. M.; H. M. Henderson; and R. J. Townsend. 1971. Biochemistry of Foods. Academic Press, New York.

Fales, H. M., and J. J. Pisano. 1962. Gas chromatography of biologically important amines. Anal. Biochem. 3:337-342.

Fales, H. M., and J. J. Pisano. 1964. The gas chromatography of amines, alkaloids, and amino acids. In H. A. Szymanski, ed. Biomedical Applications of Gas Chromatography. Plenum Press, New York.

Foy, J. M., and J. R. Parratt. 1962. Urinary excretion of 5-hydroxyindoleacetic acid in West Africans. Lancet 1:942-943.

Gale, E. F. 1940. The production of amines by bacteria: The production of tyramine by Streptococcus faecalis. Biochem. J. 34:846-852.

Gale, E. F. 1946. The bacterial amino acid decarboxylases. Adv. Enzymol. 6:1-32.

Goldberg, L. I. 1972. Cardiovascular and renal actions of dopamine: Potential clinical applications. Pharmacol. Rev. 24:1-29.

Hanington, E. 1967. Preliminary report on tyramine headache. Brit. Med. J. 2:550-551.

Heuvel, W. J. A. V.; W. L. Gardiner; and E. C. Horning. 1964. Characterization and separation of amines by gas chromatography. Anal. Chem. 36:1550-1560.

Hodge, J. V.; E. R. Nye; and G. W. Emerson. 1964. Monoamine oxidase inhibitors, broad beans, and hypertension. Lancet 1:1108.

Homola, A. D., and E. E. Dekker. 1967. Decarboxylation of γ-hydroxyglutamate by glutamate decarboxylase of Escherichia coli (ATCC 11246). Biochem. 6: 2626-2634.

Horwitz, D.; W. Lovenberg; K. Engelman; and A. Sjoerdsma. 1964. Monoamine oxidase inhibitors, tyramine, and cheese. J. Am. Med. Assoc. 188:1108.

King, H. K. 1953. The decarboxylation of valine and leucine by washed suspensions of Proteus vulgaris. Biochem. J. 54:xi.

Lerke, P.; L. Farber; and R. Adams. 1967. Bacteriology of spoilage of fish muscle: VI. Role of protein. Appl. Microbiol. 15:770-776.

Lovenberg, W. 1973. Some vaso- and psychoactive substances in food: amines, stimulants, depressants, and hallucinogens. In Toxicants Occurring Naturally in Foods. National Academy of Sciences. Washington, D.C.

Maretzki, A., and M. F. Mallette. 1962. Nutritional factors stimulating the formation of lysine decarboxylase in Escherichia coli. J. Bacteriol. 83:720-726.

McCurdy, Jr. W. H., and R. W. Reiser. 1966. Trace analysis of fatty amines by gas chromatography. Anal. Chem. 38:795-796.

McGilvery, R. W., and P. P. Cohen. 1948. The decarboxylation of l-phenylalanine by Streptococcus faecalis R. J. Biol. Chem. 174:813-816.

O'Donnell, J. F., and C. K. Mann. 1964. Gas chromatographic separation of amines and amides. Anal. Chem. 36:2097-2099.

Sandler, M.; M. B. H. Youdim; and E. Hanington. 1974. A phenylethylamine oxidizing defect in migraine. Nature 250:335-337.

Sandler, M.; M. B. H. Youdim; J. Southgate; and E. Hanington. 1970. The role of tyramine in migraine: Some possible biochemical mechanisms. In A. L. Cochrane, ed., Background to Migraine, Third Migraine Symposium. Heinemann Med. Books Ltd., London.

Sen, N. P., and P. L. McGeer. 1963. Gas chromatography of phenolic and catecholic amines as the trimethylsilyl ethers. Biochem. Biophys. Res. Com. 13:390-393.

Silverman, G. J., and F. V. Kosikowski. 1956. Amines in cheddar cheese. J. Dairy Sci. 39:1134-1141.

Soda, K., and M. Moriguchi. 1969. Crystalline lysine decarboxylase. Biochem. Biophys. Res. Com. 34:34-39.

Stahl, E., ed. 1969. Thin Layer Chromatography. Springer-Verlay, New York.

Stecher, P. G.,; M. Windholz; and D. S. Leahy, eds. 1968. The Merck Index. Merck & Co., Inc., Rahway, N.J.

Strausbauch, P. H., and E. H. Fischer. 1970. Chemical and physical properties of Escherichia coli glutamate decarboxylase. Biochem. 9:226-233.

Tabor, H. 1954. Metabolic studies on histidine, histamine, and related imidazoles. Pharmacol. Rev. 6:299-343.

Tate, S. S., and A. Meister. 1971. l-Aspartate-β-decarboxylase; structure, catalytic activities, and allosteric regulation. Adv. Enzymol. 35:503-543.

Udenfriend, S.; W. Lovenberg; and A. Sjoerdsma. 1959. Physiologically active amines in common fruits and vegetables. Arch. Biochem. Biophys. 85:487-490.

Van Veen, A. G. 1953. Fish preservation in southeast Asia. Adv. Food Res. 4:209-230.

Voigt, M. N., and R. R. Eitenmiller. 1974. Fluorescent quantitation of biologically active amines in foods with 7-chloro-4-nitrobenzofurazan (NED-Cl). J. Food Sci. 39:420-421.

Wheaton, T. A., and I. Stewart. 1965. Quantitative analysis of phenolic amines using ion-exchange chromatography. Anal. Biochem. 12:585-592.

Wilson, E. M. 1963. Crystalline l-aspartate 4-carboxylase. Biochim. Biophys. Acta 67:345-348.

APPENDIX

APPENDIX

Table A1.--Standard deviation of slope and intercept, standard error of intercept, and correlation coefficient (r) of regression equations for standard curves of amines, obtained from 4 replications.

Amines	Linear Regression Equation	S.D. slope	S.D. inter-cept	S.E. inter-cept	r
Ethanolamine	Y = 6.8500 X - 8.7400	0.0651	0.2760	0.0423	0.9999
2-Methylbutylamine	Y = 5.8257 X - 0.3067	0.0606	0.2359	0.0642	0.9998
Isoamylamine	Y = 9.3900 X - 9.5400	0.1274	0.5406	0.1623	0.9997
γ-Amino-n-butyric acid	Y = 4.4100 X - 3.7800	0.0608	0.2581	0.0370	0.9997
β-Mercaptoethylamine	Y = 3.4850 X - 2.3500	0.0450	0.2985	0.0810	0.9997
Phenylmethylamine	Y = 2.5186 X - 0.8133	0.0270	0.2103	0.0510	0.9998
β-Phenylethylamine	Y = 7.0200 X - 0.0600	0.0503	0.1669	0.0253	0.9999
Cadaverine	Y = 3.7243 X - 6.4867	0.0285	0.2218	0.0568	0.9999
Tyramine	Y = 7.8514 X - 0.7800	0.0936	0.3647	0.1534	0.9997
Synephrine	Y =12.6700 X - 8.7500	0.0480	0.1313	0.0115	1.0000
Dopamine	Y = 5.2743 X - 2.9267	0.0372	0.1448	0.0242	0.9999
Octopamine	Y = 6.4286 X - 1.5333	0.0559	0.2179	0.0548	0.9998
Tryptamine	Y = 1.8614 X - 0.0133	0.0173	0.1351	0.0210	0.9998