TYRAMINE, HISTAMINE, AND TRYPTAMINE CONTENT OF CHEESE

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Abstract

Because of the increasing knowledge of the physiological importance of biologically active amines in man and the importance of the presence of these amines in cheese, this study was done to obtain quantitative information for tyramine, tryptamine, and histamine in cheese available in the United States. The tyramine, histamine, and tryptamine contents of 156 samples of cheese purchased at retail stores were quantitated by thin-layer chromatography and fluorescence measurements of NBD-chloride derivatives of the amines. Tyramine was found in 81 of 85 Cheddar cheese samples examined. Extra-sharp, sharp, and medium Cheddar cheese samples contained average tyramine values of 0.27, 0.21, and 0.24 mg/g, respectively. Average tyramine contents were lower in mild and processed Cheddar (0.09 and 0.11 mg/g, respectively). The highest Cheddar cheese tyramine content was 0.7 mg/g. Tyramine was consistently found in all cheeses except in unripened soft cheese (Cottage). Histamine concentrations varied from nondetectable amounts to 2.6 mg/g in a Sap-Sago cheese sample. Twenty-four Cheddar cheese samples contained histamine with the highest amount being 1.3 mg/g. A domestic Blue cheese contained 2.3 mg/g. Tryptamine was uniformly low or completely absent in the Cheddar cheese samples. The highest tryptamine concentration (1.1 mg/g) was detected in a Blue cheese.

The presence of biologically active amines in food and the ability of tyramine, a pressor amine, to produce hypertensive crises in patients treated with monamine oxidase inhibitors (MAO) has been well documented. While many foods contain biologically active amines, tyramine in Cheddar cheese has been implicated most often as the causative agent in reported attacks resulting in acute hypertensive reactions in patients receiving MAO inhibitors *(1, 2, 3, 13, 16)*. Hannington *(11)* has shown that tyramine in the quantities often reported in cheese produced migraine headaches in subjects prone to such attacks and postulated that such migraine sufferers may have genetic monamine oxidase deficiences.

The tyramine content of cheese is known to be variable, ranging from almost nil to amounts approaching 3.7 mg/g *(2, 3, 5, 13)*. Histamine, while not found as frequently as tyramine, has been reported in Cheddar *(4, 17, 19)* in amounts up to 10.5 mg/100g. Swiatek and Kisza *(18)* reported histamine amounts of 50-100 mg/100 g in samples of Trappisten, Tilsiter, and Roquefort cheese, and a Gouda cheese that produced histamine intoxication was found to contain 85 mg histamine/100 g *(9)*. Formation of histamine in the Gouda cheese was traced to the presence of an unknown histidine decarboxylating *Lactobacillus* contaminant in the rennet. Tryptamine has been reported to occur in various cheese varieties including Cheddar *(17)*, and has usually been found only at low levels.

Dahlberg and Kosikowski *(6, 7, 8)* have provided extensive information on conditions leading to tyramine formation in Cheddar cheese. However, many of the concentration values reported in the literature pertain to surveys comprising small numbers of samples or values derived from single samples. This is particularly true for tyramine values as many of the quantitations were completed because the cheese was implicated in a hypertensive crisis. Because of the increasing knowledge of the physiological importance of biologically active amines in man and the importance of the presence of these amines in cheese, this study was done to gain quantitative information for tyramine, histamine, and tryptamine in cheese available in the United States.

Materials and Methods

Source of cheese

All cheese samples (156) were randomly obtained from commercial sources in the South and Midwest. Most samples were of domestic origin, but some were imported. The samples were believed to be representative of cheese available to consumers throughout the United States. After purchase, the cheeses were sealed in polyethylene bags and stored at −30 C until assayed.

Amine extraction

Tyramine, histamine, and tryptamine were extracted from the cheese samples by the method of Lovenburg and Engelman *(15)* and by the procedure of Blackwell and Mabbitt *(3)*. The extraction procedures were used interchangeably during the study and no differences were noted between the two. When both extraction procedures were used for a single sample, the amine contents were comparable (±10%). All reported values represent averages of four determinations. The procedure of Lovenburg and Engelman consisted of homogenizing 10 g of cheese in 20 ml of 0.1 N HCl in a glass homogenzier equipped with a motorized teflon coated stain-

TABLE 1. TYRAMINE CONTENTS OF VARIOUS CHEESES

Cheese		Tyramine (mg/g) Range	Average
Cheddar			
Extra-sharp	(11)[a]	0.10 - 0.60	0.27 (10)[c]
Sharp	(34)	ND[b] - 0.50	0.21 (33)
Medium	(18)	ND - 0.70	0.24 (17)
Mild	(12)	ND - 0.50	0.09 (11)
Processed	(7)	ND - 0.22	0.11 (6)
Smoked	(3)	0.07 - 0.21	0.12 (3)
Colby	(8)	0.10 - 0.56	0.21 (8)
Edam	(2)	0.30 - 0.32	0.31 (2)
Gouda	(6)	0.08 - 0.67	0.29 (6)
California Jack	(1)	0.13	—
Swiss	(6)	ND - 1.80	0.41 (5)
Roquefort or Blue	(7)	0.05 - 1.10	0.36 (7)
Camembert	(7)	0.07 - 0.21	0.12 (7)
Limburger	(1)	0.12	—
Sap-Sago	(1)	0.52	—
Romano	(1)	0.14	—
Parmesan	(1)	0.28	—
Mozzarella	(1)	0.16	—
Fontinella	(1)	0.10	—
Cottage	(3)	ND	—

[a]Number of samples examined

[b]ND = Not detectable at levels below 10 μg/g

[c]Number of positive samples

less steel pestle. The cheese slurry was centrifuged at 4 C at 12,000 × g for 10 min. The aqueous layer was decanted and adjusted to pH 10 with solid Na_2CO_3 and then saturated with excess NaCl. Fifteen milliliters of n-butanol were added and the mixture was agitated on a Genie Vortex mixer four times over a 10-min period. After centrifuging for 15 min (12,000 × g), the butanol layer was decanted and used for amine quantitation.

The procedure of Blackwell and Mabbitt consisted of homogenizing 10 g of cheese with sufficient warm distilled water to bring the total volume to 40 ml. The mixture was boiled for 5 min in a water bath and centrifuged at 4 C for 15 min (12,000 × g). The aqueous extract was decanted and filtered through Whatman No. 42 filter paper. The extract was applied to a 1 × 10 cm bed of an Amberlite weak cation exchange resin (CGC - 270, 100-200 mesh) in the sodium form. After complete passage of the extract, the column was washed with 50 ml of deionized distilled water and 30 ml of 1 N HCl. The acid eluate containing the amines was freeze-dried and dissolved in 10 ml of water:acetone (2:1 v/v) in preparation for amine quantitation.

Amine quantitation

Glass plates were coated with a 250 μ layer of silica gel (Kieselgel G. Merck, Darmstadt, Brinkman Instruments, Westbury, N. Y.). The layer was applied as a slurry consisting of 30 g of silica gel suspended in 70 ml of distilled water. The plates were dried in an oven at 100 C for 1 to 2 h and kept in a desiccator until used. The plates were reactivated at 100 C for 30 min immediately before use. The amine extracts (10 to 50 μl) were applied 2 cm from the base of the plates with quantitative capillary pipets. The solvent system used throughout the study consisted of CH_3Cl:CH_3OH:NH_4OH (12:7:1). This solvent system was chosen because of its ability to efficiently separate the three amines.

After development, the plates were air-dried and sprayed with a 0.2% NBC-Cl (7-chloro-4-nitribenzofurazan) - methanol solution and allowed to set for 24 h at 25 C *(20)*. The fluorescent amine spots were scraped from the plates and eluted from the silica gel with 5 ml of ethyl acetate. The silica gel-ethyl acetate mixture was agitated on a Genie Vortex mixer for four 30-sec intervals over a 10-min period and centrifuged for 15 min at 5000 × g to remove the suspended silica gel. Fluorescent measurements were determined on a Turner 110 Fluorometer with a primary filter No. 7-60 (365 nm) and a secondary filter No. 4 (465 nm) (G. K. Turner Assoc., Pala Alto, California). Amine contents were determined from standard curves prepared by adding known concentrations (0 - 2.5 mg/g) of tyramine, tryptamine, and histamine into a Cheddar cheese containing negligible amounts of the three amines and carrying out the extraction and the quantitation procedures as described above.

RESULTS AND DISCUSSION

The tyramine contents of the various cheeses are given in Tables 1 and 2. A total of 85 Cheddar cheese samples of various degrees of flavor development as classified by label specifications were assayed. The constancy of tyramine formation in Cheddar cheese was demonstrated by its presence in measurable quantities in 81 of the 85 Cheddar cheese samples examined. The highest tyramine concentration (0.7 mg/g) was found in a medium Cheddar cheese. The average tyramine content was highest in the extra-sharp category (0.27 mg/g) and lowest in the mild category (0.09 mg/g). Average tyramine contents of the extra-sharp, sharp, and medium Cheddar categories were 0.27 mg/g, 0.21 mg/g, and 0.24 mg/g, respectively. Although label specifications do not always accurately describe the flavor intensity or indicate the age of the cheese, the small differences found in the above Cheddar cheeses would tend to support an observation by Bullock and Irvine *(5)* that tyramine contents of Cheddar cheese did not increase significantly after a 6-month aging period. Although differences in tyramine content of extra-sharp, sharp, and medium Cheddar cheeses were slight, the lower tyramine concentration observed in the mild Cheddar category compared to the concentrations in the Cheddar cheese samples with greater flavor development follows observations of Dahlberg and Kosikowski *(6)* that tyramine concentrations in Cheddar cheese increased as the intensity of flavor increased.

Table 1 also presents the data on tyramine contents of 46 cheese samples other than Cheddar categorized by variety. Tyramine was present in detectable quantities in 42 of the samples. Tyramine was present in all varieties except in Cottage cheese where amine build-up would not be expected to oc-

TABLE 2. TYRAMINE, HISTAMINE, AND TRYPTAMINE CONTENTS OF VARIOUS IMPORTED CHEESES

Cheese		Tyramine (mg/g)	Histamine (mg/g)	Tryptamine (mg/g)
Mimolette	(1)[a]	0.28	ND	ND
Rehmkase	(1)	0.27	ND	ND
Gourmandise (Fondu Blend)	(3)	0.07-0.12 (3)[b]	ND-0.26 (2)	ND
Gjetost	(1)	0.12	ND	ND
German Blanco	(1)	0.10	0.28	ND
Cheurotin	(3)	ND[c]-0.36 (2)	ND-0.50 (1)	ND-0.23 (2)
Danbo	(1)	0.62	ND	0.13
Tybo	(1)	0.66	0.98	ND
Dofinio	(1)	0.25	ND	0.14
Graddoat	(1)	0.12	ND	ND
Norweigian Jarlsberg (Swiss Type)	(1)	ND	ND	ND
Port-Salut	(2)	0.12-0.18	ND	0.12-0.28
Reblochon	(1)	0.22	ND	0.21
Alpenjoi	(1)	0.10	ND	ND
Stilton	(1)	0.46	ND	ND
Muenster	(1)	0.14	ND	0.06
Boursault	(1)	0.11	ND	0.06
German Goldblock	(1)	0.33	ND	0.10
Brie	(2)	0.04-0.26 (2)	ND	ND

[a]Number of samples examined
[b]Number of positive samples
[c]ND = Not detectable at levels below 10 μg/g for tyramine and tryptamine and 50 μg/g for histamine.

cur. Colby, Edam, and Gouda cheese samples contained average tyramine concentrations of 0.21, 0.31, and 0.29 mg/g, respectively. These values approximate the average values found for the extra-sharp, sharp, and medium Cheddar cheese varieties. Average tyramine concentrations found for Swiss cheese and Blue and Roquefort cheese samples were 0.41 and 0.36 mg/g, respectively. The highest tyramine level found in the survey was 1.8 mg/g and was present in a Swiss cheese sample. A domestic Blue cheese contained 1.1 mg/g. All soft-ripened cheese samples (Camembert, Brie, and Limburger) contained tyramine but at levels lower than those noted in most other cheeses. Seven Camembert samples contained from 0.07 - 0.21 mg/g tyramine with an average content of 0.12 mg/g. A single Limburger sample contained 0.12 mg/g. Possible explanations for the lower levels of tyramine in soft-ripened cheese include the relatively short ripening period required for production and the rapid increase in pH of the cheese to levels above neutrality during ripening which would decrease tyrosine decarboxylase activity, since most microbial tyrosine decarboxylase enzymes demonstrate optimal activity at pH values lower than 6.0 *(10)*.

Table 2 presents data on the tyramine, histamine, and tryptamine concentrations found in several varieties of imported cheeses. For most of these varieties, the values are for single samples and were included in the study for comparative purposes. The tyramine levels observed in these cheeses were similar to the amounts found in the varieties listed in Table 1. None of the cheeses contained excessive amounts of tyramine, and the amine was present in measureable amounts in all but 2 of the samples assayed (23 out of 25).

The histamine contents of the cheese samples are given in Table 3. Histamine was present in measurable quantities in only 24 of the 85 Cheddar cheeses. The average histamine contents for the Cheddar cheese samples varied from 0.21 mg/g for the extra-sharp cheeses to non-detectable levels in the processed samples. The highest Cheddar cheese histamine content (1.3 mg/g) was found in both a sharp sample and in a mild sample. From the data, there appears to be no relationship between degree of flavor development and histamine content. It was evident that, in contrast to tyramine formation, histamine formation did not occur consistently in Cheddar cheese. It was interesting to note that none of the processed Cheddar cheeses contained histamine. Histamine has been reported to be partially heat-labile in food products *(14)* and possibly is destroyed through pasteurization of the processed cheese.

Measurable quantities of histamine were found in

TABLE 3. HISTAMINE CONTENTS OF VARIOUS CHEESES

Cheese		Histamine content (mg/g) Range	Average	
Cheddar				
Extra-sharp	(11)[a]	ND[b] - 0.8	0.21	(5)[c]
Sharp	(34)	ND - 1.3	0.11	(9)
Medium	(18)	ND - 0.9	0.14	(6)
Mild	(12)	ND - 1.3	0.19	(4)
Processed	(7)	ND	–	
Smoked	(3)	ND	–	
Colby	(8)	ND - 0.5	0.07	(3)
Edam	(2)	ND	–	
Gouda	(6)	ND - 0.45	0.075	(1)
California Jack	(1)	ND	–	
Swiss	(6)	ND	–	
Roquefort or Blue	(7)	ND - 2.3	0.5	(3)
Camembert	(7)	ND - 0.48	0.07	(1)
Limburger	(1)	ND	–	
Sap-Sago	(1)	2.6	–	
Romano	(1)	ND	–	
Parmesan	(1)	ND	–	
Mozzarella	(1)	ND	–	
Fontinella	(1)	ND	–	
Cottage	(3)	ND	–	

[a]Number of samples examined
[b]ND = Not detectable at levels below 50 μg/g
[c]Number of positive samples

only 9 of the 46 samples in Table 3 (not including Cheddar) examined in this study. The largest amount of histamine (2.6 mg/g) was found in a sample of Sap-Sago cheese. A domestic Blue cheese contained 2.3 mg/g and an imported sample of Danish Tybo cheese contained 0.98 mg/g (Table 2). Only 5 of the 25 cheeses in Table 2 contained histamine at detectable levels.

Tryptamine (Table 4) was uniformly low or completely absent in the Cheddar cheese samples. The highest level was 0.3 mg/g which was present in a sharp Cheddar cheese. The greatest tryptamine level found in this study (1.1 mg/g) was present in the domestic Blue cheese that contained 2.3 mg/g histamine. A Colby cheese sample contained 1.0 mg/g. Ten of the cheeses listed in Table 2 contained tryptamine, but the largest amount was only 0.28 mg/g which was present in a Port-Salut cheese sample.

The literature indicates that formation of biologically active amines in cheese appears to be dependent on several basic factors including sufficient ripening time for protein degradation to occur with the liberation of amino acids, and the presence of conditions necessary for formation and action of specific decarboxylases *(3, 7)*. This survey shows as previously reported by Dahlberg and Kosikowski *(6)* that tyramine concentrations generally increased with degree of flavor development in Cheddar cheese; whereas, formation of histamine and tryptamine occurred less frequently than tyramine in all of the cheese varieties examined. The tyramine levels found in Cheddar were lower than most reported earlier in the literature *(2, 3, 6, 13)* with the exception of values reported on individual Cheddar samples purchased at random by Blackwell and Mabbitt *(3)*. The highest tyramine content found in Cheddar cheese in this study was 0.7 mg/g which is somewhat lower than levels reported by Blackwell and Mabbitt *(3)* for a Cheddar cheese sample implicated in a hypertensive crisis (953 μg/g). It still must be emphasized that an individual susceptible to hypertensive crisis due to monoamine oxidase treatment or possible genetic lack of monoamine oxidase as suggested by Hannington *(11)* could be affected by ingesting cheese containing the lower levels of tyramine reported here. However, only 5 of the 85 Cheddar cheeses examined in this study contained over 0.5 mg tyramine/g cheese which was considered a dangerous dose by Blackwell and Mabbitt *(3)* for tyramine susceptible individuals consuming an average 50 g serving.

Only two samples of Cheddar cheese, one sample of Blue cheese, and the Sap-Sago cheese sample contained histamine at levels greater than 1.0 mg/g. Owing to individual differences in histamine metabolism, intake of 70-1000 mg is usually needed to cause moderate intoxication symptoms with small amounts leading to slight and often unnoticed re-

TABLE 4. TRYPTAMINE CONTENTS OF VARIOUS CHEESES

Cheese		Tryptamine (mg/g) Range	Average	
Cheddar				
Extra-sharp	(11)[a]	ND[b] - 0.1	0.02	(2)[c]
Sharp	(34)	ND - 0.3	0.04	(12)
Medium	(18)	ND - 0.1	0.02	(6)
Mild	(12)	ND - 0.2	0.03	(3)
Processed	(7)	ND	–	
Smoked	(3)	ND	–	
Colby	(8)	ND - 1.0	0.13	(2)
Edam	(2)	ND - 0.16	0.08	(1)
Gouda	(6)	ND - 0.2	0.07	(3)
California Jack	(1)	ND	–	
Swiss	(6)	ND - 0.16	0.19	(3)
Roquefort or Blue	(7)	ND - 1.1	0.20	(4)
Camembert	(7)	ND - 0.06	0.02	(2)
Limburger	(1)	0.16	–	
Sap-Sago	(1)	0.15	–	
Romano	(1)	ND	–	
Parmesan	(1)	ND	–	
Mozzarella	(1)	0.10	–	
Fontinella	(1)	ND	–	
Cottage	(3)	ND	–	

[a]Number of samples examined
[b]ND = Not detectable at levels below 10 μg/g
[c]Number of positive samples

actions *(12)*. With these dosage levels in mind and the reported intoxication of one individual resulting from ingestion of a Gouda cheese sample containing 85 mg/100 g, it appears that large portions of most cheese would be required to bring about histamine intoxication symptoms.

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SEX ATTRACTANTS AND DISEASE COMBINATION MAY BE NEW INSECT CONTROL METHOD

An entirely new method of fighting insects is being tried out at the University of Wisconsin under a grant from the Rockefeller Foundation.

It consists of luring male insects to a central location with sex attractants given off by female insects, then infecting the males with a disease organism deadly for their species, and letting them wander back into the insect world to spread the disease to others of their type.

The researchers involved are G. Mallory Boush, entomologist in the College of Agricultural and Life Sciences at the University of Wisconsin-Madison, and Wendell Burkholder who holds a joint appointment with the College and with the Agricultural Research Service of the U. S. Dept. of Agriculture.

The general idea for the procedure has been worked out during many years of basic research and it seems practical, the researchers state. But considerable work yet needs to be done in working out details, putting various parts of the scheme together, and presenting evidence of effectiveness and safety of the procedure.

They're working with beetles which infest stored food products such as grains and dry milk. Beetles called *Trogoderma inclusum* and *Trogoderma glabrum* are the specific ones they'll use in the research.

Past research has pinned down the chemical identity of the sex attractants (called pheromones) for both of these beetles, and they can be made synthetically in the chemical laboratory. Plans are to use between 5 and 50 micrograms in each "trap." The researchers figure that one microgram is about equal to the peromone produced by one female insect. This material would be mixed with the disease spores and placed in small, simple, and inexpensive disposable devices made from corrugated paper similar to that used in packing cartons.

The researchers will use a disease agent called *Mattesia trogodermae*. The insects eat the spores of

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