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Bernard F. Gibbs[a]; Inteaz Alli[a]; Catherine N. Mulligan[b]
[a] FOOD SCIENCE AND AGRICULTURAL CHEMISTRY DEPARTMENT, McGILL UNIVERSITY, ANNE DE BELLEVUE, QUEBEC, CANADA [b] FOOD BIOTECHNOLOGY DIVISION, BIVAN CONSULTANTS SUITE, MONTREAL, QUEBEC, CANADA

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TECHNICAL NOTE

Determination of Amines in Food Products

BERNARD F. GIBBS and INTEAZ ALLI
FOOD SCIENCE AND AGRICULTURAL CHEMISTRY DEPARTMENT
McGILL UNIVERSITY
MACDONALD CAMPUS
P.O. BOX 187, STE. ANNE DE BELLEVUE, QUEBEC H9X 3V9, CANADA

CATHERINE N. MULLIGAN*
FOOD BIOTECHNOLOGY DIVISION
BIVAN CONSULTANTS
SUITE 1415, 6550 SHERBROOKE ST. W., MONTREAL, QUEBEC H4B 1N6, CANADA

ABSTRACT

A method for the determination of amines in food products is described. The procedure is based on the extraction of homogenized foods by a series of acid extractions and subsequent chromatographic analysis on a cation exchanger as free amines. The method is simple, rapid, quantitative, and has been automated. The nature of the extracted basic compounds was confirmed by electrospray mass spectrometry.

INTRODUCTION

The presence of biologically active amines such as cadaverine, putrescine, histamine, and spermidine has been detected in numerous food products, animals, and plants (1). They are active during cell fission and in the synthesis of nucleotides (both RNA and DNA) and proteins (2). In food products they are usually formed by the microbial decarboxylation of amino acids. As a result, they can have aliphatic (putrescine and cadaverine), aromatic (tyramine and phenylethylamine), or heterocyclic (histamine and tryptamine) structures. Histamine, cadaverine, and putrescine are formed by enzymatic decarboxylation of histidine, lysine, and orni-

* To whom correspondence should be addressed.

thine, respectively, and collectively contribute to over 85% of the mono- and diamines found in foods (3). Putrescine can be broken down enzymatically to produce another diamine, spermidine, which in turn can also be further enzymatically reduced to spermine. Others of lesser occurrence are tyramine, tryptamine, phenylethylamine, and agmatine, whose precursors are the amino acids tyrosine, tryptophan, phenylalanine, and arginine, respectively. These amines may be used to indicate cellular toxicity within the complex system of enzymes and regulatory proteins (4).

During the ripening and spoilage of foods, the levels of histamine, cadaverine, and putrescine are increased (5). It has been hypothesized that these compounds act as agents against aging whereas ethylene seems to promote maturation leading to higher rates of fruit ripening (6). In addition, the free amines have been shown to decrease ethylene production in some tissues (7). Elevated levels have been found in spoilt fish, meats, and other products (8), and they may be involved at significant levels in human poisoning (9). Thus, it is imperative to monitor their presence in foods.

The ability to accurately quantify these low molecular weight organic bases in different matrices without interference from amino acids and other substances is thus very important. Available methods were recently reviewed by Danner (10). Most of these methods are based on thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or gas chromatography (GC) where derivatized species are formed. In general, this is necessary since amines do not show significant ultraviolet absorption or fluorescence without chemical derivatization.

We have developed a method using a strong cation exchanger, three buffers, and a triple-ramped temperature gradient. The method has been automated to a Beckman 6300 High Performance Analyzer and has been used to quantify mono- and diamines in fish, chicken, beef and cheese. The nature of the extracted basic compounds was confirmed by electrospray mass spectrometry.

EXPERIMENTAL

Chemicals

Cadaverine, putrescine, histamine, spermidine, spermine, tyramine, tryptamine, phenylethylamine, and agmatine, amino acid standard solution, ninhydrin and hydrindantin were obtained from Sigma Chemicals (St. Louis, MO). Sodium citrate dihydrate, citric acid monohydrate, phenol, and sulfosalicylic acid (all ACS grade) were purchased from Anachemia (Rouses Point, NY). Buffers A, B, and C were made to molar concentrations of 0.4, 0.7, and 1.0 and pH 3.5, 4.5, and 5.5, respectively.

Phenol (0.3%) was added to each buffer. The ninhydrin reagent was composed of 76% dimethyl sulfoxide, 22% water, 1% acetic acid, 0.02% ninhydrin, and 0.001% hydrindantin.

Standard and Sample Preparation

The standard contained 0.1 mM of each amine and 0.20 mM of the amino acid standard diluted with buffer A. Chopped samples of fish, chicken, beef, and pork (approximately 1 g) were homogenized with a tissue grinder in 2 mL of 10% sulfosalicylic acid. The samples were centrifuged at 14,000 *g* for 5 minutes. The supernatants were retained. Two more similar extractions were performed and the supernatants pooled. One percent (approximately 60 μL) was removed and lyophilized for mass spectrometry, and the remainder was diluted one-to-one with buffer A prior to injection. The column (Beckman #338076) was equilibrated with buffer A at 60°C. The chromatographic conditions are shown in Table 1.

Mass Spectrometry

Mass spectra were obtained in the positive mode on a triple stage mass spectrometer Model API-III (Sciex, Toronto, Canada). Briefly, the samples and standards were dissolved in 10% acetic acid and infused through a stainless steel capillary (100 μM I.D.). A stream of air (pneumatic nebulization) was introduced to assist in the formation of submicron droplets (11). These droplets were evaporated at the interface by nitrogen gas to produce highly charged ions which were detected by the analyzer.

The system's calibration was performed with the ammonium adduct ions of polypropylene glycol (PPG) with known mass-to-charge ratios throughout the range of the instrument (0–2470 amu). Instrument tuning, data acquisition, and data processing are controlled by a MacIntosh II computer with all software (no modifications necessary) provided by the instrument's manufacturer. Simple algorithms correlate the charges produced by these compounds to their molecular weights (12).

TABLE 1
Chromatographic Conditions for the Separation of Amines. The Temperature Gradient Used Was 1°C/min

Buffer type	A (0.4 M)	B (0.7 M)	C (1.0 M)
Time of buffer change (min)	75.0	6.0	35.0
Temperature (°C)	60.0	70.0	80.0
Time of temperature change (min)	0.0	2.0	10.0

RESULTS AND DISCUSSION

Figure 1 shows the separation of amines in the standard and some of the foods studied. The amino acids are eluted within the first 15 minutes of the chromatogram. Arginine, which is clearly resolved at 13 minutes, is used as an indicator to signal the end of the amino acid elution. This scheme was followed since the foods studied contained natural amino acids.

A mixture of nine amines was infused in the mass spectrometer at 20 pmol/μL concentration (200 pmol of the total sample was consumed). The mass spectrum is shown in Fig. 2. The protonated molecular weight (MH^+) is unambiguous for all nine species. These standards were later used to identify traces of amines in the foods studied. Figure 3 shows a fish sample extracted after being left at 2°C for 9 days.

The foods were left at room temperature or on ice (0–4°C) for several days while the buildup of metabolites were monitored. Detectable levels of cadaverine, putrescine, and histamine were not present in samples on receipt from local slaughterhouses. Fresh apple juice, light cheddar cheese, and a local light beer did not contain the major amines. Traces of spermidine (<1 ppm) were found in spoilt chicken and pork. This was not surprising since putrescine is its precursor in amine metabolism. The limit of detection was approximately 3.3 μM (approximately 1 ppm), which is 100 times less than the suggested legal limit for food products in Europe (13). The accumulation of metabolites was monitored for several days. Figure 4 shows the deterioration of a fish sample resulting in the accumulation of amines. The sample became unfit for human consumption in 9 to 12 days (at 2°C) as detected by our method. Varying amounts of amines were added to the fresh products and homogenized. The recoveries were estimated by adding the compounds dissolved in buffer to the homogenized food at final concentrations of 0.1 and 1.0 mM. These spiked samples were extracted similarly to the normal food samples. Table 2 shows the recoveries obtained. These experiments were done in triplicate with the standard deviations shown. The linear dynamic range of the method (Fig. 5) was up to 3 mM.

The column used in the experiment did not meet the Beckman specifications for amino acid separation (90% resolution between serine and threonine). This column (cost approximately $1200 US) would normally be discarded by users of the Beckman High Performance 6300 Analyzer. However, it was successfully used for over 200 injections of food samples with no decrease in resolving power or increase in backpressure. It is important to regenerate the column at 80°C with 10% sodium hydroxide for 2 column lengths after each run (approximately 5 minutes). The ramp

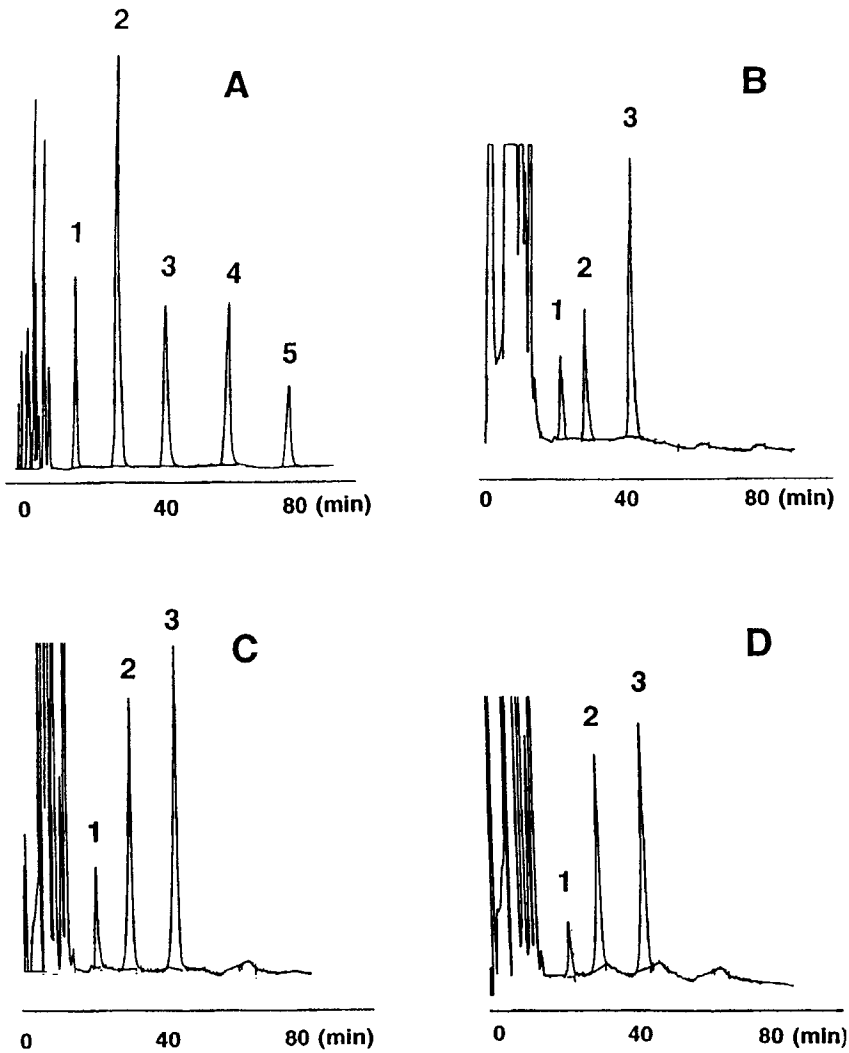

FIG. 1 Separation of amine standards and samples of foods analyzed. The peaks identified are: (1) histamine, (2) putrescine, (3) cadaverine, (4) spermidine, and (5) spermine. The concentration of the standard (A) was 0.1 mM for each of the compounds. The fish (B) was stored at 2°C for 9 days, whereas the chicken (C) and pork (D) were left at room temperature for 3 days.

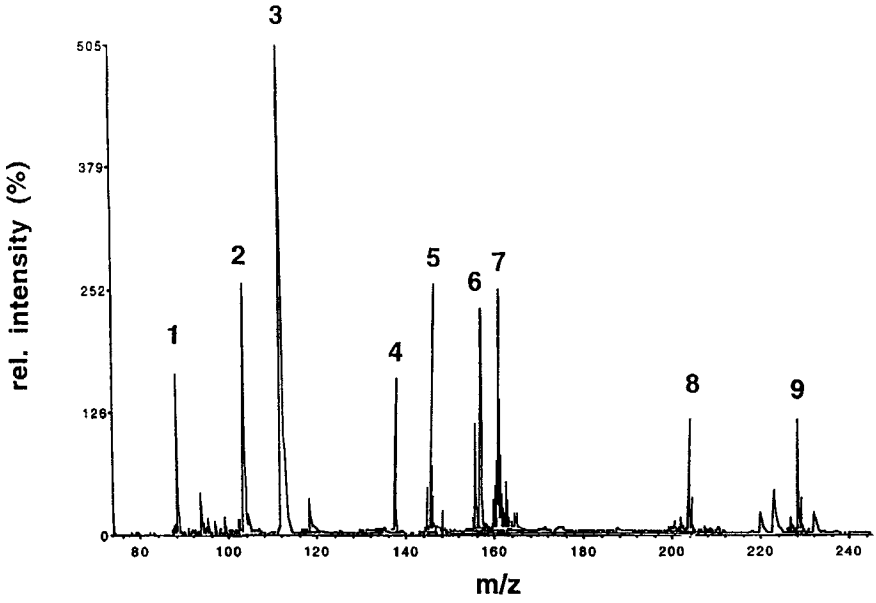

FIG. 2 Electrospray ionization mass spectrometry of a mixture of nine amines. The molecular weights obtained are within 0.1% of the theoretical monoisotopic values. These are: (1) putrescine (89.20 atomic mass units (amu)), (2) cadaverine (103.18 amu), (3) histamine (112.05 amu), (4) tyramine (138.18 amu), (5) spermidine (146.24 amu), (6) phenylethylamine (157.10 amu), (7) tryptamine (161.21 amu), (8) spermine (203.34 amu), and (9) agmatine (228.20 amu).

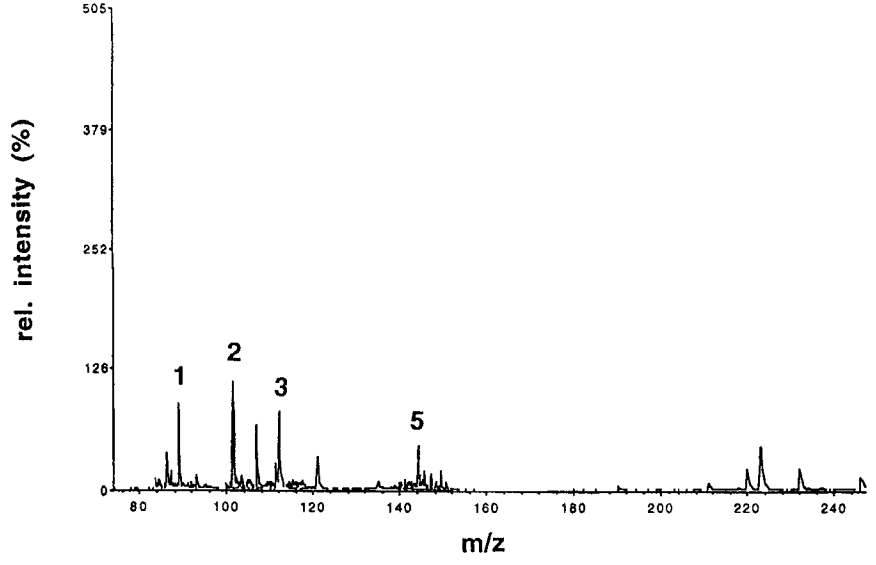

FIG. 3 Electrospray ionization mass spectrometry of a fish sample that has been left at 2°C for 9 days. Putrescine (1), cadaverine (2), histamine (3), and spermidine (5) were detectable.

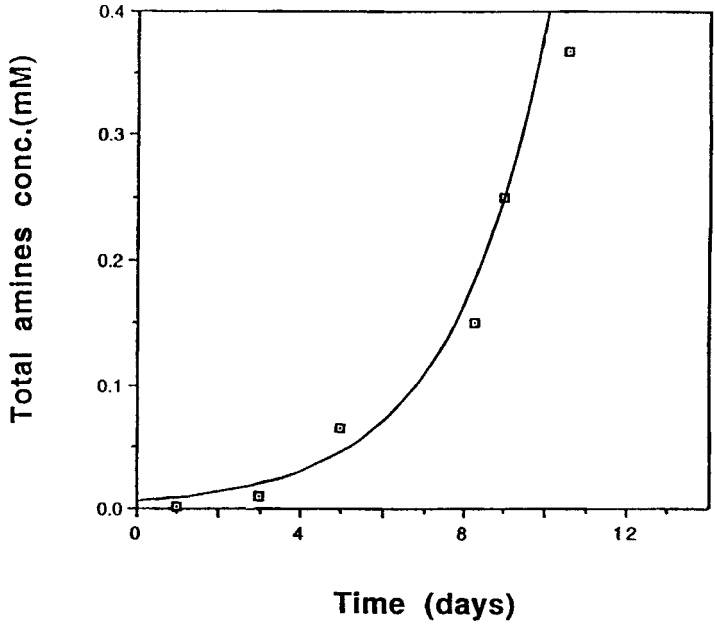

FIG. 4 The accumulation of amines in a fish sample left at 0–4°C.

program used for the chromatography was essential to avoid column shock and permanent damage to the beads. Complete resolution of tyramine and phenylethylamine was achieved when a shallower gradient was used. This approach was unnecessary in our method since cadaverine, putrescine, and histamine constitute over 85% of the mono- and diamines present in spoilt foods (3).

TABLE 2
Recoveries Obtained from Fresh Fish, Chicken, and Pork Spiked with the Amines and Extracted as Samples

Compound	Concentration (mM)	Mean recovery[a] ± standard deviation (%)
Histamine	0.1	96.0 ± 4.8
Cadaverine	0.1	101.2 ± 4.3
Putrescine	0.1	91.3 ± 2.5
Tryptamine	1.0	93.3 ± 2.9
Tyramine	1.0	103.6 ± 5.9
Spermidine	1.0	88.2 ± 4.2

[a] Triplicate determination.

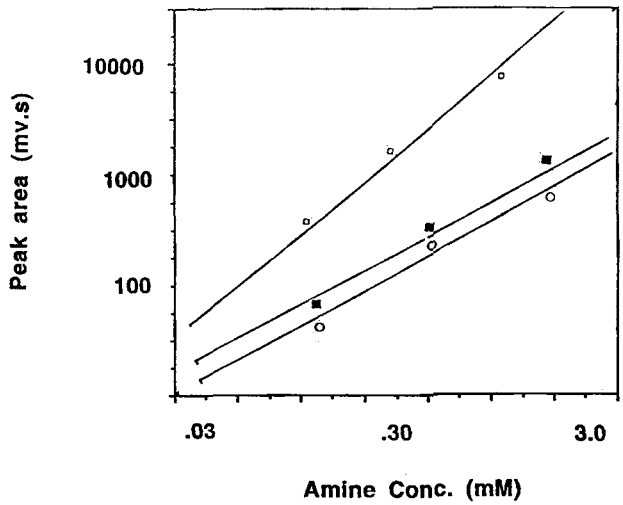

FIG. 5 Calibration curve relating the concentration of the major amines, (□) putrescine, (■) histamine, (○) cadaverine, and (●) spermidine, which varied linearly with the integrated peak areas.

Incubation (for several days or weeks) is required in the preparation of some fermented foods (e.g., cheese, beer, and fermented sausages). It is reasonable, then, to expect the presence of several kinds of microorganisms, some of which might have decarboxylase activity. The occurrence of biologically active amines is therefore sometimes expected in fermented foods. In contrast, histamine, putrescine, and histamine are not usually found in nonfermented foods, and levels increase with food degradation.

The presence of free amino acids, normally found in foods, does not interfere with this assay. They have less affinity for the column and are easily eluted. The amines, because of their highly basic nature, bind tightly to the cation exchanger, and a high salt concentration is needed to remove them.

In conclusion, we have described a method for the reliable and accurate quantitation of mono- and diamines in foods. The method showed excellent reproducibility with repeated injections and possesses good linearity over a wide range. It has been automated on a Beckman 6300 High Performance Analyzer. This procedure is sensitive and simple, and it can be used to monitor spoilage of foods in general.

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