

Development of a Gas Chromatography Method for the Estimation of Alkylamines in Foods

ANJI PENDEM, VIKAS M. PAWAR, AND SUJATHA JAYARAMAN*

Unilever R&D, Bangalore, No. 64, Main Road, Whitefield, Bangalore 560 066, India

Alkylamines are gaining importance due to their proven immunity benefits. Fruits, vegetables, and beverages are important dietary sources for alkylamines. This paper reports for the first time quantitative data on four alkylamines, ethyl-, propyl-, isopropyl-, and *sec*-butylamines, in commonly consumed fruits and vegetables. A sensitive and selective chromatography method based on derivatization with pentafluorobenzaldehyde and detection by GC-ECD is developed and validated for the analysis of alkylamines in vegetables, fruits, and tea. In vegetables and fruits, the concentrations varied from 100 to 15000 $\mu\text{g}/\text{kg}$. Propyl- and isopropylamine concentrations were significantly higher compared to other amines. Among all dietary sources, tea had the highest concentration of alkylamines (30–50 mg/kg), with ethylamine as the major component, and is the richest source for alkylamines. The stability of these alkylamines was studied under various cooking conditions, and it was observed that there is loss of alkylamines on cooking.

KEYWORDS: GC-ECD; ethylamine; propylamine; isopropylamine; *sec*-butylamine; vegetables; fruits; tea; pentafluorobenzaldehyde

INTRODUCTION

Alkylamines such as ethylamine, propylamine, isopropylamine, and *sec*-butylamine are volatile compounds found in many plant materials and are gaining importance due to their proven immunity benefits (1–8). Dietary ingestion of plant products rich in alkylamines and their precursors may contribute to the health properties of these foods. Hence, quantification of alkylamines in commonly consumed vegetables, fruits, and beverages is of huge interest. The key challenge in quantification is developing a sensitive and selective method for alkylamines.

Several analytical methods using high-performance liquid chromatography (HPLC) and gas chromatography (GC) have been reported for the analysis of alkylamines in water (9, 10), wine (11–16), body fluids (17, 18), and foods (19). In the case of HPLC methods, alkylamines are derivatized with orthophthalaldehyde, dansyl chloride, or fluorescamine and then quantified with UV-visible, fluorescence, or electrochemical detectors (11–13). The detection limit of alkylamines by HPLC methods is 100 ng/L. Alkylamines are analyzed by GC directly or after derivatization. Several derivatization agents such as benzenesulfonyl chloride, trifluoroacetic anhydride, and pentafluorobenzylamines (10, 16) are used to detect alkylamines as low as 1 ng/L in water, plasma, urine, and wine. To our knowledge, there is no published literature on detailed analysis of alkylamines in commonly consumed vegetables and fruits. Alkylamine analysis is reported in wine, grapes, and apple (16, 20, 21).

In this paper, we discuss the development of a method for the analysis of alkylamines in commonly consumed vegetables and fruits. The method involves derivatization of alkylamines with

pentafluorobenzaldehyde (PFB) to corresponding pentafluorobenzylamines followed by liquid–liquid extraction and analysis by gas chromatography–electron capture detector (GC-ECD). Advantages of PFB derivatization with GC-ECD detection method over other methods are higher sensitivity, selectivity, and better resolution. The method has been optimized and validated. We have estimated ethylamine, propylamine, isopropylamine, and *sec*-butylamine concentrations in 10 commonly consumed vegetables and 5 common fruits that constitute the daily palate of Indian consumers. This method has also been applied to black tea and fresh green leaf to estimate ethylamine and other alkylamines.

MATERIALS AND METHODS

Chemicals. Ethylamine (70%), propylamine (99%), isopropylamine (99.5%), *sec*-butylamine (99%), pentafluorobenzaldehyde (PFB), and polyvinylpyrrolidone (PVPP) were procured from Sigma-Aldrich. Sodium hydroxide, anhydrous sodium sulfate, and acetonitrile were procured from Merck. Hexane (GC grade) was procured from Labscan Analytical Sciences, Thailand. For all preparations, Milli-Q water was used. Vegetables purchased locally were carrot (*Daucus carota*), cabbage (*Lysichiton americanum*), potato (*Solanum tuberosum*), capsicum (*Capsicum annuum*), beet root (*Armoracia rusticana*), bitter melon (*Momordica charantia*), tomato (*Solanum lycopersicum*), brinjal (eggplant) (*Solanum longena*), spinach (*Spinacia oleracea*), and cauliflower (*Brassic oleracea*). Fruits such as banana (*Asimina tetramera*), apple (*Malus domestica*), grape (*Coccoloba uvifera*), orange (*Citrus sinensis*), and sapodilla (*Manikara zapota*) as well as black tea were purchased locally. Green tea (*Camellia sinensis*) leaf was procured from southern India.

Extraction of Alkylamines from Vegetables and Fruits. As alkylamines are volatile, all sample preparations were carried out at low temperatures. One hundred grams of sample was homogenized by cutting vegetables/fruits into small pieces followed by powdering with liquid nitrogen. One gram of powdered sample was used for extraction. Fifty milliliters of water was added to the cold sample ($\sim 2^\circ\text{C}$) and kept under

*Author to whom correspondence should be addressed (phone +91 80 39831067; fax +9180 28453086; e-mail sujatha.jayaraman@unilever.com).

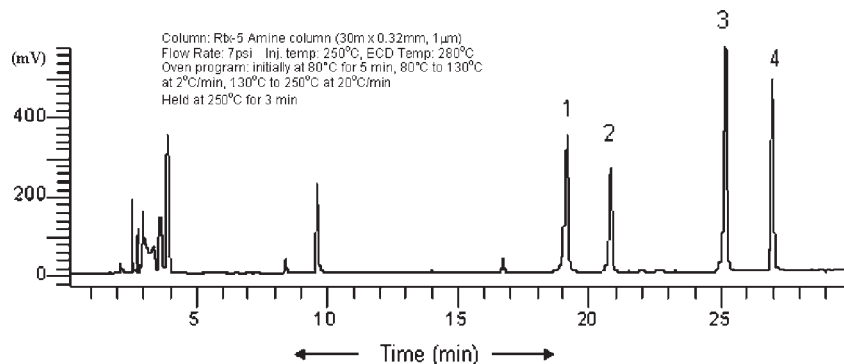


Figure 1. GC-ECD chromatogram for four standards. Peaks: 1, ethylamine; 2, isopropylamine; 3, propylamine; 4, *sec*-butylamine.

stirring in a cold room at 4 °C (15 min) for alkylamine extraction. The supernatant was collected. With residue the extraction procedure was repeated until no alkylamine was detected in the supernatant (three extractions). Extracts were pooled together and made up to a final volume of 100 mL. PVPP resin was added to the water extract to remove hydrophobic colored compounds such as polyphenols and chlorophyll. Suspended solids (if any) were removed by centrifugation at 10000 rpm for 10 min. Supernatant was used for PFB derivatization.

Extraction of Alkylamines from Tea. One and a half grams of green tea leaf was weighed and cut into small pieces. Frozen tea leaves were homogenized. To this sample was added 25 mL of water to extract amines. The mixture was centrifuged at 5000 rpm for 15 min to remove suspended material, and the supernatant was collected. The procedure was repeated in triplicates with the residue. All water extracts were pooled to make a final volume of 50 mL. Polyphenols were removed by PVPP treatment. The supernatant was collected after centrifugation was collected and used for derivatization. In the case of black tea, 1.0 g of black tea was weighed and transferred to a mortar and pestle for grinding and homogenization. To this sample was added 25 mL of water to extract amines at room temperature, and the remaining extraction procedure was the same as that for green tea leaf.

Procedure for PFB Derivatization of Amines. The concentration of reaction mixture, time, and temperature for 100% completion of derivatization were optimized. In 8 mL of sample solution, 1 mL of 0.1 N NaOH solution was added (pH 12). To this was added 1 mL of PFB reagent. This mixture was kept at room temperature for 30 min (optimized), which yields PFB-imines. Once the reaction was complete (as evaluated by TLC), the reaction mixture was extracted with 2 mL of hexane in duplicate. The volume of hexane and the number of extractions were optimized to get >99% recovery. Hexane extracts were pooled together. Unreacted PFB was removed by washing the hexane layer with 1 mL of 0.1 N NaOH. The NaOH layer was discarded. The hexane extract was dried over anhydrous sodium sulfate, decanted to a volume of 5 mL. This extract was used for GC injection.

Cooking of Vegetables. To study the effect of the cooking conditions on the concentrations of the alkylamines, the three most common ways of cooking were chosen: (i) open heating in water, (ii) microwave cooking, and (iii) pressure cooking. Potato, carrot, and cabbage were taken and washed under running water. For all types of cooking, the vegetable to water ratio was 1:3. In the case of boiling, vegetables were cut and cooked in water in separate containers for 30 min. In the case of microwave cooking, vegetables were cooked using water in a microwave oven for 20 min, whereas in the pressure cooker vegetables were cooked for 20 min. After boiling, vegetables were decanted, and cooked vegetables were used for analysis of alkylamines. For microwave cooking, a microwave oven from BPL-SANYO was used (model BMO 700T), with microwave power of 1.2 kW and microwave frequency of 2450 MHz.

Apparatus and Chromatographic Conditions. A Perkin-Elmer (model Clarus 500) GC with ECD and mass spectrometer (MS) detector was used for all PFB-imine analyses. Separation was achieved with an Rt-5 Amine column (30 m × 0.32 mm, 1 µm) from Restek. Carrier gas (He) flow rate was maintained at 7 psi with split ratio of 1:10. The injector and ECD detector were maintained at 250 and 280 °C, respectively. One microliter of the sample was injected for each run. The oven temperature

program was held at 80 °C for 5 min and heated to 130 °C at a rate of 2 °C/min. The temperature was further increased from 130 to 250 °C at a heating rate of 20 °C/min and held at 250 °C with transfer line temperature maintained at 200 °C. MS was operated at full scan (m/z 50–500).

Method Validation. An external calibration method was used to quantify ethylamine, propylamine, isopropylamine, and *sec*-butylamine in samples. A calibration curve was generated for the range of 250–5000 µg/kg after derivatization of standards. Each standard was run in triplicate. The GC-ECD method was validated for various parameters such as extraction efficiency, precision, linearity, quantification range, limit of detection (LOD), and limit of quantification (LOQ) for ethylamine, propylamine, isopropylamine, and *sec*-butylamine.

RESULTS AND DISCUSSION

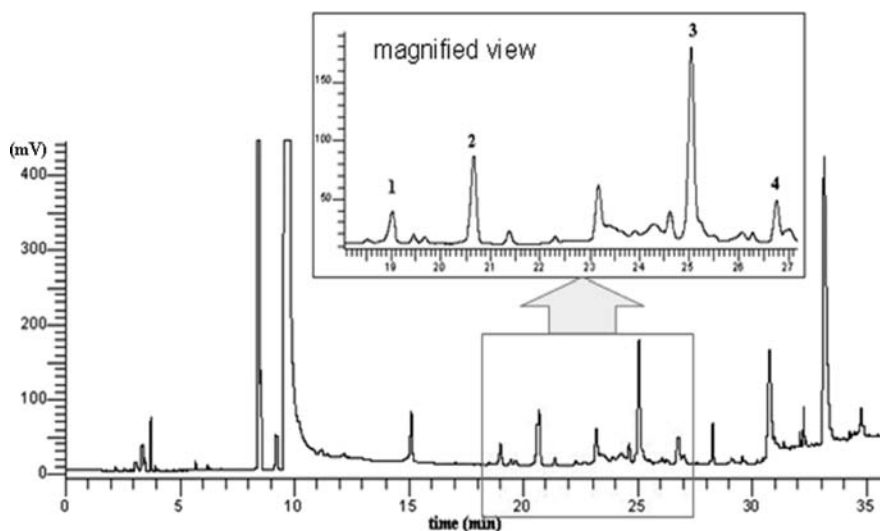
Method Development. HPLC methods were evaluated for alkylamine analysis in fruits and vegetables. HPLC methods had limitations to analyze alkylamines in vegetables and fruits due to poor resolution of amino acids and amines. Hence, we chose GC over HPLC separation. A GC-ECD method, which has a reported detection limit of 1 ng/L and minimum matrix interference especially from amino acids, was adapted for this application. We started with identical GC conditions described by Ngim et al. with ECD detector in which a DB-5 column (nonpolar GC column) had been used (16). Under the reported GC conditions, resolution of the alkylamines that are of interest to us was poor. Hence, the method required significant modifications. As the PFB-imines are polarizable, we used RTX amine, a midpolar GC column rather than the nonpolar DB5 column. Due to better interaction, we obtained a superior resolution for the derivatized alkylamines. Also, the oven program was optimized for rapid and better resolution. The GC-ECD chromatogram for analysis of ethylamine, propylamine, isopropylamine, and *sec*-butylamine is shown in **Figure 1**. All of the amines are well resolved with good peak shapes. Retention times for alkylamines with this method are given in **Table 1**.

GC-ECD Method Validation. The GC-ECD method is validated, and the linearity range for quantification, recovery, LOD, and LOQ for each alkylamine in the vegetable extract are given in **Table 1**. For all of the alkylamines analyzed, the linear range was from 250 to 5000 µg/kg. On a weight by weight basis the detection limit of the method was 50–70 µg/kg in fruits and vegetables. Cabbage was used to find the percentage recovery. The percentage recovery obtained was >90% for the spike concentrations of 365 µg/kg for ethylamine, 380 µg/kg for isopropylamine, 300 µg/kg for propylamine, and 500 µg/kg for *sec*-butylamine. The LOQ was done in the matrix-matched sample through

Table 1. Method Validation Data^a

amine	retention time (min)	LOD ^b	LOQ ^b	% recovery	linearity range ^b	linearity (R^2) ^c
ethylamine	19.13 ± 0.12	48 ± 4.5	151 ± 5.6	107 ± 2.2	250–5000	0.9994
isopropylamine	20.79 ± 0.15	68 ± 2.0	213 ± 2.6	104 ± 1.9	250–5000	0.9993
propylamine	25.18 ± 0.09	52 ± 0.7	147 ± 3.9	89 ± 2.1	250–5000	0.9988
sec-butylamine	26.97 ± 0.1	58 ± 1.5	198 ± 5.0	93 ± 3.5	250–5000	0.9962

^a % CV for LOD and LOQ was in the range of 0.7–5.6%. Percentage recovery studies were done on cabbage with spike concentrations of 365 $\mu\text{g}/\text{kg}$ for ethylamine, 380 $\mu\text{g}/\text{kg}$ for isopropylamine, 300 $\mu\text{g}/\text{kg}$ for propylamine, and 500 $\mu\text{g}/\text{kg}$ for sec-butylamine. ^b Values for LOD, LOQ, and range are in $\mu\text{g}/\text{kg}$ (on dry weight basis). ^c R^2 is obtained from linear regression coefficient of three replicates

**Figure 2.** Representative GC-ECD chromatogram for carrot. Peaks: 1, ethylamine; 2, isopropylamine; 3, propylamine; 4, sec-butylamine.**Table 2.** Alkylamine Quantities in Vegetables

vegetable	concentration ^a ($\mu\text{g}/\text{kg}$)				total amines
	ethylamine	isopropylamine	propylamine	sec-butylamine	
carrot	514 ± 10	2617 ± 5	1997 ± 26	586 ± 4	5711 ± 45
capsicum	205 ± 33	4100 ± 14	2104 ± 13	911 ± 13	7320 ± 73
cabbage	300 ± 25	1588 ± 9	5647 ± 200	406 ± 10	7914 ± 244
potato	10 ± 0	128 ± 0	774 ± 1	1 ± 0	913 ± 1
beet root	1073 ± 26	571 ± 22	1052 ± 7	135 ± 2	2831 ± 57
bitter melon	147 ± 9	1044 ± 10	2891 ± 146	226 ± 1	4308 ± 166
tomato	306 ± 27	1407 ± 4	2977 ± 109	376 ± 5	5066 ± 140
brinjal	45 ± 4	<LOD ^b	2815 ± 74	<LOD	2860 ± 78
cauliflower	103 ± 5	<LOD	5436 ± 29	<LOD	5539 ± 33
spinach	372 ± 27	<LOD	553 ± 85	<LOD	925 ± 106

^a All values are expressed on dry weight basis. Each value is an average value over four analyses. ^b <LOD indicates concentrations of alkylamine below the detection limit.

serial dilution, and it was taken as the concentration at which the signal-to-noise ratio is 6; it was 150–200 $\mu\text{g}/\text{kg}$ for the alkylamines.

Quantification of Alkylamines in Vegetables. With the optimized derivatization and chromatographic conditions, vegetable and fruit samples were analyzed. A representative GC chromatogram for the alkylamines in the vegetable extract is shown in **Figure 2**. Retention times for alkylamines in vegetable extracts are observed to be the same as of standards, and the peak identity was confirmed from gas chromatography–mass spectrometry (GC-MS). From the external calibration curve, the concentrations of individual alkylamines were calculated. Quantities of each alkylamine in vegetables under study are given in **Table 2**. Total alkylamine concentrations in vegetables varied from 900 to 8000 $\mu\text{g}/\text{kg}$ of dry weight of vegetable. Potato had the lowest amount of alkylamines, whereas cabbage had the highest concentration. Most of the vegetables had all

four alkylamines. It is interesting to observe that propylamine and isopropylamine are the major components in many vegetables studied in this paper.

Ethylamine concentrations in the vegetables were between 1 and 10% of total alkylamine content except for spinach and beet root, which had 40%. All the vegetables had high concentrations of propylamine varying from 29 to 98% of total alkylamine with the lowest in capsicum and the highest for spinach and brinjal. For vegetables in which isopropylamine was detected, its concentration varied from 9 to 56% of total alkylamines, with potato having lower concentration and capsicum having higher concentration of isopropylamine. sec-Butylamine was not detected in cauliflower, spinach, and brinjal, whereas its percentage on the basis of total alkylamine varied from 5 to 35%.

If the total alkylamine contents across different classes of vegetables are compared on the basis of the edible part, the fruit and flower class of vegetables has higher alkylamine concentration

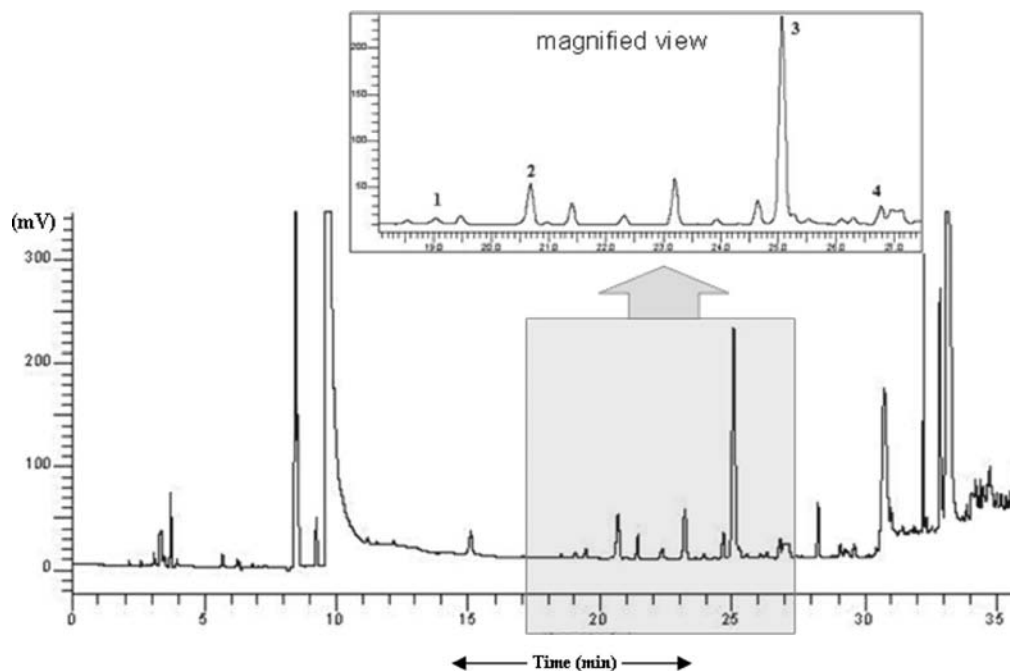


Figure 3. Representative GC-ECD chromatogram for banana. Peaks: 1, ethylamine; 2, isopropylamine; 3, propylamine; 4, *sec*-butylamine.

Table 3. Alkylamine Quantities in Fruits

fruit	concentration ^a ($\mu\text{g}/\text{kg}$)				total amines
	ethylamine	isopropylamine	propylamine	<i>sec</i> -butylamine	
banana	39 \pm 10	514 \pm 35	944 \pm 19	97 \pm 22	1594 \pm 88
apple	54 \pm 6	71 \pm 1	951 \pm 2	273 \pm 7	1349 \pm 16
grape	679 \pm 31	929 \pm 46	9548 \pm 55	3667 \pm 81	14823 \pm 195
orange	19 \pm 0	<LOD ^b	1228 \pm 384	<LOD	1247 \pm 384
sapodilla	24 \pm 3	<LOD	1120 \pm 56	491 \pm 5	1635 \pm 64

^a The values are expressed on dry weight basis. Each value is an average value over four analyses. ^b <LOD indicates concentrations of alkylamine below the detection limit.

and the leaf and root class has lower alkylamine concentration. The source of alkylamines in vegetables is mainly amino acids, and if this is the major source, then we expect a correlation between amino acid concentrations in vegetables and alkylamines. Ethylamine is formed by the decarboxylation of alanine (22). Alanine concentrations in vegetables such as cauliflower, cabbage, carrot, beet root, and potato vary from 50 to 98 mg/100 g of vegetable (23). In our analysis, ethylamine concentration varied between 1 and 100 $\mu\text{g}/100$ g. Ethylamine concentrations measured are very low compared to alanine concentrations in vegetables. Propylamine is a metabolic intermediate in the biosynthesis of polyamines such as spermidine and spermine with methionine as an initial precursor (24). Hence, higher concentrations of propylamine in vegetables can come from higher concentrations of methionine. Methionine concentrations in vegetables (23) such as spinach, cauliflower, and potato are > 25 mg/100 g, whereas carrot, tomato, and cabbage have < 25 mg/100 g, and there appeared to be a correlation between methionine and propylamine. The biogenesis of isopropylamine and *sec*-butylamine is not well understood, and hence their precursors are unknown. Apart from biological source, there could be alternate sources for alkylamines in these food materials. Especially for propylamine, isopropylamine, and butylamine pesticide residues could be a possible external source as they are used as adjuvants and antifungal chemicals (25, 26). Among all of the vegetables, the best sources of ethylamine, isopropylamine, and *sec*-butylamine are beet root, cabbage, and capsicum, respectively.

Quantification of Alkylamines in Fruits. Five fruits, banana, apple, grape, orange, and sapodilla, were analyzed for alkylamine content. A GC-ECD chromatogram is shown in **Figure 3**. Quantities of each alkylamine in fruits under study are shown in **Table 3**. All of the values are on a dry weight basis. Total alkylamines concentrations in fruits varied from 1200 to 15000 $\mu\text{g}/\text{kg}$. All four alkylamines were detected in most fruits except orange and sapodilla. The total alkylamine content is 10 times higher in grapes compared to other fruits. Hence, grapes are the richest source for alkylamines.

Ethylamine content on a total alkylamine basis in fruits varied from 1 to 5%. Isopropylamine concentrations were between 6 and 32%. Banana had the highest isopropylamine content. Percentage of propylamine in fruits varied from 59 to 98%, with orange being highest, whereas banana had the lowest percentage. In the case of *sec*-butylamine, its concentrations per total alkylamine varied from 6 to 30% of total amines. For *sec*-butylamine, sapodilla had the highest percentage of *sec*-butylamine per total alkylamine and banana had the least.

As in vegetables, the source of alkylamines in fruits is mainly amino acids, and if amino acids are the major source, then there should be a correlation between amino acid concentrations and alkylamines in fruits. Alanine concentrations in the apple, grape, and banana are 81, 90, and 103 mg, respectively, per 100 g of dry weight (23). The variation observed in ethylamine content in fruits is much greater compared to the reported difference in alanine content in fruits. Higher concentrations of propylamine

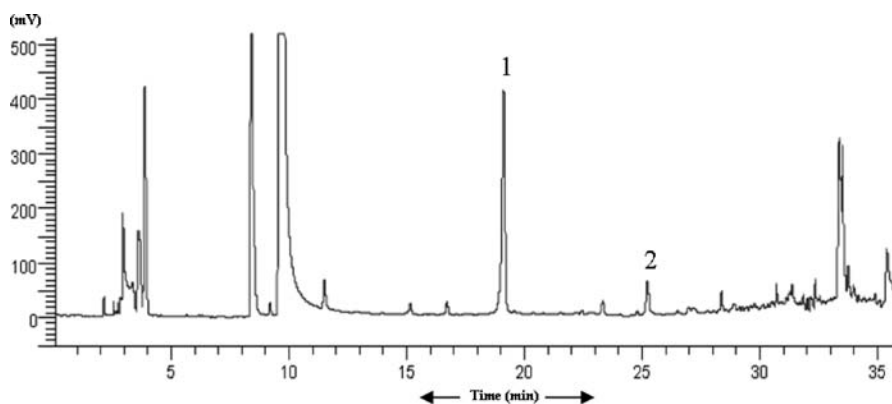


Figure 4. Representative GC-ECD chromatogram for black tea. Peaks: 1, ethylamine; 2, propylamine.

Table 4. Alkylamine Concentration in Tea

tea ^b	concentration ^a ($\mu\text{g}/\text{kg}$)				
	ethylamine	isopropylamine	propylamine	sec-butylamine	total amines
green tea	10501 \pm 129	780 \pm 35	18536 \pm 40	662 \pm 117	30479 \pm 361
black tea	41952 \pm 938	308 \pm 32	4365 \pm 33	190 \pm 1	46815 \pm 1004

^a The values are expressed on dry weight basis. Each value is an average value over four analyses. ^b Green tea samples were procured from southern India, and black tea was purchased locally.

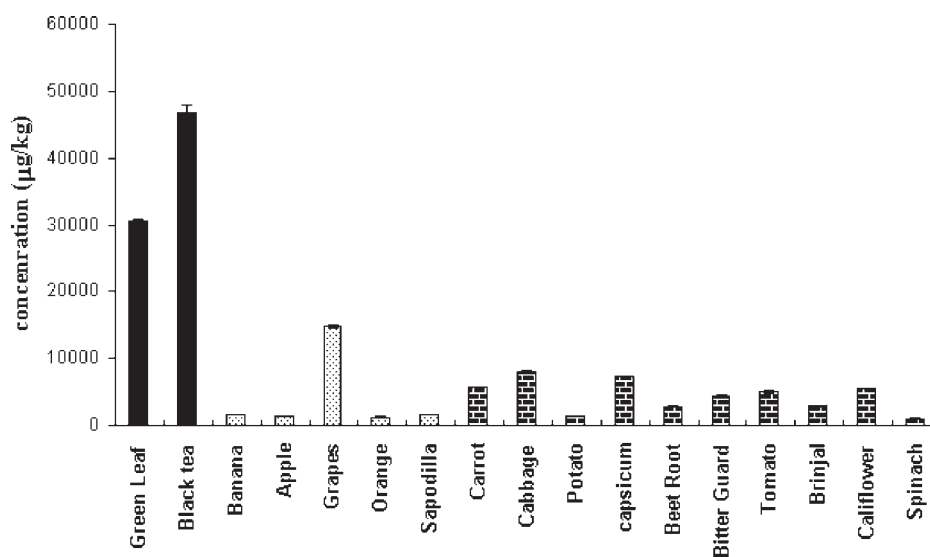


Figure 5. Comparison of total alkylamine contents in vegetables, fruits, and beverages.

can come from higher concentrations of its precursor, methionine. Methionine concentrations in apple, grape, and banana are reported to be 14, 31, and 37 mg/100 g of dry weight, respectively. This does not show any correlation to propylamine concentrations in those fruits. Similar to vegetables, the biogenesis of isopropylamine and *sec*-butylamine is not well understood, and hence their precursors are unknown. For all alkylamines in fruits, no correlation could be observed between alkylamines and their respective amino acid concentrations. This suggests that there could be alternate sources for alkylamines. Especially for propylamine, isopropylamine, and butylamine, pesticides residues could be a possible external source as they are used as adjuvants and in antifungal chemicals (25, 26).

Quantification of Alkylamines in Tea. Apart from vegetables and fruits, both black tea and green tea leaf are reported as good sources for alkylamines. Hence, alkylamine concentration is analyzed from black and green teas. The GC chromatogram

for tea is shown in **Figure 4**. In the case of fresh tea leaf, as expected, ethylamine concentrations are found to be much higher compared to other amines (**Table 4**). Also, total amine concentrations are also higher compared to any other vegetables or fruits. This could be explained as due to a higher concentration of theanine in tea, as during theanine biosynthesis ethylamine is generated. Compared to green tea leaf, black tea has 30% more total amine content. Higher concentrations of ethylamine observed in black tea than in green leaf further confirm that ethylamine is formed from theanine biosynthesis during black tea processing.

Comparison of Alkylamines in Vegetables, Fruits, and Beverages. Total alkylamines content is plotted for all of the samples used in this study (**Figure 5**). Black tea and green tea have much higher concentrations of alkylamines than any other vegetables and fruits. Hence, tea is a rich source for alkylamines. In vegetables, cabbage and capsicum are better sources for alkylamines.

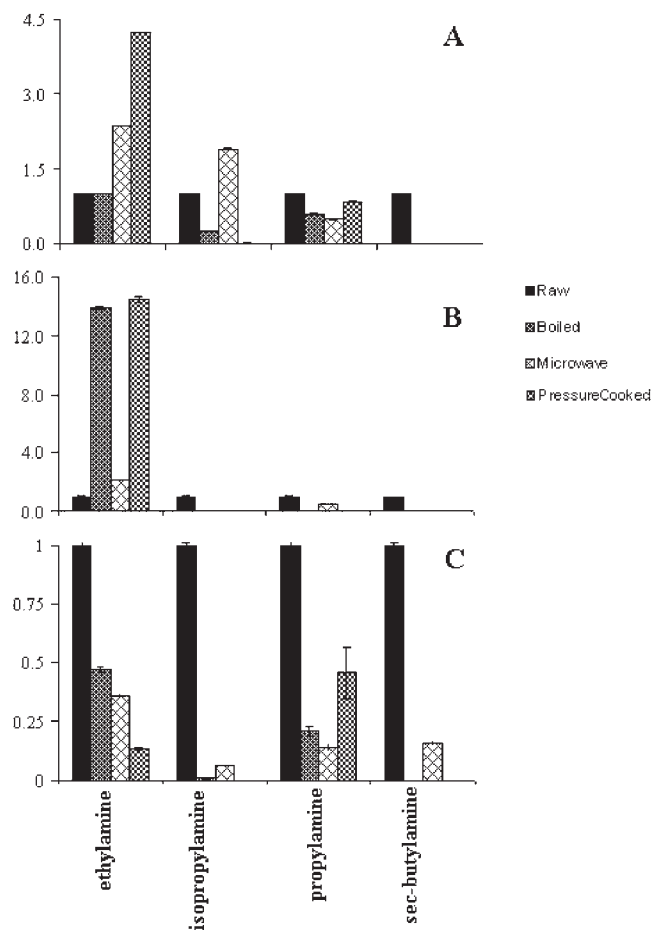


Figure 6. Effect of cooking on primary alkylamine concentrations in vegetables: (A) potato; (B) carrot; (C) cabbage.

Effect of Cooking on Alkylamine Concentrations in Vegetables.

The bioavailability of alkylamine from these foods will depend on their stability during cooking. Hence, their stability during cooking was evaluated. For this study potato, capsicum, and carrot were used. Ethylamine and isopropylamine concentrations were quantified under various cooking conditions, and data are presented in Figure 6. Cooking by open boiling, microwave, and pressure cooking was evaluated in this study. As expected, there was reduction in the concentration of all alkylamines on cooking in cabbage. In potato and carrot it was interesting to observe ethylamine concentrations increased dramatically on cooking and the type of cooking had an effect on the extent of increase. However, propylamine, isopropylamine, and *sec*-butylamine concentrations decreased on cooking. For potato loss in total alkylamines concentrations varied from 13 to 50%, with pressure cooking having minimum loss and microwave cooking maximum loss. For carrot, ~65% of total amines were lost during microwave cooking, whereas open boiling and pressure cooking showed enhancement in total alkylamines content by 50 and 60%, respectively. There was severe loss of total alkylamines during cooking of cabbage. More than 66% was lost during pressure cooking, and more than 86% was lost during open boiling and microwave cooking. This study suggests cooking led to generation as well as degradation of alkylamines.

A novel GC-ECD method has been developed and optimized for the analysis of the four amines with LOQs of 150 $\mu\text{g}/\text{kg}$ for ethylamine, 210 $\mu\text{g}/\text{kg}$ for isopropylamine, 150 $\mu\text{g}/\text{kg}$ for propylamine, and 200 $\mu\text{g}/\text{kg}$ for *sec*-butylamine in fruits and vegetables. Total alkylamines concentrations for vegetables vary from 0.92 to

7.3 mg/kg. For the fruits the range is 1.2–14.5 mg/kg. Both green tea leaf and black tea have much higher concentrations of alkylamines compared to fruits and vegetables (~40 mg/kg of dry weight). Hence, a beverage such as tea was found to be the best source for alkylamines relative to commonly used fruits or vegetables. During cooking of the vegetables, ethylamine and propylamine are generated, whereas secondary amines are lost.

LITERATURE CITED

- (1) Groh, V.; Steinle, A.; Bauer, S.; Spies, T. Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells. *Science* **1998**, *279*, 1737–1740.
- (2) Fisch, P.; Meuer, E.; Pende, D. Control of B cell lymphoma recognition via natural killer inhibitory receptors implies a role for human V γ 9V δ 2 T cells in tumor immunity. *Eur. J. Immunol.* **1997**, *27*, 3368–3379.
- (3) Wilhelm, M.; Kunzmann, V.; Eckstein, S.; Reimer, P.; Weissinger, F.; Ruediger, T.; Tony, H. P. $\gamma\delta$ T cells for immune therapy of patients with lymphoid malignancies. *Blood* **2003**, *102*, 200–206.
- (4) Thompson, K.; Roja-Navea, J.; Rogers, M. J. Alkylamines cause V γ 9V δ 2 T cell activation and proliferation by inhibiting the mevalonate pathway. *Blood* **2006**, *107*, 651–654.
- (5) Bukowski, J. F.; Morita, C. T.; Brenner, M. B. Human $\gamma\delta$ T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. *Immunity* **1999**, *11*, 57–65.
- (6) Wang, L.; Kamath, A.; Das, H.; Li, L.; Bukowski, J. F. Antibacterial effect of human V γ 2V δ 2 T cells in vivo. *J. Clin. Invest.* **2001**, *108*, 1349–1357.
- (7) Kamath, A. B.; Wang, L.; Das, H. Antigens in tea-beverage prime human V γ 2V δ 2 T cells in vitro and in vivo for memory and nonmemory antibacterial cytokine responses. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *10*, 6009–6014.
- (8) Thompson, K.; Mönkkönen, H.; Mönkkönen, J.; Rogers, M. Nitrogen-containing bisphosphonates and alkylamines activate V γ 9V δ 2 T cells through a common mechanism involving inhibition of the mevalonate pathway. *Bone* **2000**, *38*, 63–63.
- (9) Ábalos, M.; Bayona, J. M.; Ventura, F. Development of a solid-phase micro extraction GC-NPD procedure for the determination of free volatile amines in wastewater and sewage-polluted waters. *Anal. Chem.* **1999**, *71*, 3531–3537.
- (10) Avery, M. J.; Junk, G. A. Gas chromatography/mass spectrometry determination of water-soluble primary amines as their pentafluorobenzaldehyde imines. *Anal. Chem.* **1985**, *57*, 790–792.
- (11) Ibe, A.; Saito, K.; Nakazato, M. Quantitative determination of amines in wine by liquid chromatography. *J. Assoc. Off. Anal. Chem.* **1991**, *7*, 695–698.
- (12) Lehtonen, R. Isolation and HPLC determination of amines in wine. *Z. Lebensm. Unters. Forsch.* **1986**, *183*, 177–181.
- (13) Busto, O.; Valero, Y.; Guasch, J.; Borrull, F. Solid-phase extraction applied to the determination of biogenic amines in wines by HPLC. *Chromatographia* **1994**, *38*, 571–578.
- (14) Lehtonen, P. Determination of amines and amino acids in wine – a review. *Am. J. Enol. Vitic.* **1996**, *47*, 127–133.
- (15) Daudt, C. D.; Ough, C. S. A method for detecting volatile amines in grapes and wines. *Am. J. Enol. Vitic.* **1980**, *3*, 356–359.
- (16) Ngim, K.; Ebeler, S.; Lew, M. E.; Crosby, D. G.; Wong, J. W. Optimized procedures for analyzing primary alkyl amines in wines by pentafluorobenzaldehyde derivatization and GC-MS. *J. Agric. Food Chem.* **2000**, *48*, 3311–3316.
- (17) Avery, M. J.; Junk, G. A. Procedure for the determination of amines in small urine samples. *J. Chromatogr.* **1987**, *420*, 379–384.
- (18) Kusch, P.; Knupp, G.; Hergarten, M.; Kozupa, M.; Majchrzak, M. Identification of corrosion inhibiting long-chain primary alkyl amines by gas chromatography and gas chromatography–mass spectrometry. *Int. J. Mass Spectrom.* **2006**, *263*, 45–53.
- (19) Takashi, H.; Yukimasa, M.; Yukio, M. Glass capillary gas chromatography of secondary amines in foods with flame photometric detection after derivatization with benzenesulfonyl chloride. *Agric. Biol. Chem.* **1981**, *45*, 2237–2243.

- (20) Hartmann, T. Detection of *n*-butylamine in apples. *Experientia* **1967**, *23*, 680–681.
- (21) Singh, G.; Rippon, L. E.; Gilbert, W. S.; Ahmad, N. *sec*-Butylamine residues in citrus, pome fruits and bananas from post-harvest treatments. *Aust. J. Exp. Agric. Anim. Husb.* **1979**, *19*, 118–121.
- (22) Denga, W.; Ogitab, S.; Ashiharaa, H. Z. Ethylamine content and theanine biosynthesis in different organs of *Camellia sinensis* seedlings. *Z. Naturforsch.* **2009**, *64*, 387–390.
- (23) Bremer, H. J.; Anninos, A.; Schulz, B. Amino acid composition of food products used in the treatment of patients with disorders of the amino acid and protein metabolism. *Eur. J. Pediatr.* **1996**, *155*, 108–114.
- (24) Ram, K. S.; Seymour, S. C. Propylamine transferases in Chinese cabbage leaves. *Plant Physiol.* **1984**, *74*, 645–649.
- (25) Abu-Qare, A. W.; Duncan, H. J. Photodegradation of the herbicide EPTC and the safener dichlormid, alone and in combination. *Chemosphere* **2002**, *46*, 1183–1189.
- (26) Muller, T. C.; Main, C. L.; Thompson, M. A.; Steckel, L. E. Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentrations on the control of various weeds. *Weed Technol.* **2006**, *20*, 164–171.

Received for review February 6, 2010. Revised manuscript received June 23, 2010. Accepted July 12, 2010.