

Analysis of Neuroactive Amines in Fermented Beverages Using a Portable Microchip Capillary Electrophoresis System

Christine N. Jayarajah, Alison M. Skelley,[†] Angela D. Fortner, and Richard A. Mathies*

Department of Chemistry, University of California, Berkeley, California 94720

A portable microfabricated capillary electrophoresis (CE) instrument is used for the determination of neurologically active biogenic amines, especially tyramine and histamine, in fermented beverages. The target molecules are labeled on their primary amino groups with fluorescamine in a 10-min reaction, and the samples analyzed directly, producing a detailed electropherogram in only 120 s on a microfabricated glass CE device containing 21.4-cm-long separation channels. Tyramine was found mainly in red wines at <1–3.4 mg/L, while the histamine content of these samples ranged from 1.8 to 19 mg/L. The highest levels of histamine (20–40 mg/L) were found in sake. The analysis of samples drawn from grape crush through malolactic fermentation in four varieties of zinfandel red wines revealed that histamine and tyramine are produced during yeast and malolactic fermentation, respectively. Following malolactic fermentation, the histamine content in these samples ranged from 3.3 to 30 mg/L, and the tyramine content ranged from 1.0 to 3.0 mg/L. This highly sensitive and rapid lab-on-a-chip analysis method establishes the feasibility of monitoring neurologically active amine content and potentially other chemically and allergenically important molecules in our food supply.

Tyramine and histamine, produced by the decarboxylation of tyrosine and histidine, are among the most harmful of the biogenic amines found in fermented beverages (Figure 1). These amines, produced as degradation products resulting from microbial activity, are found widely in fermented foods and beverages, meat, fish, and dairy products.^{1–7} Biogenic amines such as histamine, tyramine, and phenylethylamine are known to induce nausea,

headaches, and respiratory disorders in sensitive individuals, particularly when accompanied by alcohol and acetaldehyde.⁸ These amines are normally metabolized by amine oxidases to keep their steady-state concentrations low. However, for individuals with reduced monoamine oxidase (MAO) activity or expression and for individuals taking MAO inhibitors, ingestion of foods containing large amounts of tyramine can lead to transient hypertension, hypertensive crisis, and panic attacks.⁹ This response occurs because tyramine that is not deaminated is converted to octopamine when taken up in sympathetic nerve terminals where it displaces norepinephrine (NE) from storage vesicles. A portion of this NE diffuses out of the nerve to react with receptors causing hypertension and other sympathomimetic effects.¹⁰ Analogously, histamine in wine can induce headaches in patients suffering from reduced or lack of diamine oxidase activity. The treatment of choice for patients with histamine or tyramine intolerance and chronic headache is a histamine- and tyramine-free diet.¹¹ For these reasons, the development of a fast, accurate, point-of-consumption (POC) method to measure biogenic amine concentrations in foods would be valuable.

Previous methods to determine the biogenic amine content in foods involve conventional chromatographic separations with extensive and complex derivatization protocols and sample processing. High-performance liquid chromatography (HPLC) methods typically include pre- or postcolumn derivatization and fluorometric detection of the corresponding derivatives.¹² The commonly used derivatization reagent is *o*-phthalaldehyde in the presence of 2-mercaptoethanol.^{13,14} A recent method for the simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion in wine and beer samples as aminoenone derivatives involves reaction with the derivatization reagent diethyl ethoxymethylenemalonate in methanolic alkaline medium.¹⁵ Millan et al. have developed a more rapid liquid chromatographic-

* To whom correspondence should be addressed. Phone: (510) 642-4192. Fax: (510) 642-3599. E-mail: rich@zinc.cchem.berkeley.edu.

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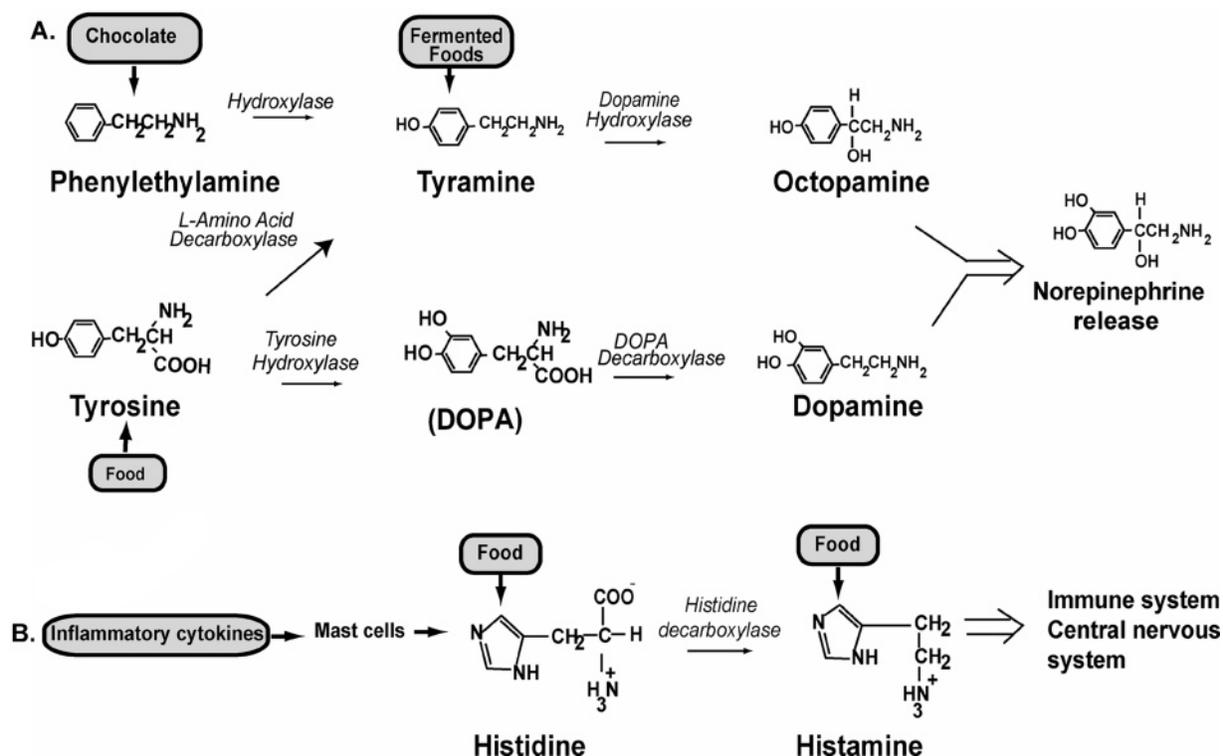


Figure 1. Metabolic pathways that mediate biogenic amine responses. (A) Tyramine is generated from the decarboxylation of tyrosine and from excess plasma levels of phenylalanine and phenyl ethylamine. Monoamine oxidases (MAO) catalyze the oxidative deamination of biogenic amines, reducing the circulating amounts of pressor amines such as tyramine, norepinephrine, epinephrine, and dopamine. Tyramine that is not deaminated can produce octopamine, which displaces norepinephrine from storage vesicles causing hypertension and other sympathomimetic physiological effects. (B) Histamine, the decarboxylation product of histidine, enters the immune and central nervous systems directly from food intake or when released by mast cells in response to allergic reactions, causing edema, itching, hypotension, and bronchoconstriction.

electrospray ionization ion trap mass spectrometry method for the routine analysis of eight of the most oenologically important biogenic amines.¹⁶ Separations of wine samples were accomplished within 20 min, and concentrations for tyramine ranged from 0.03 to 3.20 mg/L while those of histamine ranged from 0.40 to 8.22 mg/L. Such HPLC and mass spectrometry analysis methods are slow, cumbersome, and require large, expensive instrumentation that is not feasible for POC analysis.

Capillary electrophoresis (CE) is an attractive method for biogenic amine analysis because it is fast and can be reduced to a compact micro total analysis system.^{17–23} An electrophoretic method for the quantification of biogenic amines based on in-capillary derivatization with 1,2-naphthoquinone-4-sulfonate was proposed by Garcia-Villar et al.²⁴ In order to increase the

sensitivity, a selective preconcentration of the analytes by field-amplified stacking is achieved in this method. A direct automatic determination of biogenic amines in wine by flow injection-capillary electrophoresis-mass spectrometry has been proposed by Santos et al.²⁵ The authors ascribed the higher concentrations of biogenic amines found in red wine (histamine ~7 mg/L, tyramine ~2 mg/L) compared to white wine (histamine ~2 mg/L, tyramine ~1 mg/L), to the greater significance of malolactic fermentation in red wine. Male and Luong described the derivatization, stabilization, and detection of biogenic amines by cyclodextrin-modified capillary electrophoresis laser-induced fluorescence detection.²⁶ These methods establish the utility of conventional CE for biogenic amine analysis.

The Mars Organic Analyzer (MOA) is a portable chemical analysis device that is a part of the Urey instrument selected for the 2013 ESA ExoMars mission (www.astrobiology.berkeley.edu). The MOA detects primary amine-containing biomarkers such as amino acids, amines, amino sugars, and nucleobases by performing fluorescamine labeling followed by microchip capillary electrophoresis.^{27,28} This portable instrument has been tested in sites as remote as the Atacama Desert, Chile, providing rapid, robust

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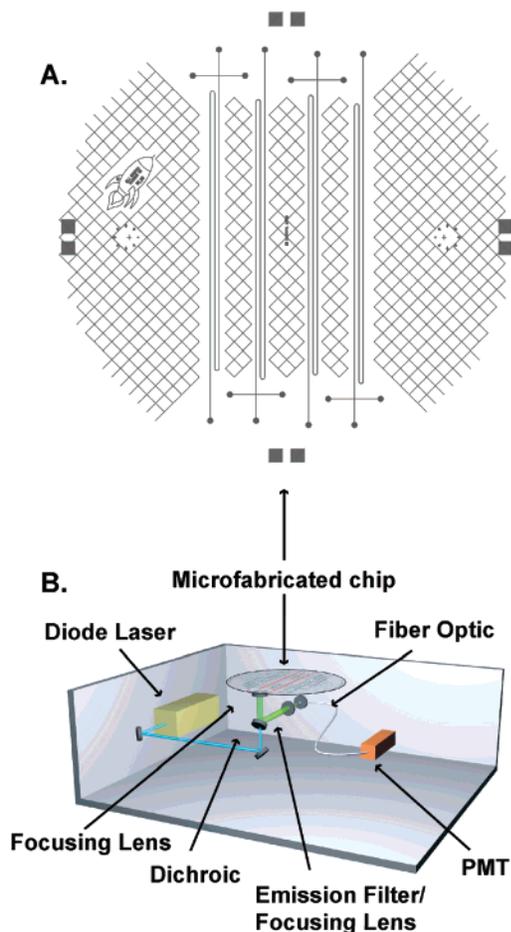


Figure 2. Schematic diagram of the microchip capillary electrophoresis chip and instrument adapted from ref 27. (A) The microfabricated chip consists of four folded separation channels 21.4 cm in length, each with a 0.6-cm-long, 70- μm -wide injection channel placed 0.6 cm from the anode reservoir. The crosshatch marks are included in the chip design for improved bonding. (B) This microchip is placed against the planar face of a composite objective through which the confocal excitation and fluorescence detection is performed. Electrophoresis power supplies, a thermoelectric cooler for temperature control, and pneumatic valve actuators are also contained within the instrument. The 11-kg instrument measures 10 \times 12 \times 4 in.

analysis and high detection sensitivity.²⁹ Given this capability, we reasoned that the MOA might also be useful for the terrestrial analysis of bioamines of relevance to our food and beverage supply.

To test the utility of the MOA for this purpose, we examine here the bioamines in a variety of fermented beverages, focusing on the concentrations of the most neurologically active components, tyramine and histamine. Liquid beverage samples are labeled with fluorescamine in a 10-min reaction and then separated in minutes on a glass microfabricated CE device followed by laser-induced fluorescence detection. The chromatograms reveal a rich fingerprint of the bioamines and amino acids in these beverages and provide direct quantitative analysis of the tyramine and histamine concentrations. A wide variety of beverages including wine, beer, and sake are analyzed for their tyramine and histamine

concentrations. Furthermore, we examine four varieties of zinfandel wine from grape crush through malolactic (ML) fermentation to determine the stage at which histamine and tyramine appear in the wine-making process. These results demonstrate the utility of the MOA for analyzing fermented beverages for their active bioamine components and establish the validity of our portable microdevices for point-of-consumption analysis.

MATERIALS AND METHODS

Microfabrication. Previously described protocols were followed to fabricate the microchip devices as summarized here.^{27,28,30} The microfabricated chip design used in this work is shown in part A of Figure 2. Borofloat wafers (10 cm in diameter) protected with a sacrificial layer of amorphous Si, were coated with photoresist, patterned through a chrome mask using a contact aligner, and developed to define folded separation channels 21.4 cm in length. The exposed Si was removed with SF₆, and 70- μm -wide channels were etched 21 μm deep with 49% HF for 3 min. These channels have a 0.6-cm-long, 70- μm -wide injection channel placed 0.6 cm from the anode reservoir. Diamond-tipped drill bits (1.8-mm diameter) were used to drill reservoirs on the etched chip. In order to increase the reservoir volume, 3-mm-diameter holes were cut into a 0.125-in.-thick PDMS sheet that was applied over the drilled holes.

Samples. Red zinfandel wine samples were obtained from Rosenblum Cellars, CA, and other bottled wine samples were purchased from local stores. Sake samples were obtained from Takara Sake Inc., CA, and beer samples were obtained from Pyramid Ale House, CA, and a local store. Samples from the wine-making process from grape crush to press were provided by Rosenblum Cellars, CA. These samples were taken at crush before and after the addition of SO₂, at the end of cold soak, 2, 4, 6, 8, and 10 days after inoculation with yeast, at press, and after bottling.

Amine and Amino Acid Standard. Tyramine and histamine (Sigma-Aldrich, St. Louis, MO) 0.2 mM stock solutions were prepared in an 8 mM pH 10.4 Na₂CO₃/NaHCO₃ buffer. Amino acid standards were prepared from individual 1 mM stock solutions in water (Sigma-Aldrich). Equimolar amounts of valine (Val), serine (Ser), and glutamic acid (Glu) were combined, dried down, and dissolved in 50 μL of 8 mM pH 10.4 Na₂CO₃/NaHCO₃ buffer. A bioamine standard was made by combining the solution containing the amino acids with the tyramine and histamine stock solutions at a volume ratio of 5:3:2 to yield 0.06 mM tyramine, 0.04 mM histamine, and 0.1 mM each of Val, Ser, and Glu.

Labeling. To label the samples for fluorescence detection, 10 μL of buffer and 30 μL of 1 mM fluorescamine dissolved in DMSO were added to 10 μL of sample and allowed to react at room temperature for \sim 15 min. This labeled solution was then diluted 10-fold with the running buffer (8 mM pH 10.4 Na₂CO₃/NaHCO₃ buffer) and loaded in the sample reservoir for CE separation. The spiked samples were labeled using the same protocol, but the buffer in the preceding process was replaced with 10 μL of the amine and amino acid standard solution. The sake samples were further diluted 5-fold (net dilution from original sample 250-fold) prior to loading.

Instrumentation. All samples were analyzed on a portable microchip CE instrument called the Mars Organic Analyzer that

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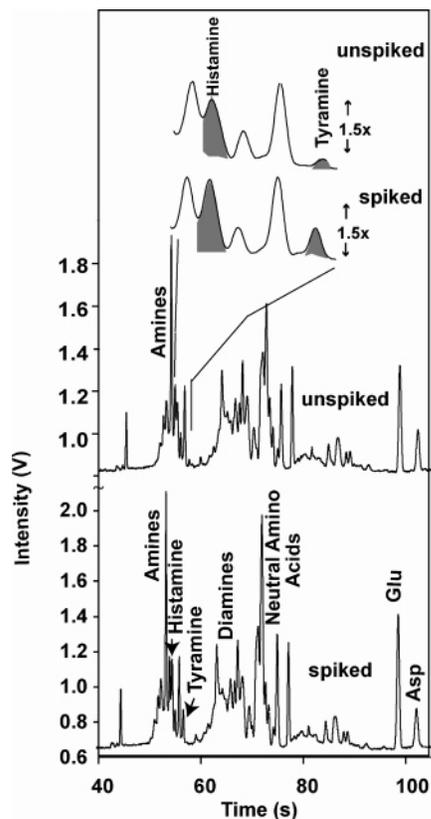


Figure 3. Analysis of a zinfandel red wine and a sample spiked with a standard containing $1.2 \mu\text{M}$ tyramine, $0.8 \mu\text{M}$ histamine, $2 \mu\text{M}$ valine, $2 \mu\text{M}$ serine, and $2 \mu\text{M}$ glutamic acid at a 50-fold dilution. The inset shows an expanded region of both the spiked and unspiked samples in the region where the bioactive amines appear. These traces were obtained at pH 10.4.

includes the laser excitation, optical detection system, and electrophoresis power supplies.²⁷ A schematic diagram of the confocal excitation and detection optics is shown in Figure 2, part B. Briefly, a 400-nm diode laser (Crystalaser, Reno, NV) excites the sample through a 430-nm dichroic beam splitter and an objective lens. The bottom of the microchip is placed against the planar face of the composite objective, which has a numerical aperture of ~ 0.9 . The excitation light is focused 0.7 mm from the interface to form a 10–20 μm spot in the channel. The fluorescence gathered by this lens is focused into a 100- μm -diameter fiber-optic-coupled photomultiplier for confocal detection. Three miniature high-voltage power supplies provide the -15 - and -3 -kV electrophoresis potentials, and one HV solid-state switch enables floating of the anode. A manifold is used to clamp the microdevice to the instrument and to provide electrical and pneumatic connections. The MOA weighs ~ 11 kg, the dimensions are 10 in. length \times 12 in. width \times 4 in. height, and the power consumption is ~ 10 W.

Procedures for Separation and Detection. Samples were injected by applying -2.5 kV at the waste reservoir and grounding the sample and cathode reservoirs, while floating the anode during the 10-s injection. The sample and waste reservoirs were then held at -2.65 kV, the anode at 0 V, and the cathode at -15 kV to perform CE separations. The channel was rinsed with 0.1 M NaOH followed by doubly distilled water before and between runs.

Data Analysis. The spiked and unspiked traces were aligned in time using Adobe Illustrator to identify the tyramine and

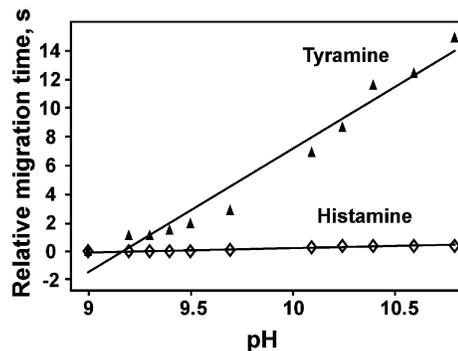


Figure 4. Relative migration time of tyramine and histamine detected 7 cm from the injection point on the 21.4-cm-long channel as a function of buffer pH. The mobility differences in seconds are for tyramine relative to histamine to show that tyramine shifts away from histamine as the pH is increased. The mobility of histamine is shown relative to the isopropylamine, which does not vary as a function of pH. A buffer pH of 10.4 was chosen for subsequent analysis as the tyramine peak is adequately shifted away from the histamine peak and is easily resolved because it migrates to a clean region of the electropherogram between the amines and the diamines.

histamine peaks. Following this, peak integration was carried out using Grams32 (Thermo Galactic, Salem, NH) in order to obtain peak areas to calculate the tyramine and histamine concentrations.

RESULTS

All beverage samples were analyzed for tyramine and histamine content by labeling with fluorescamine followed by microchip capillary electrophoresis. Mobilities and concentrations were determined by spiking the samples with a standard containing a known concentration of tyramine and histamine, along with valine, serine, and glutamic acid as markers. Figure 3 presents a typical separation of a wine sample along with expanded traces to better display the tyramine and histamine regions. This chromatogram provides a distinctly rich fingerprint of the many amine-containing compounds present in wine. The nitrogen component of wine is important to the fermentation process and exists in musts and wine in different forms: amino acids, polypeptides, proteins, amines, ammonia, nitrates, and vitamins.

A pH of 10.4 was chosen for the analysis to keep the tyramine and histamine peaks distinct and the tyramine peak in a clear region of the chromatogram (Figure 3). The tyramine peak is shifted from the congested amine region to longer migration times as the pH is changed from 8.5 to 10. The mobilities of tyramine and histamine were determined in the pH range of 9–11, using isopropylamine as a stationary marker (Figure 4). The tyramine migration time increases with rising pH and shifts away from histamine, which remains relatively stationary. The deprotonation of the phenolic hydroxy group ($\text{p}K_a = 10.4$) on tyramine is the likely cause of this shift.

Various beer, sake, and wine samples representing different fermentation procedures were analyzed for tyramine and histamine content to explore the utility of our method and apparatus as well as the range of bioamine concentrations in typical beverages. Representative chromatograms for red wine (zinfandel), white wine (chardonnay), beer (Guinness Draught), and Sake (Ginjo) are presented in Figure 5. Since the fluorescence signals for the sake samples were significantly higher, they were diluted

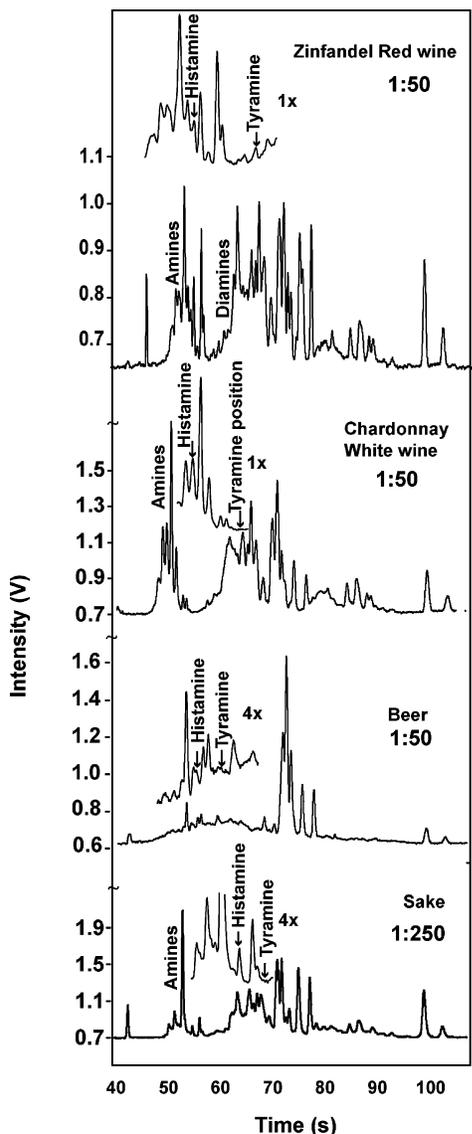


Figure 5. Comparison of the bioamine electropherograms for different fermented beverages including red wine (Annette's Reserve zinfandel), white wine (chardonnay), beer (Guinness Draught), and sake (Ginjo). The wine and beer samples were diluted 50-fold and sake was diluted 250-fold for the analyses. The insets have been expanded by the indicated factor.

5-fold more than the beer and wine samples. For samples with undetectable tyramine levels such as chardonnay and sake, the tyramine positions were determined by spiking and marked. The red wine has a very complex spectrum of amines, diamines, and late-eluting larger and more highly charged amines. The white wine has similar components, but the electropherogram is not as complex. The beer samples exhibit a dramatically simpler spectrum with intense peaks in the neutral amino acid region. On the other hand, the sake samples are rich in complex amines that are more concentrated than in the red wine after accounting for the dilution factor. Tyramine was found in red wine in freshly opened samples from less than 1 to 3.4 mg/L, while the highest level of histamine was found in sake at 21–44 mg/L. Variations in amine and amino acid content were found within different varieties of red wines as well, as shown in Figure 6. The electropherogram for pinot noir (Tyr <1 mg/L) was much less

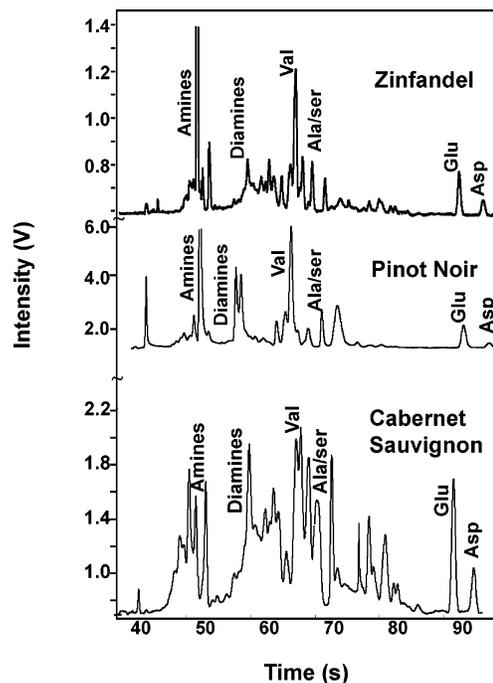


Figure 6. Electropherograms for three different red wine samples (unspiked), all at 50-fold dilution. The traces were aligned in time using the monoamines, valine and glutamic acid peaks.

Table 1. Tyramine and Histamine Content in Fermented Beverages^a

beverage	content (mg/L)	
	tyramine	histamine
merlot	3.4 ± 0.7	<5
cabernet sauvignon	2.8 ± 0.6	4 ± 2
Annette's Reserve	2 ± 1	13 ± 2
black muscat	1.2 ± 0.3	1.8 ± 0.2
chardonnay white wine	nd	3.7 ± 0.1
Sho Chiku Bai (SCB) sake	nd	44 ± 6
SCB organic sake	nd	23 ± 10
Koshu Plum sake	nd	35 ± 3
SCB nigori filtered sake	nd	21 ± 3
SCB Ginjo sake	nd	25 ± 10
Keenan Syrah at press	nd	8 ± 8
Petit Syrah at press	nd	11 ± 5
Keenan Syrah post ML	1.4 ± 0.8	3.5 ± 0.9
Petit Syrah post ML	0.9 ± 0.4	3 ± 1
Harris Kratka post ML ^b	3.0 ± 0.4	30 ± 9
Maggies post ML	1.0 ± 0.6	3 ± 3
Harris Kratka bottled sample	2.6 ± 0.9	19 ± 13
Maggies bottled sample	0.3 ± 0.2	11 ± 4

^a The average values represent experiments repeated with three labeled solutions injected twice, corresponding to six runs. nd, not detected ≤0.1 mg/L; ML, malolactic fermentation. ^b The post ML sample was reanalyzed after 6 months during which time the samples were stored frozen at -20 °C, revealing higher levels of histamine and tyramine, as well as cadaverine, putrescine, and other overlapping amines.

complex compared to zinfandel and especially cabernet sauvignon. Furthermore, the ratio of intensities of the amine peaks to neutral and acidic amino acid peaks differs in the three varieties of wine.

The tyramine and histamine content of these different wine, beer, and sake samples is summarized in Table 1. Both tyramine and histamine were found in all red wine samples, while detectable levels of only histamine were found in white wine and sake samples. Within newly opened red wine samples, the lowest level

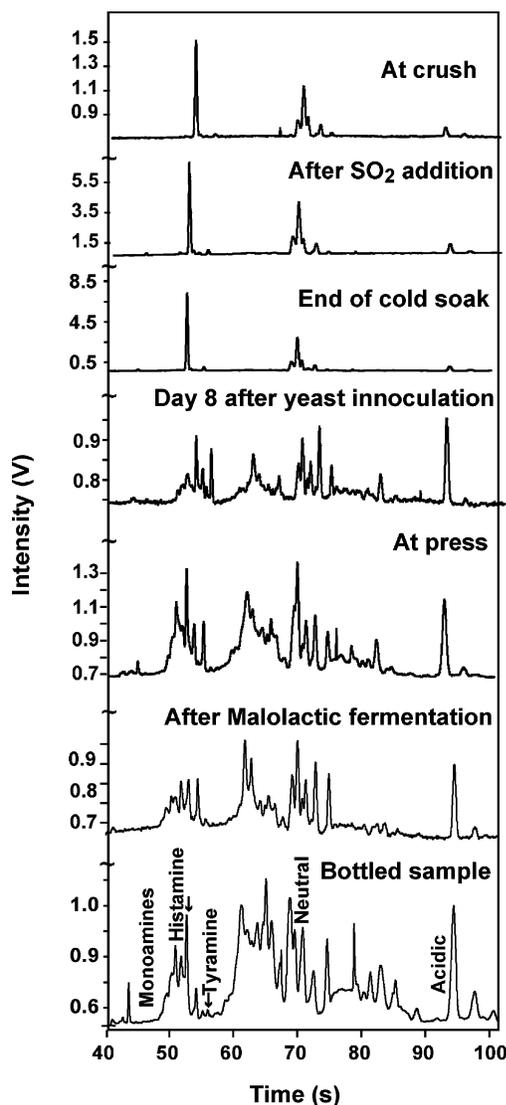


Figure 7. Analysis of red zinfandel wine from grape crush through malolactic fermentation and 3 months after it was bottled. All samples were diluted 50-fold, and aligned in time using the monoamines, valine and glutamic acid peaks.

of tyramine was found in dessert wine at 1.2 ± 0.3 mg/L, and the highest level was found in merlot at 3.4 ± 0.7 mg/L (Table 1). The histamine content in these red wine samples ranged from 1.8 ± 0.2 mg/L in a dessert wine to 19 ± 13 mg/L in a zinfandel.

In order to determine the stages at which the biogenic amines form during wine making, samples were analyzed from grape crush through malolactic fermentation to bottling for four different wine grape varieties. At crush, SO_2 is added as an antimicrobial and an antioxidant agent, and ammonium salts are added to increase the nutrient (i.e., nitrogen) supply.³¹ The reduction of sugar concentration during yeast fermentation is accompanied by an increase in yeast population. During ML fermentation, malolactic bacteria carry on the transformation of malic acid to lactic acid. As shown in Figure 7, significantly fewer amines and amino acids were found in all samples obtained from grape crush, with no detectable levels of histamine or tyramine. The amine and

amino acid content did not change noticeably in samples from grape crush through the end of cold soak. The large peak to the left of the chromatogram arises from the ammonium salts that are added. A significant decrease in the ammonia peak and the appearance of amines and amino acids were observed during yeast fermentation. Histamine appeared in all samples during yeast fermentation, and continued to grow during malolactic fermentation (Table 1). Since the emerging histamine peaks in the samples drawn at press significantly overlapped with an adjoining peak to the left, the standard deviations for the corresponding histamine peaks were relatively large. The histamine content on the tenth day of yeast fermentation acquired prior to the sample at press for Keenan Syrah was 1.4 ± 0.3 mg/L. For the other three varieties, the growth of histamine at this stage was significantly overlapping with adjacent peaks. Detectable levels of tyramine were not found in any samples prior to the malolactic fermentation. The post ML Harris Krata zinfandel sample contained 3.0 ± 0.4 mg/L tyramine and 30 ± 9 mg/L histamine. This variety of zinfandel contained higher levels of tyramine and histamine, compared to the other three varieties that were analyzed (Table 1). Following completion of fermentation, the wine is filtered with a sterile $0.45\text{-}\mu\text{m}$ filter to remove any remaining yeast or bacteria and bottled. The tyramine and histamine content did not change notably 3 months after bottling, although there was growth of other amines and amino acids that contributed to a much more complex electropherogram. The Maggie's zinfandel sample that was analyzed a month after it had been bottled did not show any further increase in tyramine content beyond the malolactic fermentation stage, although a slight increase in histamine content was noted. Longer time-course aging experiments were not within the scope of this work. We do not believe the grape variety to be the primary factor that influences the tyramine and histamine content, as the different protocols employed in the primary and secondary fermentation may all contribute to differences in these amine concentrations.

DISCUSSION

The development of point-of-analysis and point-of-care medical and genetic analysis systems using microchips is presently expanding rapidly.^{22,23,32} Since diet and nutrition are also key elements that determine one's state of health, a more comprehensive knowledge of the content of our food supply is indispensable. This constitutes a critical opportunity for lab-on-a-chip microdevices: the samples are accessible, no regulatory approvals are required, and a POC analysis device would be highly valuable if it operated in the 5–30-min time frame. More specifically, biogenic amines such as tyramine often appear in conjunction with food intoxication.⁷ Food-borne chemical intoxication in humans, such as scombroid or histamine fish poisoning, causes skin rashes and disturbances of the gastrointestinal tract and is treated with antihistamines. More recently, pet food suspected to be contaminated with melamine, which can cause kidney failure in dogs and cats, was recalled.^{33,34} Establishing the feasibility of point-of-consumption as well as point-of-manufacture chemical analysis of foods is clearly of high importance.

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(34) <http://www.fda.gov/oc/opacom/hottopics/petfood.html>.

Microbial fermentation or spoilage of protein through processing, ripening, and storage of fermented foodstuffs including cheese, fish, meat products, wine, beer, and sauerkraut typically generates biogenic amines. Histamine and tyramine, the decarboxylation products of histidine and tyrosine, are the major biogenic amines in wine. Putrescine and cadaverine are two diamine homologues formed by the decarboxylation of the amino acids arginine and lysine, respectively, which are also of significance in wine.^{2,35} An efficient method to measure biogenic amine concentrations in foods is valuable because of the potentially adverse health effects discussed below.

Biogenic amines such as histamine and tyramine are normally metabolized by monoamine oxidase (MAO). MAOs catalyze the oxidative deamination of biogenic amines and lower circulating amounts of pressor amines such as tyramine, norepinephrine, epinephrine, and dopamine (Figure 1). While multiple forms of human brain mitochondrial MAO have been reported, many mammalian tissues contain two major forms of the enzyme that differ in their substrate specificities and sensitivities to inhibitors.^{36–38} MAO A and B are the major intracellular enzymes in the central nervous system and peripheral tissues that catalyze the oxidative deamination of neuroactive and vasoactive amines.³⁹

Tyramine has been frequently considered a substrate for both forms of MAO, albeit with different kinetic parameters.³⁸ The role of MAOs in the pathophysiology of depression and in neurodegenerative disorders such as Parkinson's disease has led to their being targeted for pharmacologic drugs. The hypotensive effect that results from the administration of inhibitors of MAO may be explained by the false transmitter hypothesis.⁴⁰ In this case, the tyramine that is synthesized in the gastrointestinal tract as a result of the action of bacterial tyrosine decarboxylase may be absorbed systemically. It is transported into the adrenergic nerve terminal, β -hydroxylated to octopamine, and stored in the vesicles with a gradual displacement of norepinephrine. Consequently, stimulation results in the release of a relatively small amount of norepinephrine and a fraction of octopamine, which has relatively little ability to activate adrenergic receptors, causing a functional impairment of sympathetic nerve transmission. Despite this functional impairment, patients receiving MAO inhibitors may experience severe hypertensive crisis if they ingest fermented foods containing significant quantities of tyramine. The ingested tyramine is adsorbed rapidly and reaches the systemic circulation in high concentration, resulting in a massive release of norepinephrine, which can trigger hypertension.⁴⁰ As new MAO inhibitors were developed for treating depression, tyramine-rich foods were observed to cause a serious hypertensive crisis leading to

cerebrovascular accidents, hyperpyrexia, and potentially death. Consequently, it is important for individuals who suffer from a dietary tyramine intolerance syndrome or those on MAO inhibitors to pay attention to their dietary intake of tyramine.⁹ A clinically significant level, as it relates to the severity of blood pressure, is considered to be 6 mg in one or two servings that can cause a mild adverse condition.¹ For histamine, 5 mg/L is noted to induce headaches when 500 mL of wine is consumed.⁴¹ However, the definition of a clinically significant level will depend on the population sampled and their genotypic variations as well as medications, such as MAO inhibitors, consumed by individuals.¹¹

In this work, we have demonstrated the sensitive and rapid analyses of tyramine and histamine in fermented beverages, as well as monitoring the onset of these toxic amines in the wine production process. Following a simple and direct labeling protocol, we are able to complete chromatographic separations of amines and amino acids in fermented beverages rapidly, in only 3 min. A comparison of different fermentation products, wine, beer, and sake, reveals the corresponding levels of tyramine and histamine. We show that tyramine is mainly present in red wine and is produced during malolactic fermentation. While histamine is produced during yeast fermentation, it continues to grow in secondary fermentation and storage. It will be interesting to identify factors in the wine fermentation process that influence the formation of tyramine and histamine; a more thorough study on the effect of aging is also called for.

Portable devices such as the MOA offer the advantages of rapid and sensitive analyses, which can be of value to the manufacturer and to the consumer. Using the MOA, the manufacturer can determine the concentrations of bioactive and other key amines during the fermentation process to aid in improving and regulating protocols. This work also needs to be extended toward case studies of tyramine and histamine response to quantify the impact of biogenic amines and to correlate this response with different genotypes. A consumer who has knowledge of individual sensitivity to these chemical substances can thus take informed preventive measures. In addition, the application of these devices in the food industry can be extended to the monitoring of food and food ingredients to detect the appearance of contaminants or adulterants. This work thus points to the development of portable, and eventually handheld point-of-consumption devices to test food, water, and beverages prior to ingestion. We have shown here that the MOA chip and optical system can be miniaturized effectively; the principal remaining challenges are to miniaturize the electronics contained within the instrument and to develop convenient software for automated peak identification and quantification.

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