High-Performance Liquid Chromatographic Method for Determination of Biogenic Amines in Feedstuffs, Complete Feeds, and Animal Tissues

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Numerous methods to analyze biogenic amines in biological materials have been described. A versatile and rapid methodology to analyze these compounds in feedstuffs, complete feeds, and animal tissues, however, has not been reported. The current method was developed to address this need. Biogenic amines in feedstuffs, complete animal feeds, and animal tissues were extracted with 10% trichloroacetic acid, reacted with *O*-phthaladehyde using high-performance liquid chromatographic employing a cation exchange column. Detection limits were 50 pmol/mL for tyramine, histamine, putrescine, and spermine; 40 pmol/mL for cadaverine; and 25 pmol/mL for spermidine. Extraction efficiency of biogenic amines in feedstuffs, duodenum, liver, ileum + jejunum, and whole shrimp and shrimp hepatopancreas ranged between 99–105, 93–135, 80–85, 65–102, 88–98, and 88–97%, respectively. It can be concluded that the current method can be applied to individual feedstuffs, complete feeds, and animal tissues for the rapid and accurate determination of concentration of biogenic amines.

INTRODUCTION

Biogenic amines are naturally occurring compounds found in low levels in animal tissues and plants. High concentrations of these substances in feedstuffs or foods can arise from the decarboxylation of free amino acids through the action of bacteria and can be used as spoilage indicators (Brink et al., 1990; Sander et al., 1996; Veciana-Nogues et al., 1997).

Biogenic amines can be aliphatic (putrescine, cadaverine, spermidine, spermine), aromatic (tyramine, phenyletylamine), or heterocyclic (histamine) in nature (Brink et al., 1990; Kobayashi et al., 1992). Cellular concentrations of the mammalian polyamines (putrescine, spermine, and spermidine) have been correlated with anabolic processes such as DNA, RNA, and protein synthesis; cellular growth; membrane stabilization; and promotion of tissue amino acid uptake (Seiler, 1992).

The toxicity of diets containing soybean protein was reduced when supplemental putrescine was fed to calves and chicks (Grant et al., 1989; Mogridge et al., 1996). A growth-promoting effect has been reported when shrimp were fed diets supplemented with spermine or histamine + cadaverine (Tapia-Salazar et al., 1999). A chemoattractant effect of biogenic amines has also been reported in crayfish and shrimp fed diets supplemented with low levels of cadaverine and histamine + cadav-

erine (Mendoza et al., 1997; Tapia-Salazar et al.). The presence of biogenic amines in feedstuffs, however, has also proven toxic. The feeding of diets rich in histamine resulted in poor growth performance, decreased feed intake, and gizzard erosion in chickens (Harry et al., 1975; Osuna, 1985). Cowey and Cho (1992) reported reduced feed consumption in rainbow trout fed diets supplemented with putrescine while Fairgrieve et al. (1994) observed intestinal damage in rainbow trout fed diets rich in histamine.

Numerous methods have been published for the analysis of biogenic amines in biological materials. These include high-performance liquid chromatography (HPLC; Linares et al., 1998), capillary electrophoresis (Arce et al., 1998), ion chromatography integrated pulse amperometric detection (Draisci et al., 1998), gas–liquid chromatography (GLC; Kim et al., 1997), micellar electrokinetic chromatography (Rodriguez et al., 1996) and thin-layer chromatography (TLC; Shalaby, 1994). A versatile and rapid methodology to analyze biogenic amines in whole animal feeds, individual feedstuffs, and animal tissues, however, has been not described. The current method was developed to address this need.

EXPERIMENTAL PROCEDURES

Reagents. *O*-Phthaladehyde (OPA; 3% potassium hydroxide, 3% boric acid, 94% water, pH = 10.40), thiofluor, and the eluents K130 (0.7% potassium chloride, 4% 2-propanol, 0.5% potassium hydroxide, 95% water, pH = 13.00), K563 (5% potassium chloride, 4% 2-propanol, 0.9% potassium phosphate, dibasic, 0.3% acetic acid, 90% water, pH = 5.63), and K600 (11% 2-propanol, 0.9% potassium phosphate, dibasic, 0.3% acetic acid, 88% water, pH = 6.00) were obtained from Pickering Laboratories (Vancouver, BC). Purified reference

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Table 1. Extraction Efficiency (%)

amine	feedstuffs	duodenum	liver	ileum + jejunum	whole shrimp	shrimp hepatopancreas
tyramine	99 ± 4[a]	nd[b]	nd	nd	nd	nd
histamine	92 ± 3	120 ± 16	80 ± 8	83 ± 8	91 ± 24	88 ± 3
putrescine	101 ± 5	93 ± 14	85 ± 2	65 ± 4	98 ± 34	94 ± 1
cadaverine	105 ± 4	135 ± 17	85 ± 1	102 ± 8	88 ± 25	97 ± 1
spermidine	100 ± 2	111 ± 13	82 ± 1	66 ± 4	95 ± 17	94 ± 1
spermine	98 ± 4	128 ± 6	81 ± 1	84 ± 1	95 ± 3	93 ± 4

[a] Below detection limit. [b] Values are means ± SD.

standards for tyramine (Tyr, free base), histamine (His, dihydrochorlide), putrescine (Put, dihydrochloride), cadaverine (Cad, dihydrochloride), spermidine (Spd, trihydrochloride), and spermine (Spm, trihydrochloride) were purchased from Sigma Chemical Co. (Oakville, ON). Brij 35 was obtained from Fisher Scientific Ltd., Whitby, ON.

HPLC Apparatus. The unit employed was a Varian 9010 solvent delivery system with gradient capability equipped with a Varian 9100 autosampler with a 100 μl syringe and a 50 μl sample loop (Varian Canada Inc., Mississauga). An ALKION (4 × 150 mm) cation exchange column complete with an ALKION guard column was utillized (Pickering Laboratories, Vancouver, BC) and fitted with a heated (40 °C) PCX 5200 postcolumn reaction module for derivatization with OPA. Peaks were detected by a Varian 9070 fluorescence detector (excitation wavelength 330 nm; emission wavelength 466 nm). Quantification was obtained using a Varian Star Chromatography Workstation (V 4.51).

Sample Preparation. Feedstuffs and complete diets were ground to a fine powder using a Micro-Mill grinder (Fisher Scientific, model 08-335). Turkey tissues (liver, muscle, intestine) were stored at −80 °C prior to homogenization. Whole shrimp tissue and shrimp hepatopancreas samples were frozen in liquid nitrogen, lyophilized, and then ground using a micro Wiley mill grinder and a hand spatula.

Extraction Procedure. Samples of feedstuffs or complete diets (2 g) were combined with 50 mL of 10% trichloroacetic acid (TCA) and were blended (Sorval Omni mixer model 17150) for 5 min. Frozen tissue (2 g) and 10 mL of 10% TCA were blended using a Sorval Omni mixer model 5000 for 2 min. Whole shrimp (75 mg) were homogenized (Sorval Omni mixer model 5000) for 2 min after being combined with 2 mL of 10% TCA. Shrimp hepatopancreas samples (10 mg) were combined with 300 μL of 10% TCA and blended for 30 s using a Fisher Scientific sonicator (model 300) equipped with a micro tip. A 1-mL aliquot was transferred to a 1.5-mL microcentrifuge tube (Fisher Scientific, model 72.690) and centrifuged (Fisher Scientific, model 235C) for 10 min at 13600g. Samples were diluted with 10% TCA in ratios of 1:1 for plant feedstuffs, whole shrimp, and diets unsupplemented with biogenic amines; 1:2, for turkey muscle tissue from birds fed diets unsupplemented with biogenic amines; 1:7 to 1:10, for samples of turkey duodenum, ileum + jejunum, and liver; 1:10, for shrimp hepatopancreas tissue; 1:20, for feedstuffs of animal origin and diets supplemented with low concentrations of biogenic amines; and 1:100, for diets supplemented with higher concentrations of biogenic amines. The diluted samples were then transferred to 2-mL glass HPLC autoinjector vials (Supelco, Inc., Oakville, ON) and capped with PFTE/rubber lined snap-caps (Supelco, model 2−4757).

Chromatography. An elution gradient was used as follows: 0−6.00 min, K600; 6.01−25.00 min, K563; 25.01−27.00, K130 and 27.01−30.00, K600. Flow rate was 0.8 mL/min. A calibration curve (1, 2.5, 5, 10, and 25 nmol/ml) was established daily. Retention times were 3.2, 6.5, 8.2, 9.5, 14.6, and 20.1 min for Tyr, His, Put, Cad, Spd, and Spm, respectively. Detection limits were 50 pmol/mL for Tyr, His, Put, and Spm; 40 pmol/mL for Cad; and 25 pmol/mL for Spd. Tissue samples contained large quantities of free amino acids which were detected and eluted primarily in the solvent front. It was necessary, therefore, to alter the excitation and emission wavelengths to 430 and 600 nm respectively from 1.00 to 2.60

min after injection. They were subsequently returned to 330 and 466 nm, respectively.

Extraction Efficiency. Extraction efficiency was determined by combining samples with known standards and then carrying out the appropriate extraction procedure followed by chromatography.

RESULTS AND DISCUSSION

The current methodology was developed to overcome deficiencies in other published methods particularly when applied to individual feedstuffs and complete animal feeds. An example of this problem was the GLC technique used in our laboratory for determination of biogenic amines, precursor amino acids, and metabolites in animal tissues. This employed *N*-heptafluorobutyryl-isobutyl derivatives (Lindqvist and Mäenpää, 1982) using a modification (Smith, 1990) of the electron capture detection capillary GLC technique of Bedford et al. (1987). This technique could not be applied to animal feedstuffs as too many interfering compounds were also derivatized.

The efficiency of extraction of biogenic amines from feedstuffs and animal tissues is given in Table 1. Extraction efficiency from feedstuffs was ∼100% with exception of histamine which was extracted with ∼92% efficiency. Extraction efficiency from animal tissues was generally lower and ranged from ∼80% for histamine from liver to ∼98% for putrescine from whole shrimp. The duodenum was exceptional with extraction efficiencies of over 100% for all amines with the exception of putrescine. The reason for this discrepancy is not clear. All values were considered high enough to ensure reliability.

Large quantities of free amino acids were not retained on the column and were eluted in the solvent front, exceeding the capacity of the detector and interfering with the resolution of tyramine. To avoid such overloading, the excitation and emission wavelengths used for the first 2.6 min of the sample elution program were 430 and 600 nm, respectively. The wavelengths were subsequently changed to 330 and 466 nm for the balance of the elution program.

The elution pattern of known standards of biogenic amines is given in Figure 1. Tyramine and histamine eluted first followed by putrescine, cadaverine, spermidine, and spermine. Sample chromatograms for feedstuffs (citrus pulp and poultry byproduct meals), experimental shrimp diets (control and putrescine supplemented), and shrimp tissues (whole shrimp and hepatopancreas) are given in Figures 2, 3, and 4, respectively.

Biogenic amine concentrations found in feedstuffs, complete diets, and animal tissues are given in Table 2. Bardocz et al. (1993) reported polyamine content of a variety of foods available at an institutional cafeteria. Fermented foods contained the highest concentrations of polyamines but citrus fruits (oranges and grapefruit
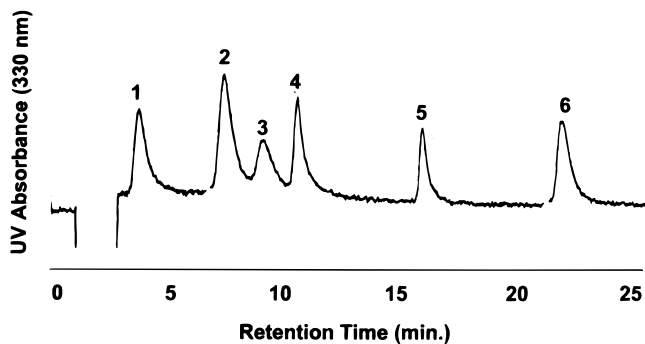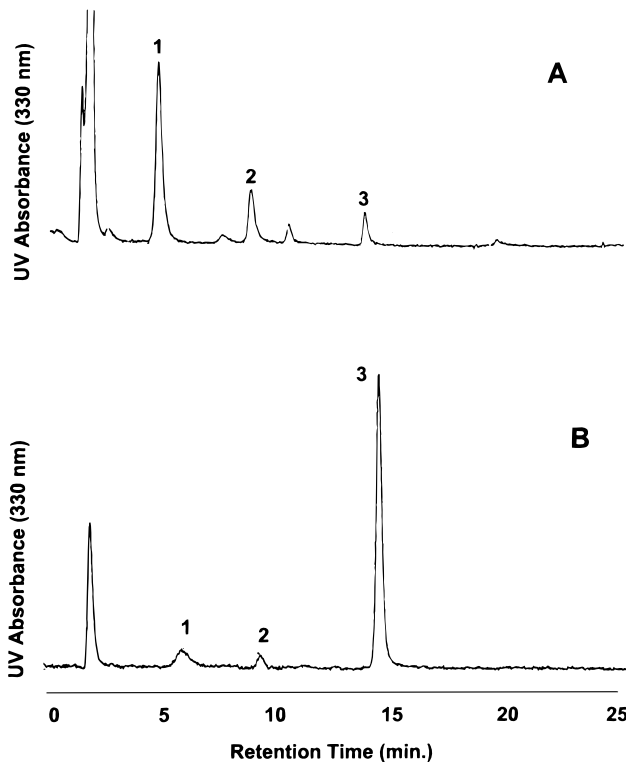
Figure 1. High-performance liquid chromatogram of known standards of biogenic amines: (1) tyramine, (2) histamine, (3) putrescine, (4) cadaverine, (5) spermidine, and (6) spermine.
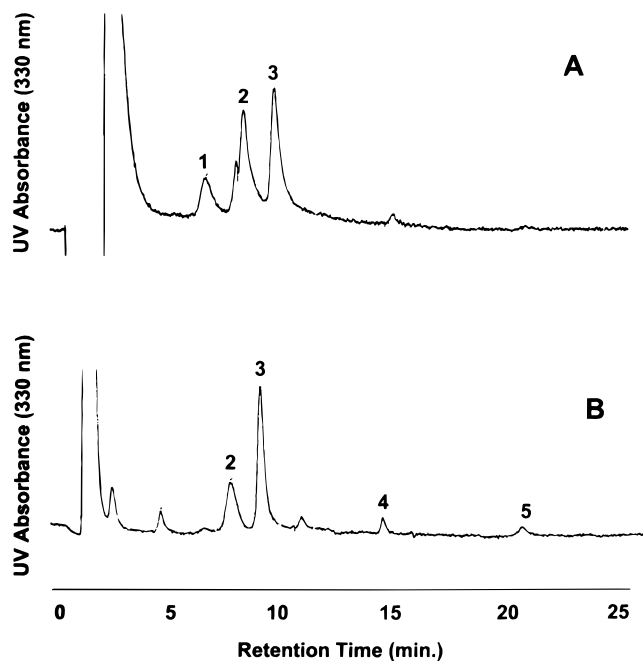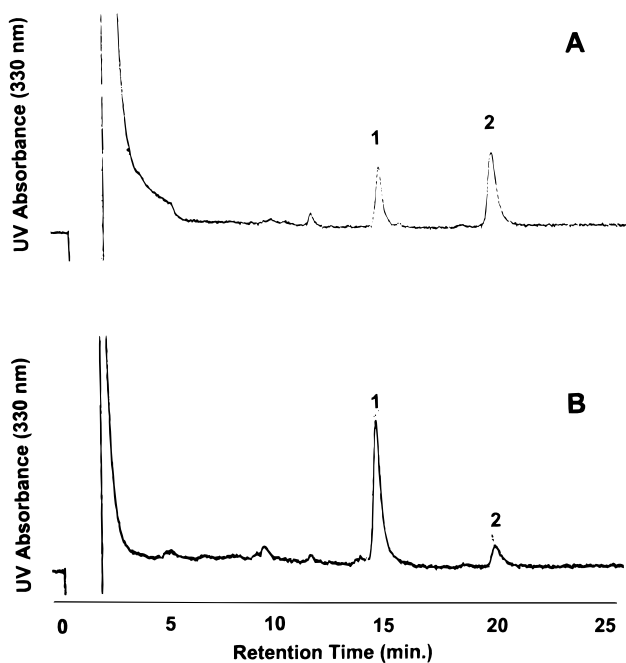

Figure 2. High-performance liquid chromatogram of feedstuffs: (A) distillers' dried grains and (B) poultry byproduct meal [(1) histamine, (2) putrescine, (3) cadaverine, (4) spermidine, and (5) spermine].

Figure 3. High-performance liquid chromatogram of shrimp diets: (A) unsupplemented diet and (B) diet supplemented with spermidine [(1) histamine, (2) cadaverine, and (3) spermidine].

Figure 4. High-performance liquid chromatogram of shrimp tissues: (A) whole shrimp without hepatopancreas, (B) shrimp hepatopancreas [(1) spermidine and (2) spermine].

juice) contained large amounts of putrescine (1.3 and 1.1 mg kg^{-1}, respectively). In the current study, dehydrated cubes of citrus pulp were found to contain 65 mg of putrescine kg^{-1} confirming the potential for such feedstuffs to serve as dietary putrescine sources for livestock feeds. Bardocz et al. (1993) reported putrescine concentrations of 0.03 mg of putrescine kg^{-1} of chicken which is somewhat less than the 21 mg kg^{-1} found in turkey muscle in the current study.

Analysis of poultry byproduct meal indicated the presence of significant amounts of cadaverine which is likely due to the presence of spoilage microorganisms. The concentration of 413 mg kg^{-1} is in close agreement with the report of Sander et al. (1996) who reported 541 mg of cadaverine kg^{-1} of a similar product. The only biogenic amine found in feathermeal, another rendered product, was cadaverine which again likely reflects the action of spoilage microorganisms. Fishmeal 1, manufactured from fresh fish, contained far less biogenic amine than fishmeal 2, manufactured from stale fish. This was particularly so for cadaverine (142 and 2631 mg kg^{-1}, respectively). Aksnes and Mundheim (1997) reported that fishmeal manufactured from stale herring contained 1580 mg of cadaverine kg^{-1}.

The application of the HPLC method to whole animal feeds is illustrated by the determination of biogenic amines in control and supplemented diets. Control broiler chicken diets contained 16 mg of putrescine kg^{-1} while diets supplemented with 2000 mg of putrescine kg^{-1} contained 1546 mg kg^{-1}. Control and supplemented (4000 mg of putrescine kg^{-1}) turkey diets were determined to contain 22 and 3353 mg of putrescine kg^{-1}, respectively. Unsupplemented shrimp diets contained

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Table 2. Biogenic Amine Content of Feedstuffs, Complete Diets, and Animal Tissues

	Tyr[a]	Hist	Putr	Cad	Spd	Spm
Feedstuffs (mg kg^{-1})						
apple pommace	nd[b]	nd	nd	nd	nd	nd
barley	nd	nd	14	nd	32	27
brewers' grains	357	131	nd	nd	nd	nd
citrus pulp	nd	422	65	nd	nd	nd
corn	nd	nd	6	nd	34	20
distillers' solubles, freeze-dried	nd	159	594	351	34	nd
distillers' dried grains	nd	5	53	16	23	10
feathermeal	nd	nd	nd	54	nd	nd
fishmeal 1	27	nd	13	142	21	11
fishmeal 2	1142	nd	1358	2631	nd	nd
malted rye	nd	nd	40	nd	35	7
malted barley	nd	nd	104	nd	57	17
meat and bone meal	nd	nd	nd	64	nd	nd
poultry byproduct meal	145	nd	273	413	53	nd
rye	nd	nd	9	nd	33	15
Complete Diets (mg kg^{-1})						
broiler diet unsupplemented	nd	nd	16	nd	87	20
broiler diet supplemented	nd	nd	1546	nd	75	nd
shrimp diet unsupplemented	nd	492	20	142	88	25
shrimp diet supplemented	nd	nd	4500	nd	nd	nd
shrimp diet supplemented	nd	nd	nd	nd	4602	nd
shrimp diet supplemented	nd	nd	nd	nd	nd	4143
turkey diet unsupplemented	nd	nd	22	19	nd	24
turkey diet supplemented	nd	nd	3353	nd	nd	nd
Turkey Tissue (μg mg^{-1} of wet tissue)						
duodenum	nd	nd	102	nd	68	120
ileum + jejunum	nd	nd	nd	nd	nd	nd
liver	nd	nd	21	nd	74	203
muscle	nd	nd	13	nd	16	51
Shrimp Tissue (μg mg^{-1} of dry tissue)						
whole	nd	nd	nd	2	22	50
hepatopancreas	nd	nd	45	70	198	332

[a] Abbreviations: Tyr, tyramine; Hist, histamine; Putr, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine. [b] Below detection limit.

20, 88, and 25 mg kg^{-1} of putrescine, spermidine, and spermine. Diets supplemented with 4400 mg of putrescine kg^{-1} were analyzed to contain 4500 mg of putrescine kg^{-1}. Supplementation with spermidine at 4500 mg kg^{-1} resulted in determined levels of 4602 mg of spermidine kg^{-1} and the diet supplemented with spermine at 4600 mg kg^{-1} was found to contain 4143 mg kg^{-1} spermine. These results confirm the accuracy of the current method for determination of biogenic amines in complete animal feeds.

It can be concluded that the current method can be applied to individual feedstuffs, complete feeds, and animal tissues for the rapid and accurate determination of concentration of biogenic amines.

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