

Biogenic amines in traditional alcoholic beverages produced in Nigeria

O.O. Lasekan *, W.O. Lasekan

Department of Food Science and Technology, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria

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Abstract

The biogenic amines, tyramine, putrescine, cadaverine, 2-phenylethylamine, histamine and tryptamine were determined in seven traditional alcoholic beverages produced in Nigeria. They were derivatised with dansylchloride and analysed using a high-performance liquid chromatographic method. Total amine content ranged from 0.10 to 2.38 µg/ml, with 'agadagidi' beer containing the highest level and 'pinto' the lowest. The levels of amines in the tested samples were, 0–0.5 µg/ml for tryptamine, 0–0.7 µg/ml for 2-phenylethylamine, 0–0.8 µg/ml for putrescine, 0–0.5 µg/ml for cadaverine, 0–0.5 µg/ml for histamine and 0–0.6 µg/ml for tyramine. These levels seem unlikely to have adverse effects on human health. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Many ethnic groups use diverse agricultural products in preparing fermented alcoholic beverages in Nigeria. These beverages are mostly consumed for nutritional, ceremonial and medicinal purposes (Sanni, 1989). Some of these beverages are 'burukutu' beer (a fermented sorghum product), 'sekete' beer (a fermented maize product), palm wine, 'agadagidi' (a fermented plantain product), 'pito' and 'pinto' beverages (sour maize liquor) and ginger beer. The associated micro-organisms in these beverages are yeast, bacteria and mould, while the fermentation process is by chance inoculation.

Alcoholic beverages, like other fermented foods, may contain biogenic amines as a result of decarboxylation of certain amino acids by the micro-organisms used in their production. Biogenic amines have been found to occur in a wide variety of foods such as wines, cheese, fishery products and other fermented foods (Maga, 1978; Smith, 1980). The biogenic amine contents of must (Buteau & Diutshaefer, 1984) and wine (Baucom, Tabacchi, Cottrell & Richmond, 1986) have been studied. Amino acid decarboxylation is the most common mode of synthesis of these amines (Rice & Koehler, 1976). The major factors leading to the formation of the

amines and their type are probably the availability of free amino acids and the presence of certain bacteria able to decarboxylate amino acids.

An extensive but controversial literature (Maga, 1978; Smith, 1980) exists concerning the relevance of biogenic amines in foods to diet-induced migraine. Lovenberg (1974) suggested that biogenic amines are either psychoactive or vasoactive and may cause problems to some consumers. Under normal circumstances in man, exogenous amines absorbed from food are rapidly detoxified by mono-or-di-amine oxidase or by conjugation (Tatyanenko, Gvozdev, Gvozdev, Lebedeva, Vorobyoz, Gorkin, 1971). However, high levels of these amines constitute a possible danger to patients treated with mono-amine oxidase inhibitors for depressive illness, since the pathway for inactivation of amines after ingestion is blocked, giving rise to hypertensive crisis (Stockley, 1973).

In view of the possible harmful effects of these amines, their concentration in foods deserves careful investigation. Whilst the production and chemical compositions of 'burukutu' beer (Faparusi, 1970), 'pito' (Ekundayo, 1969), 'sekete' and 'agadagidi' (Sanni, 1989; Sanni & Oso, 1988) have been studied, no study has been undertaken to evaluate their biogenic amines. The present study evaluates the biogenic amine contents of seven traditional alcoholic beverages consumed in Western Nigeria.

* Corresponding author.

2. Materials and methods

Four (4) bottles of each local beverage (Palm wine, 'burukutu', 'sekete', 'pito', 'pinto', 'agadagidi' and ginger) were purchased from local markets in Abeokuta, Nigeria. 2-Phenylethylamine, putrescine dihydrochloride, histamine dihydrochloride, tryptamine hydrochloride, tyramine hydrochloride and dansylchloride were obtained from Sigma Chemical Co. Acetone, methanol, acetonitrile and ethylacetate were LC grade. Other reagents employed in this study were GR grade from E. Merck Co., Germany.

2.1. Preparation of standard amine solution

Histamine dihydrochloride (165.7 mg), tyramine hydrochloride (126.7 mg), 2-phenylethylamine (130.1 mg), putrescine dihydrochloride (182.9 mg), cadaverine dihydrochloride (171.4 mg), and tryptamine hydrochloride (122.8 mg) were dissolved in 10 ml of deionised water. The final concentration of amine (free base) was 10 mg/ml solution.

2.2. Preparation of dansyl chloride solution

Dansyl chloride (100 mg) was dissolved in 10 ml of acetone to make a final concentration of 10 mg dansyl chloride per ml acetone.

2.3. Dansylation of standard amine solution

The procedure of Buteau and Diutshaever (1984) was adopted. Standard amine solution (25 μ l) was pipetted into a screw-capped vial. To the vial, 3 ml of 40 g/l sodium carbonate solution and 1.5 ml of dansyl chloride solution were added, and the mixture was stirred in the dark at 40°C for 2 h. Acetone was then removed using nitrogen gas. Ethyl acetate (1.5 ml) was added to the mixture and mixed thoroughly, followed by centrifuging at 1650 \times g for 1 min. The supernatant was placed in a volumetric flask and the volume was made up to 5 ml with further extracts of the residue. The complete extract was then filtered through a Millipore filter (pore size 0.45 μ m) before HPLC analysis.

2.4. HPLC analysis of amine standards and construction of standard curves

HPLC was in a Lichrosorb RP-18 reverse-phase column (7 μ m, 25 cm \times 4 mm id. E. Merck) on a Water Associates ALC/GPC 244 liquid chromatograph equipped with a Model 6000 A solvent delivery system, a Model U6K Universal Injector and a Lambda-max model 480 spectrophotometer set at 254 nm. The recorder was a Shimadzu Chromatopac C-RIB data processor. An isocratic elution system with a mixture of acetonitrile:

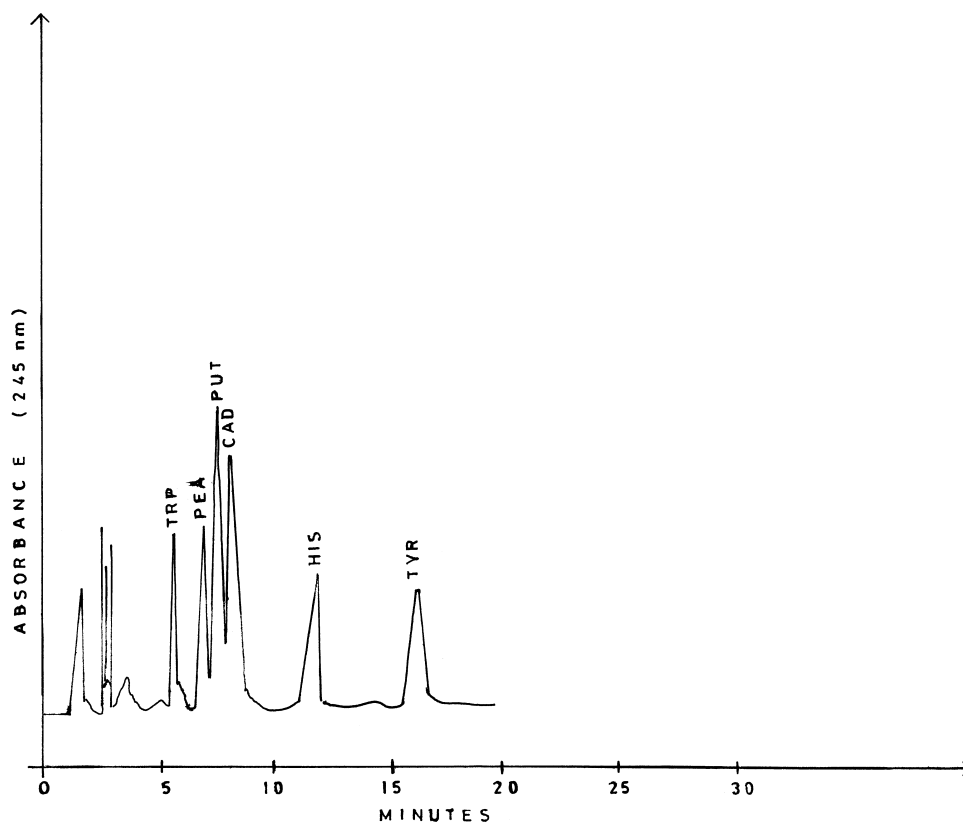


Fig. 1. HPLC chromatogram of dansylated biogenic amines: tryptamine, TRP 2-phenylethylamine, PEA: putrescine, PUT: cadaverine, CAD: histamine, HIS: tyramine, TYP.

methanol:water (1:2:1 v:v:v) was used. The flow rate was 1.5 ml/min.

For construction of standard curves, various concentrations of dansylated amine standards were analysed and relative peak area was plotted against amine concentration as described by Yen (1986).

2.5. Beverage dansylation

Each sample (10 ml) was concentrated to about 0.5 ml prior to the dansylation procedure. Dansylation was conducted in duplicate following the procedure for the amine standards above. HPLC analysis of dansylated samples was carried out as described for asinine standards.

2.6. Determination of recovery of amines

Recoveries of biogenic amines in six spiked samples were determined by adding 25 ppm amines to the alcoholic beverages.

3. Results and discussion

The HPLC chromatograms of the dansylated amine standards and that obtained from 'agadagidi' beer with and without spiked amines are shown in Figs. 1 and 2, respectively. A linear relationship was obtained between peak area and concentration of each amine. A correlation coefficient > 0.97 was obtained with every standard

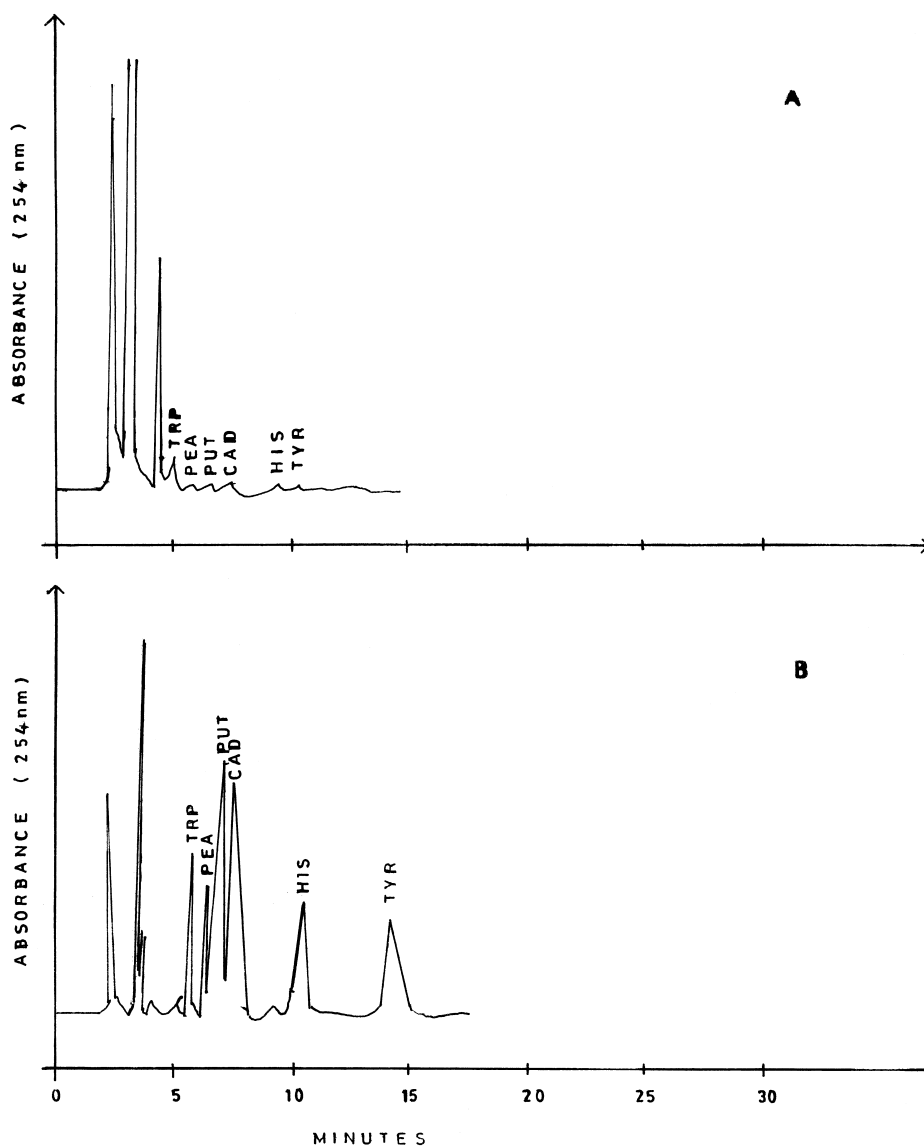


Fig. 2. Typical HPLC chromatograms obtained from 'agadagidi' beer with and without added biogenic amines. (a) control; (b) spiked with amines, injection vol. equivalent 50 μ g of each amine spiked.

Table 1
Amine content^a (µg/ml) of alcoholic beverages produced in Nigeria

Samples	Alcohol content (%)	Amines ^c						Total
		TRP	PEA	PUT	CAD	HIS	TYR	
Burukutu	3.0	0.02±0.01 (0.01–0.02)	0.3±0.1 (ND–0.4)	0.4±0.2 (0.2–0.4)	0.2±0.1 (ND–0.4)	0.03±0.01 (ND–0.4)	0.1±0.1 (ND–0.4)	(0.21–2.2)
Sekete	2.8	0.12±0.04 (0.10–0.30)	0.03±0.01 (ND–0.2)	ND	0.04±0.01 (0.02–0.07)	0.02±0.01 (ND–0.04)	0.1±0.1 (ND–0.3)	(0.12–0.91)
Agadagidi	3.5	0.29±0.05 (0.2–0.50)	0.4±0.02 (ND–0.7)	0.1±0.02 (ND–0.3)	0.06±0.03 (0.01–0.08)	0.1±0.01 (ND–0.3)	0.35±0.01 (ND–0.5)	(0.21–2.38)
Pito	2.5	0.13±0.02 (0.1–0.3)	0.2±0.1 (0.1–0.3)	ND ^b	0.2±0.02 (ND–0.5)	0.02±0.01 (ND–0.04)	0.1±0.1 (ND–0.3)	(0.2–1.44)
Pinto	2.4	0.10±0.01 (ND–0.3)	0.2±0.1 (0.1–0.5)	ND	0.1±0.1 (ND–0.2)	0.1±0.1 (ND–0.4)	0.1±0.01 (ND–0.3)	(0.1–1.7)
Ginger	2.0	0.12±0.08 (0.1–0.4)	0.2±0.1 (ND–0.3)	0.2±0.2 (0.1–0.6)	0.2±0.1 (0.1–0.4)	0.1±0.1 (ND–0.3)	0.1±0.01 (ND–0.2)	(0.30–0.2.2)
Palm wine	4.0	0.02±0.01 (0.01–0.03)	0.1±0.1 (ND–0.2)	0.3±0.1 (ND–0.5)	0.2±0.1 (0.1–0.5)	0.3±0.1 (0.2–0.5)	0.1±0.2 (0.1–0.6)	(0.41–2.13)

^a Mean ± S.D., range respectively.

^b ND: Not detected.

^c Abbreviations of amines are the same as in Fig. 1.

curve. The average recoveries of the amines (tyramine, 2-phenyl-ethylamine, tryptamine, putrescine, cadaverine, and histamine) were greater than 96% in all cases.

Variations in amine content of the beverages are shown in Table 1. With the exception of putrescine, all the other amines were detected in all samples. The total amine content ranged from 0.10 µg/ml for 'pinto' beverage to 2.38 µg/ml for 'agadagidi' beer. The highest level of tryptamine and 2-phenylethylamine were found in 'agadagidi' beer (0.29 and 0.4 µg/ml). 2-Phenylethylamine, which is a product of the decarboxylation of phenylalanine, is a more potent migraine initiator (Yen & Chandra, 1988). Sandler, Youdin and Hanington (1974) postulated that 3 mg of 2-phenylethylamine were sufficient to produce migraine. Whilst 'agadagidi' beer had the highest level of this amine (0.4 µg/ml), this is not high enough to cause migraine. For instance, if a consumer drinks a litre of 'agadagidi', the concentration of 2-phenylethylamine will translate to approximately 0.4 mg/l. This level of amine is still quite lower than the 3 mg required to produce migraine (Sandler et al.).

The highest level of putrescine found was in 'burukutu' beer (0.4 µg/ml). On the other hand, 'pinto' beverage had the highest concentration of cadaverine (0.3 µg/ml). Warthesen, Scanlan, Bills and Libbey (1975) postulated that putrescine and cadaverine upon heating may give rise to pyrrolidine and piperidine, which can then react with nitrite under acidic conditions to form heterocyclic carcinogenic *N*-nitroso compounds, nitrosopyrrolidine and nitrosopiperidine, respectively.

The histamine level was generally low in all the samples, with palm wine showing the highest level (0.3 µg/ml). Higher levels of histamine up to 100 mg/100 g

sample, have been shown to produce no clinical symptoms (Arnold & Brown, 1978).

The highest level of tyramine was also found in the 'agadagidi' beer (0.35 µg/ml). 'Sekete', 'Pito' and palm wine had the same level of tyramine (0.2 µg/ml).

The levels of tyramine in the samples are quite low compared with those reported by Yen and Chandra (1988) for 'Mg ka py' liqueur. Blackwell and Mabbit (1965) reported that ingestion of 6 mg of tyramine could produce a hypersensitivity reaction when taken concurrently with monoamine oxidase inhibitor drugs.

Finally, the wide variation noticed in the amine content of the alcoholic beverages could be ascribed to the varied raw materials and micro-organisms used for their fermentation.

4. Conclusion

A wide variation in the biogenic amines (tyramine, 2-phenylethylamine; tryptamine, putrescine, cadaverine and histamine) was noticed in seven traditional alcoholic drinks produced in Nigeria. The concentrations of the amines were generally low in all the samples and it is expected that this will have no adverse effects on human health.

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