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SHORT COMMUNICATION

THE EFFECT OF STORAGE ON THE FORMATION OF BIOGENIC AMINES IN WINES BREWED IN NIGERIA

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ABSTRACT. The effect of two storage conditions on the formation of biogenic amines in wines brewed in Nigeria has been examined. The amines were determined as their N-benzamides using a high performance liquid chromatographic (HPLC) technique. Changes in the concentration of biogenic amines at the two storage conditions during the six-week monitoring period reveals that levels of monoamines-histamine and tryptamine increased, while diamines-putrescine and cadaverine decreased. No significant differences were observed between wine types and amine concentration. Analysis of covariance at $\alpha = 0.05$ showed that formation of biogenic amines in wines is depended on the wine type, storage condition, and the duration of storage after opening. The result showed that high concentration of biogenic amines especially the monoamines may be encountered during long term storage of wines.

INTRODUCTION

Biogenic amines are a group of natural antinutritional and possibly toxic compounds widespread in foods [1]. They arise mainly from microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes by amino acid transaminases. The most common monoamines-histamine, tyramine and tryptamine are derived from histidine, tyrosine and tryptophan, while the diamines-putrescine and cadaverine are formed by microbial decarboxylation of lysine and ornithine, respectively.

Biogenic amines in low concentrations are essential for many physiological functions, while high concentrations may cause some deleterious effects. They function psychoactively and/or vasoactively. Psychoactive amines affect the neurotransmitters in the central nervous system. The vasoactive amines act either directly or indirectly on the vascular system as vasoconstrictors (e.g. tyramine) or vasodilators (e.g. histamine). A number of symptoms occur following excessive oral intake of biogenic amines. Toxicity strongly depends on the efficiency of detoxification, which may vary considerably between individuals and is affected by several factors. Normal intake of the biogenic amines are metabolized in the intestinal tract by a fairly efficient detoxification system based on the activities of monoamine oxidase (EC 1.4.3.4) and diamine oxidase (EC 1.4.3.6) [2].

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In non-fermented foods, the biogenic amines appear as a result of undesirable microbial activity. Very high level of histamine are found in spoiled fish, a number of biogenic amines occur in some well-matured cheese, fish and meat products, as well as in some fruits and vegetables such as spinach and tomato. Medium levels of biogenic amines are usually observed in fermented products such as soya sauce, beer and wine [1]. The occurrence and formation of biogenic amines, including spermidine and spermine formed from putrescine were studied in beers produced in Italy [3], Spain [4], and Germany [5].

Wine is consumed in considerable quantities in Nigeria. Statistical consumption per capita was however unavailable at the Federal Ministry of Trade and Industries. Also, background levels of biogenic amines in Nigerian wines are unavailable. Thus, wine could represent an important source of intake of biogenic amines. Therefore, the aim of this research was to present the values for concentration of four biogenic amines in Nigerian fruit wines and the effect of storage conditions on their formation after opening.

EXPERIMENTAL

Sample collection. Six brands of highly consumed non-alcoholic fruit wines were purchased from supermarkets in Port Harcourt: a major industrial city in Southern Nigeria.

Storage conditions. Test wine samples were opened and analyzed for the four biogenic amines as described below, and then stored at two conditions. The first condition was storage in the dark. Both dark brown and clear glass bottles of wine were warped with black cellophane wrappers and kept in a cupboard in the laboratory, while the second condition was by placing the wines on the shelf. The two conditions enable us to study the exclusion of light during storage.

Determination of biogenic amines. Determination of amines in the samples were carried out after forming their N-benzamide derivative with benzoylchloride using a high performance liquid chromatographic (HPLC) technique with isocratic elution. Histamine was determined in 35 cm^3 of wine degassed under vacuum, to which 0.5 cm^3 of 1.0 M 1,7-diaminoheptane was added as an internal standard. The mixture was made alkaline with 2.15 M sodium hydroxide solution followed by the addition of 1.0 cm^3 of 0.078 M benzoylchloride. The procedure was continued with acidification to pH 6.0 with 60% perchloric acid, addition of a 2.0 cm^3 of 1.55 mM sodium diphosphate buffer and 0.15 g sodium chloride and mixed. The N-benzamides formed were extracted into 10 cm^3 of diethylether. A 5.0 cm^3 of the extract were evaporated with warm air stream and the residue was dissolved in 0.4 cm^3 of mobile phase (71% v/v) methanol in water. A 10 μL aliquot of the extract was then injected for HPLC analysis.

Tryptamine, putrescine and cadaverine were determined in 40 cm^3 of degassed wine. A 0.5 cm^3 solution of 1.0 M 1,6-diaminohexane was added as an internal standard. The following procedure was the same as described above, but only 1.0 cm^3 diethyl ether was evaporated and dissolved in 0.3 cm^3 of 63.5% (v/v) methanol in water. The injected volume was again 10 μL.

Separation of N-benzamides was carried out using a high performance liquid chromatography HPP 4001 on an HPLC Column SGX-C_{18} 3 mm. The pressure was 150 MPa with a flow rate of 0.5 cm^3 min^{-1}. The detection was made at 254 nm. The peak area were integrated by using an Apex integrator. The retention times were 10.1, 8.4, 6.5, and 7.4 min for histamine, tryptamine, putrescines, and cadaverine, respectively.

Statistical data analysis. The analytical data were calculated from the calibration values for the individual amines designed for concentration ranges of 5-30 mg/L. The correlation coefficients for the amine calibration curves ranged between 0.997 and 0.999. The detection limits were very similar for all the amines, with values of about 0.3 mg/L. Reproducibility of the analytical procedure was tested by parallel analyses of seven samples from one bottle of wine. Relative standard deviations were 8.5%, 11.4%, 7.6%, and 2.7% at mean concentrations of 0.4, 1.5, 5.9, and 10.5 mg/L for histamine, tryptamine, putrescine, and cadaverine, respectively, for dark glass bottles and 10.7%, 15.5%, 6.4%, and 3.2% at mean concentration of 0.6, 1.01, 8.6, and 15.7 mg/L for histamine, typtamine, putrescine, and cadaverine, respectively, for clear glass bottles.

To assess the relationships between the brand of wines, the storage conditions and the concentration of biogenic amines during the monitoring periods, the analysis of covariance (ANACOVA) at $\alpha = 0.05$ was applied. The homogeneity of the ANACOVA was tested by the F_{max} test [6]. Consequently, parametric single factor ANACOVA was performed using the combined linear regression model-analysis of variance procedure [7] for variables that cannot be held fixed but can be measured. A null hypothesis and its alternative were tested:

1. H_o: there are no differences in the average level of biogenic amine formation between the two storage conditions during the six-week monitoring period among the six brands of wine;
2. There are differences in the average level of biogenic amine formation in the two storage conditions among the six brands of wine. Reject the null hypothesis, if $F_{cal} > 3.59$.

Reagents. All the chemicals used were of analytical grade. Chromatographic determination was carried out within a week after forming the derivatives. The samples for HPLC analyses were stored at -15 °C.

RESULTS AND DISCUSSION

Data on the concentration of biogenic amines in the six brands of non-alcoholic wines bottled in dark and clear glass bottles from six Nigerian wineries are summarized in Tables 1 and 2, respectively. Storage of wine, either table, dessert or non alcoholic fruit wines in fridge, on wine shelf or dinning table for a longer period after opening is a popular habit in Nigeria, partly because of the high cost of the commodity. The wines under investigation were stored at two conditions: representing cupboard and shelf for six weeks and tested for changes in biogenic amine concentration at 2-weekly intervals.

Table 1. The concentration (mg/L) of biogenic amines in the different brand of wines in dark bottles .

	Biogenic amines			
Wine brand	His	Try	Put	Cad
A	0.6 ± 1.7	0.9 ± 1.5	8.5 ± 9.9	13.3 ± 19.3
B	0.6 ± 1.3	1.0 ± 1.9	7.2 ± 9.5	12.8 ± 16.9
C	0.4 ± 0.7	1.7 ± 3.5	9.1 ± 12.3	13.5 ± 18.5
D	0.5 ± 1.0	1.7 ± 3.5	11.0 ± 14.6	14.1 ± 19.8
E	1.0 ± 2.1	1.5 ± 2.8	8.6 ± 9.6	13.9 ± 17.6
F	0.4 ± 0.7	0.9 ± 1.6	8.1 ± 7.0	12.4 ± 17.9

*Results are based on zero week measurement which represents the time of opening the wine bottles.
His = histamine; Try = tryptamine; Put = putrescine; Cad = Cadaverine.

Table 2. The concentration (mg/L) of biogenic amines in the different brand of wines in clear bottles .

Biogenic amines				
Wine brand	His	Try	Put	Cad
A	0.2 ± 1.3	1.1 ± 1.9	8.6 ± 8.4	12.3 ± 18.3
B	0.7 ± 1.7	1.2 ± 2.1	7.8 ± 10.6	12.8 ± 17.3
C	0.3 ± 0.7	2.0 ± 3.7	10.5 ± 13.4	13.2 ± 18.5
D	0.5 ± 1.5	0.8 ± 1.3	7.2 ± 8.8	12.6 ± 12.7
E	1.0 ± 2.4	1.4 ± 2.1	9.9 ± 14.6	14.3 ± 20.1
F	0.5 ± 1.3	1.9 ± 3.6	9.6 ± 13.2	13.4 ± 19.3

Time dependent amine concentration changes during storage are shown in Figure 1. The uniformity of biogenic amine concentrations within a brand of wine was tested by parallel analysis of seven bottles from one brand. Relative standard deviations were 9.4%, 12.5%, 6.0%, and 4.4% at mean concentrations of 0.9, 1.1, 8.4, and 12.4 mg/L for histamine, tryptamine, putrescine, and cadaverine, respectively. The values of the deviation are comparable with those observed in testing the reproducibility of the analytical procedure. Thus, one bottle was used as a representative sample of a brand of wine.

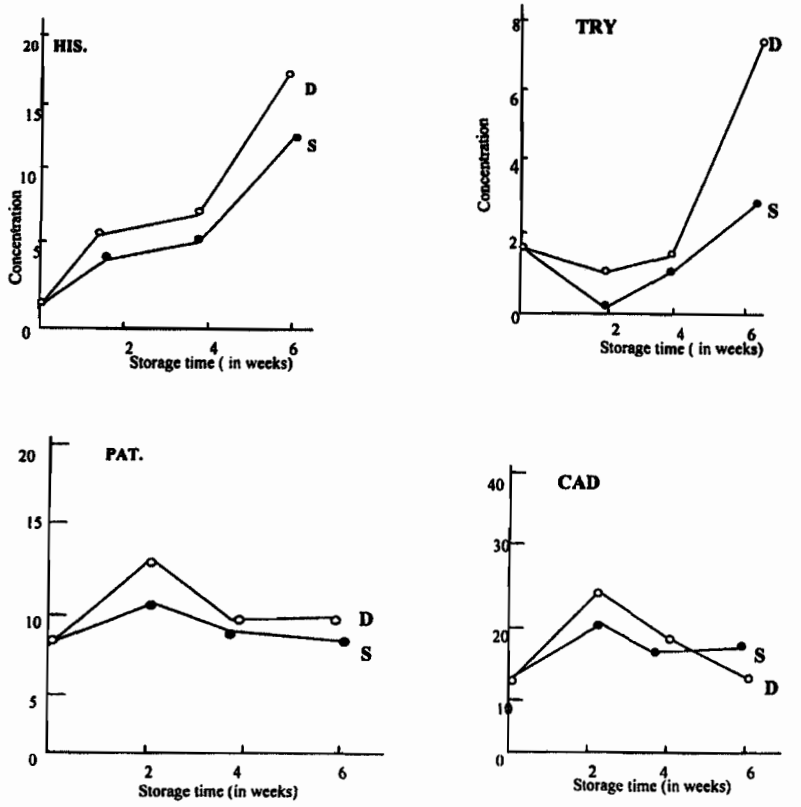

Figure 1. Changes in biogenic amine concentration during storage (• = stored in dark, ○ = stored on the shelf).

The levels of histamine in our sample ranged between 0.36-0.98 mg/L and 0.30-0.96 mg/L for wines in dark brown and clear glass bottles, respectively. The percent difference in histamine concentrations among brands was between 10.56-28.74% for dark brown glass bottles and 8.98-28.74% for wines in clear glass bottles.

Tryptamine concentrations in dark brown and clear bottles among the brands of wines ranged between 0.90-1.67 mg/L and 0.78-2.00 mg/L, respectively, with percent distribution of 11.76-21.77% and 9.26-23.75%, respectively.

Wines in dark brown glass bottles contained a purtrescine concentration of 7.21-10.95 mg/L among the different brands with a percent distribution of 13-74-20.87%, while those in clear bottles contained between 13.59-18.72 mg/L putrescine levels with a percentage distribution of 9.26-23.75%.

Cadaverine levels in dark brown glass bottle ranged from 12.80-13.85 mg/L with a percent distribution of between 15.48-17.67%, while levels in clear glass bottle ranged between 12.30-14.30 mg/L. The percentage distribution of cadaverine in clear glass bottle ranged from 15.67-18.22%.

The percentage distribution of biogenic amine concentration among the brands of wine tested in dark brown or clear bottles showed that light does not have a significant effect on the formation of the amines during storage.

Comparable results among brands were also reported elsewhere [5]. However, absolute concentration level seem to be characteristic of a winery, and to a limited extent of the different types of wines produced within this winery. Similar conclusions were reached for histamine and tyramine in Spanish beers [4], and for tyramine, cadaverine and, to a limited extent, tryptamine in German beers [5].

Changes in the amine concentration during the monitoring period (Table 3) showed that the levels of the monoamines-histamine and tryptamine increases with storage while that of the diamines-putrescine and cadaverine decreases with storage.

Table 3. Average concentration of biogenic amine at the two storage conditions (mean±sd in mg/L).

Biogenic Amine	Storage time* (in weeks)	Storage conditions Dark	Shelf
His	0	0.6 ± 1.1	0.7 ± 1.1
	2	3.2 ± 0.2	4.4 ± 0.5
	4	4.3 ± 0.7	5.7 ± 0.5
	6	12.0 ± 1.3	15.5 ± 0.9
Try	0	1.6 ± 1.8	1.6 ± 1.8
	2	0.3 ± 0.03	1.3 ± 0.3
	4	1.5 ± 0.3	1.6 ± 0.2
	6	2.3 ± 0.2	7.7 ± 1.1
Put	0	9.4 ± 8.9	9.4 ± 8.9
	2	10.5 ± 0.5	13.6 ± 1.4
	4	8.3 ± 0.4	9.4 ± 0.0
	6	6.6 ± 0.6	8.9 ± 0.6
Cad	0	13.4 ± 13.0	13.4 ± 13.0
	2	24.3 ± 0.8	29.9 ± 0.7
	4	21.3 ± 1.2	22.7 ± 1.2
	6	17.9 ± 2.3	14.8 ± 1.4

*Storage time of zero indicate the time of opening the wine bottle.

Analysis of covariance (ANACOVA) at $\alpha = 0.05$ of the relationship between wine types, biogenic amine formation and the two storage conditions during the six-weeks monitoring period showed that F_{cal} (2.63) < $F_{0.05}$ (3.59) which enables the acceptance of the null hypothesis. In other words, we can conclude, that formation of biogenic amines in wines is dependent on the wine type, storage condition and the duration of storage.

About 99% of samples contained less than 2 mg/L of histamie and nearly 80% of samples had a tryptamine level below 3 mg/L while 10 mg/L for putrescine is exceeded in nearly 30% sample. The maximum levels determined in the wines tested were 1.0, 2.0, 11.0, and 14.3 mg/L for histamine, tryptamine, putrescine, and cadaverine, respectively.

These values compared well with data reported for numerous European and Cuban wines and beers [5,8], however, increased levels of putrescine and cadaverines were observed in some Nigerian wines. The concentrations reported in European wines are within the range 0.2-8.0 mg/L whereas the upper value was exceeded in about one-third of our samples.

The observed amine concentrations can be considered as harmless for most wine consumers, however, biogenic amine concentrations, especially the monoamines may increase significantly, during long-term storage.

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