Measurement of Histamine in California Wines

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A chemical fluorescence method had been adapted for the measurement of histamine in grape juice and wine. The standard deviation of the method, based on duplicate or quadruplicate samples of 18 wines, was ±0.35 mg per l. The average value of these replicate samples was 2.39 mg per l. California wines are generally low in histamine; of about 300 samples of wines measured, only seven samples exceeded 5 mg per l., and three, 10 mg per l. Fruit wines averaged 0.56 mg per l., dessert wines 2.60 mg per l., and 253 table wines (including champagne) averaged 1.80 mg per l.; all of these are well below the level that would normally be considered physiologically important when ingested. The source of the histamine was not definitely determined. The effect of malolactic fermentation, fermentation temperature, and bentonite fining did not significantly affect the average amount of histamine in the commercial wine samples.

Marquardt and Werringloer (1965) measured 100 different wines and found up to 22 mg per l. of histamine. They found none in unfermented grape juice (50 samples). Further, they reported that only lactobacilli or colibacilli would induce histamine formation from histidine, and that yeast would not. Recently Quevauviller and Mazière (1969) reported contrary results. They found that histamine was formed during the alcoholic fermentation by the yeast. In measuring 60 French wines they found up to 30 mg per l. of histamine present, and noted higher amounts in red wines. They quoted 8 mg per l. as a level which may induce headaches when large amounts are ingested. Other investigators have reported on the histamine content of wines and other beverages. A summary of their results is given in Table I. Burgundy wines averaged in excess of 15 mg per l. Other European wines had values for histamine generally lower.

This report gives an improved method and the analytical results obtained from analyzing approximately 300 California wines and associated materials, and includes some pertinent histories of the wines.

APPARATUS AND MATERIALS

Fluorometric determinations were made with a Turner Model 111 using the high sensitivity sample holder. A matched set of borosilicate glass, 12 mm × 75 mm cuvettes, was selected. The primary filter used was a narrow pass C.S. #7-60 with an optimum activation wavelength of 360 mμ. The secondary filter chosen was a sharp cut C.S. #3 which cut off all transmission below 440 mμ. Minimum slit width was employed. (The use of a sharp cut filter C.S. 2A increases test sensitivity by about 2½ times.)

Diamond Shamrock Chemical Companies ES-63, Duolite cross-linked polystyrene, 16 to 50-mesh phosphonic acid cationic resin, and Dowex 1-X8 anionic resin were used for separations.

Glass thistle tubes 5 × 300 mm with a 15 ml reservoir and plugged with glass wool and half filled with resin were used for the cation resin exchangers. The anion exchanger was a 10 × 150 mm tube plugged with glass wool and one-third filled with the resin.

The ortho-phthaldialdehyde (OPT) was obtained from the Aldrich Chemical Co. The histidine, histamine, spermidine, spermine, putrescine, serotonin, and tyramine were purchased from the National Biochemical Corp. Deionized water was used for all dilutions and ion exchange work.

Wines were obtained directly from the bottling winery. Visits were made to these wineries and winery personnel interviewed to obtain information concerning the source of the raw product and the practices employed. Most of the active bottling wineries in the state were visited, and samples and histories were obtained. The wines and other materials were stored at 11° C until analyses were made.

METHODS AND PROCEDURES

The method of Shore *et al.* (1959), as modified by Michaelson and Coffman (1969), was altered slightly for these determinations. Samples of known histamine, histidine, and spermidine were neutralized with NaOH to pH 6.0 and passed through the cation exchanger which had previously been washed with base and acid and ethanol and charged with 0.2 *M* phosphate buffer. Samples were washed with 25 ml of 0.02*N* HNO_3 and eluted with 25 ml of 0.1*N* HNO_3, then with 25 ml of 0.2*N* HNO_3. Careful elution studies indicated that histidine could not be separated from the histamine and that spermidine did not completely separate from the histamine under our column conditions. The histidine was separated from the other two components by passing a portion of the eluate, after neutralization to pH 6.0, through the anion column, which had been charged with 1*N* NaOH and thoroughly washed. The histamine and spermidine pass through the column, while the histidine is retained.

The determination of malolactic fermentation was evidence of lack of malic acid in the wine. This was done by paper chromatography of the wines by the method of Kunkee (1968).

RESULTS AND DISCUSSION

Measurements were made to determine the fluorophore intensity of other amines relative to the intensity of the histamine–OPT fluorophore. The only ones of consequence measured were those of spermidine and histidine. The relative intensities on a molar basis were 1:39:390 for spermidine, histidine, and histamine. This is in good agreement with the report of Anton and Sayre (1969). Other amines tested were spermine, putrescine, tyramine, and serotonin.

Recovery studies were made by adding a known amount at various concentration combinations of histamine and spermidine to four different wines. About 90% of the histamine was recovered from the 0.1 and 0.2*N* HNO_3 eluates after

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Table I. Summary of Recent Histamine Analyses

Reference	Histamine mg per l. Range	Average	Number of samples	Type of material	Method
Marquardt and Werringloer (1965)	0–22	...	100	European, North African, Chilean wines	Chromatography
Diemair and Diemair (1966)	10–20	...	...	German white table	
Millies (1966)	0.4–1.9	1.0	13	European red and white grape juice	Thin-layer chromatography
	...	1.7	13	European red and white wine	
	1.2–1.3	...	2	Currant juice	
	1.9	...	1	Sour cherry juice	
Schuller *et al.* (1967)	0–10	0.85	33	German—misc. wine	Physiochemical
de Saint-Blanquat and Derache (1968)	0.05–9.3	1.8	12	French—misc. wine	Ion exchange and fluorescent
Jakob (1968)	0.6–20	...	7	European—misc. wine	Physiochemical
Mayer and Pause (1969)	7–8	...	2	Swiss red and white wine	Gel-electrophoresis
Blackwell *et al.* (1969)	0.21–2.83 mg per g	...	8	English yeast	Chromatography
Maier (1969)	...	1.0	14	German wine	Thin-layer electrophoresis
Puputti and Suomalainen (1969)	0.3–0.5	...	3	European wines	Thin-layer chromatography
Quevauviller and Mazière (1969)	0.1–30	5.4	60	French wines	Physiochemical
Granerus *et al.* (1969)	0.04–15.6	4.9	13	European wines	Ion exchange, biochemical

Table II. Estimate of the Standard Deviation of the Histamine Measurements

Wine Variety and Type	Histamine mg per l.
Sauvignon vert, White table	1.3, 1.5
Pinot blanc, White table	0.3, 0.5
Semillon, White table	3.5, 3.9
White Riesling, White table	2.5, 2.3
Mixed blend, White table	1.6, 1.6, 0.6, 1.5
Pinot noir, Red table	3.0, 3.5
Cabernet Sauvignon, Red table	1.9, 1.1, 1.7, 1.8
Cabernet Sauvignon, Red table	1.7, 1.8
Cabernet Sauvignon, Red table	1.6, 1.0
Zinfandel, Red table	2.1, 2.0
Mixed blend, Red table	3.5, 4.5
Zinfandel, Red table	0.9, 1.5
Petite Sirah, Red table	1.5, 1.6, 1.5, 1.1
Petite Sirah, Red table	3.3, 3.6
Carignane, Red table	2.9, 2.4
Concord, Red table	5.2, 4.6
Mixed blend, White dessert	2.9, 3.0
Mixed blend, Red dessert	2.7, 2.9
Average	= 2.39
Standard deviation	= ±0.35

anion exchange. In these measurements about $^1/_3$ of the histamine was recovered from the 0.2*N* eluate portion. It was calculated that about $^1/_6$ of the histidine carried over into this eluate portion. Because of the histamine carryover and the large fluorophore response difference, the spermidine could not be adequately measured. Hence the total response of the ion-exchanged eluates of the 0.1*N* and 0.2*N* elutions was used to calculate only the histamine. This will induce a positive error of a small magnitude. Castelli and Rossoni (1968) report the total concentration of spermidine in growing yeast cells, after 12 hr, at about 900 μg per g. If all of this spermidine were released into a wine, it would amount roughly to 0.9 mg per l. or an amount that would not influence the reading significantly.

With these limitations in mind, a standard curve was prepared for histamine by taking water solutions through both the cation and anion exchanges and developing the fluorophore as suggested by Michaelson and Coffman (1969) with the following slight changes: 1 ml of the final eluate added to 0.1 ml 4*N* NaOH, 0.05 ml of 0.5% OPT in methanol, and after 3 min of fluorophore development 0.2 ml of 3.5*N* H_3PO_4 added to give the fluorescent form of the fluorophore. Two minutes time was inadequate under the conditions of our laboratory. Anton and Sayre (1969) also use a longer time for the reaction. The ion exchange column blanks were equivalent to about 0.5 mg per l. of histamine.

A number of the samples were analyzed several times to get a measure of the variation in the results. Table II lists these repeated samples and notes the precision of a single measurement (standard deviation = ±0.35). The coefficient of variation was 14.6%. Another determination was made on a set of data consisting of four samples, each of which was put through three different columns and the eluates each analyzed in duplicate. The error data were portioned to determine the distribution of the variation between column and between fluorophore development and measurement. The latter accounted for $^1/_3$ of the variation. The coefficient of variation for these total data was 11.7%.

A summary of results obtained by the histamine analysis of the California wines is shown in Table III. The wines were arranged into groups, first by type, then by area from which gathered and, if sufficient samples, by variety. The pertinent histories of the wines are included, as well as the malolactic fermentation measurement results.

The differences by wine types are not large. Fruit wines were lower in histamine than were the grape wines. This may be due to the source as well as to the generally lesser amount of fermentation and the dilution practices involved in fruit wine manufacture. Millies (1966) found currant and sour cherry juices to have significant amounts of histamine. The next lowest type was champagne. Only five samples were analyzed. White table and red table wines did not vary significantly overall. This, despite the difference in % of malolactic fermentations. Dessert wines of approximately 20% alcohol were higher than the table wines or the sherry wines. Rose wines were not greatly different from the other table wines.

There were no differences that might be attributed directly to geographical areas. Other differences seemed independent

Table III. Summary of Certain History and the Histamine Measurements of Some California Wines

Type	Area[a]	Variety	Number of samples	Histamine mg per l. Range	Histamine mg per l. Average	Malolactic % undergone	Bentonite lb per 1000 gal average	Fermentation temperature °F average
White table	North coast	Miscellaneous	27	0.8–3.1	1.34	18	3	60
	South coast	Miscellaneous	24	0.8–7.8	1.88	33	2	65
	San Joaquin Valley	Miscellaneous	20	0.3–4.0	1.50	5	6	65
	Other	Miscellaneous	8	1.5–11.4	3.68	12	6	65
	Total		79		1.78	18.7		
Rose	Miscellaneous	Miscellaneous	11	0.6–2.8	1.68	9	3.5	62
Red table	North coast	Pinot noir	10	0.7–6.6	2.41	80	2	...
	South coast	Pinot noir	11	0.4–7.0	2.18	73	2	70
	North coast	Cabernet Sauvignon	12	0.3–2.3	0.98	75	1.5	...
	South coast	Cabernet Sauvignon	14	0.3–3.8	1.26	93	2.0	75
	North coast	Zinfandel	17	0.2–5.0	1.60	76	0.5	75
	North coast	Miscellaneous	27	0.6–5.0	1.60	78	0.8	72
	South coast	Miscellaneous	24	0.3–7.2	1.92	87	2.8	78
	Lodi	Miscellaneous	14	0.6–4.9	2.20	14	7	73
	San Joaquin Valley	Miscellaneous	19	0.6–2.1	1.13	60	3.5	...
	Other	Miscellaneous	10	1.0–15.5	4.53	50	5.2	80
	Total		158		1.85	70.4		
Sherry	San Joaquin Valley	Miscellaneous	12	0.6–3.1	1.41	8	7.5	...
Dessert wine	San Joaquin Valley	Miscellaneous	16	1.5–4.6	2.60	0	5.9	
Champagne	Other	Miscellaneous	5	0.3–1.9	1.00	...	2.0	...
Fruit wines	Other	Miscellaneous	12	0.1–1.3	0.56	0	4.0	70

[a] North coast refers to areas north of San Francisco and exclusive of the Sacramento Valley. South coast refers similarly to the areas south of San Francisco and exclusive of the San Joaquin Valley. Lodi is an area of the San Joaquin Valley, south of Sacramento and exposed to some extra climatic effects. Other areas included sources not specifically described.

of the area. Certain wineries did have consistently higher histamine values than others. This probably relates to process methods. As noted by Quevauviller and Mazière (1969), the time of fermentation on the skin seems important.

The only varietal wine samples collected in sufficient numbers to measure for two areas were "Pinot noir" and "Cabernet Sauvignon." These two varieties were significantly different in their histamine levels. The Cabernet Sauvignon had the lowest histamine content of the varieties measured. This is in contrast to statements attributed to Styler (1969).

In the wine samples measured for histamine, only one of the 79 white table wines was over 5 mg per l. and one over 10 mg per l.; of the 158 red table wine samples, six were over 5 mg per l. and two over 10 mg per l. Of the other 45 miscellaneous types of wine measured, none exceeded 5 mg per l.

Two other related products were measured. Three grape juice concentrate samples were found to contain an average of 34.5 mg per l. of histamine. The normal concentrate, if reconstituted with water, has about three to four times the original concentrate volume. This still would average fairly high values of histamine. The highest concentrate measured was 55.7 mg per l. The other product was two samples of wine concentrate. This is wine with the alcohol and other volatiles removed. These samples contained 16.0 and 23.4 mg per l. of histamine. The concentration information was unavailable. The product is used sparingly and probably no more than 200 ml is used at one time on food during preparation; this also would not be ingested by one person alone.

Jakob (1968) showed bentonite fining to reduce the histamine content. From the histories gathered on the amount of bentonite used, there seems to be no significant relationship. However, his work indicated 1000 mg per l. of bentonite was needed to reduce the histamine by one-half. This amount is large compared to what is usually used commercially (1 lb per 1000 gal = 120 mg per l.), as can be seen in Table III.

Table IV. Effect of Source of Grape Material on Histamine Content[a]

Sample number	Juice	Alcohol extract[b]	Wine[c]
1	4.6	2.5	0.3
2	10.6	4.0	16.1
3	10.1	3.3	1.3

[a] As mg per l. [b] 20% ethanol extract of grapes after dejuicing. [c] Fermented on the seeds and skins to dryness at 70° F, then held at 0° C until analysis.

No significant Spearman Rank correlations could be demonstrated between the number of wines which had undergone malolactic fermentation and the histamine levels of these wines.

The possibility exists, however, that in the few wines which are high in histamine, the levels can be reduced to lower levels. Trials with adsorbents and with yeast refermentations are distinct possibilities that could be further investigated to find methods of histamine reduction.

The data reported in Table IV, showing the histamine content for juice, alcohol extract of the seeds and skins, and on the wine (fermented to dryness on the seeds and skins) demonstrate one possible source of high histamine in wine and the effect of yeast growth and fermentation in reducing the histamine. These samples had been prepared for another experiment in 1968 and had been held in cold storage for over 1 year prior to analysis. The second sample was extremely high in total nitrogen in both the juice and the wine. The other two wines were not high in total nitrogen. This suggests perhaps that, as with other materials—for example, proline in wines (Ough, 1968), the presence of an overabundance of nitrogen would spare such compounds as histamine from use by the yeast. Also, if the histidine level were high it would perhaps induce the formation of histidine decarboxylase.

A scatter plot of histamine *vs.* the total nitrogen of the commercial wine samples further suggests the validity of this line of reasoning. A correlation coefficient of +0.60 was calculated for the 286 data pairs. Considering the possibilities of treatment effects on such a miscellaneous selection of samples, this is a very indicative value.

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