

Histamine and Tyramine Content of South African Wine¹

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The histamine content of 184 wines and tyramine content of 156 wines, produced in South Africa was measured. The histamine and tyramine content of the wine was found to be similar to those of wines produced in other countries. The average histamine content of South African red wines that had undergone malo-lactic fermentation was more than double that of red wines that had not undergone malo-lactic fermentation. All the red wines containing relatively large amounts of histamine had pH's above 3,7. Six selected strains of malo-lactic bacteria were tested for their ability to form histamine and tyramine in white and red wine. No histamine or tyramine was formed.

Biogenic amines are usually bacterial degradation products of the corresponding amino acid and may be formed in almost all foods in varying amounts. A comprehensive review of their occurrence in a wide range of foods has recently been published by Smith (1981).

The biogenic amine content of alcoholic beverages has been much publicised as there has been evidence that these compounds could be responsible for some of the physiological changes that have been reported after the consumption of certain alcoholic beverages (Ienistea, 1971; Zee, *et al.*, 1983). Lowenberg, *et al.*, (1981) however, concluded that ingested alcohol stimulates the liberation of endogenous histamine and that histamine in wine has no effect on the human plasma histamine level.

The histamine content and to a lesser extent tyramine and other biogenic amines of European and American wines, has been determined (Subden, *et al* 1979; Zee *et al.*, 1983). There have been no studies, however, on the histamine and tyramine content of South African wines.

Although bacteria responsible for malo-lactic fermentation have been indicated to form amines (Aerny, 1982), there is still a lack of information concerning the specific micro-organisms responsible for, and conditions favouring the formation of the amines during vinification.

METHODS AND MATERIALS

Analytical methods: The extraction and column chromatography procedure used by Yamamoto, Wakabayashi & Makita (1980) for the gas-liquid chromatographic determination of tyramine in fermented food products was applied in the extraction, purification and concentration of histamine and tyramine in must and wine samples.

The following modifications to the method were used: Instead of adding a constant volume of 6 N HCl to the 50 ml portion of the must or wine sample, the pH of the sample was adjusted to 0,8 with the HCl. A two percent perchloric acid solution was found to be sufficient to transfer the oily residue into a 25 ml volumetric flask.

The method suggested by Lindroth & Mopper (1979) for the HPLC determination of subpicomole amounts of

amino acids by percolumn fluorescence derivitisation with o-phthaldialdehyde (OPT) was used as a basis for the development of the method for the separation and quantification of histamine and tyramine in the prepared samples.

One hundred microlitres of OPT reagent was added to 100 μ l of the prepared sample in a 5 ml glass tube. The mixture was allowed to react for exactly one minute before injection onto the HPLC column.

Histamine dihydrochloride, tyramine hydrochloride and OPT were obtained from Sigma Chemical Co. Standard solutions of histamine and tyramine (as free base) were prepared in water, and aliquots were taken for the preparation of the calibration curve and for the calculation of the recovery rate from the wine samples. All other chemicals were reagent grade unless otherwise stated.

Analysis was performed on a Varian Model 5 000 liquid chromatograph, equipped with a Varian Fluorichrom fluorescence detector and a Varian Vista 401 chromatography data system. The HPLC parameters used for the separation and detection of histamine and tyramine are summarised in Table 1.

TABLE 1

HPLC parameters used for the separation and detection of histamine and tyramine

Parameter	
Column:	Micro Pack MCH-5 (4 mm ID \times 30 cm)
Column temperature:	30 °C
Mobile phase: Methanol:	65%
Phosphate buffer (pH 6,78):	35%
Flow rate:	0,7 ml. min. ⁻¹
Detector: Excitation wavelength:	360 nm
Emmission wavelength:	460 nm

Figure 1 illustrates the degree of separation of histamine and tyramine from interfering compounds and the retention times of the amines. The procedure used for extracting histamine and tyramine was such that their concentrations

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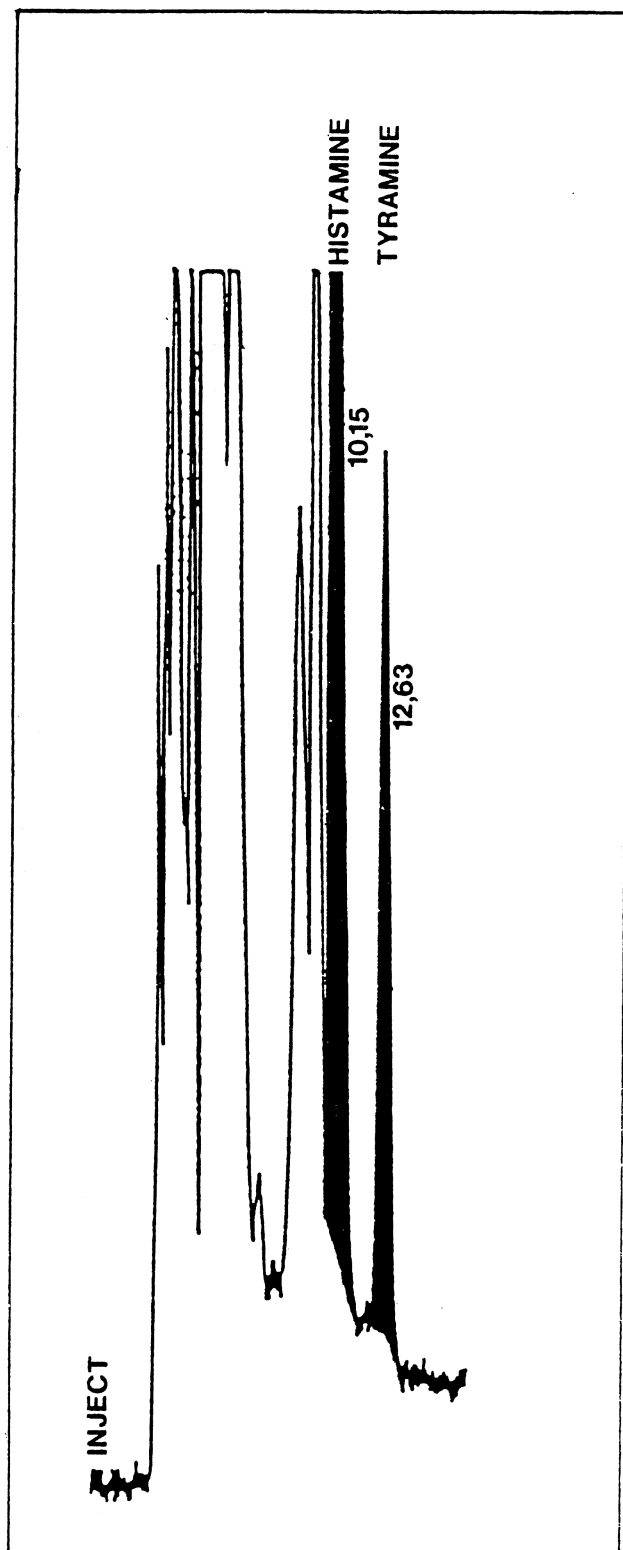


FIG. 1

Chromatogram of a wine containing $2,5 \text{ mg.l}^{-1}$ of histamine and $0,8 \text{ mg.l}^{-1}$ of tyramine.

were in a range that could be readily analysed by the liquid chromatographic procedure (Figure 1).

The mean average percentage recovery of histamine ($2,54 \text{ mg.l}^{-1}$) and tyramine ($2,96 \text{ mg.l}^{-1}$) was 99,2 and 83,8

TABLE 2

Mean, average percentage recovery, standard deviation and coefficient of variation for histamine and tyramine

Amine	Mean ¹	Mean Average Percentage Recovery	Standard Deviation	Coefficient of Variation
Histamine	2,52	99,21	0,12	4,64
Tyramine	2,48	83,78	0,08	3,26

¹ = 5 replicates

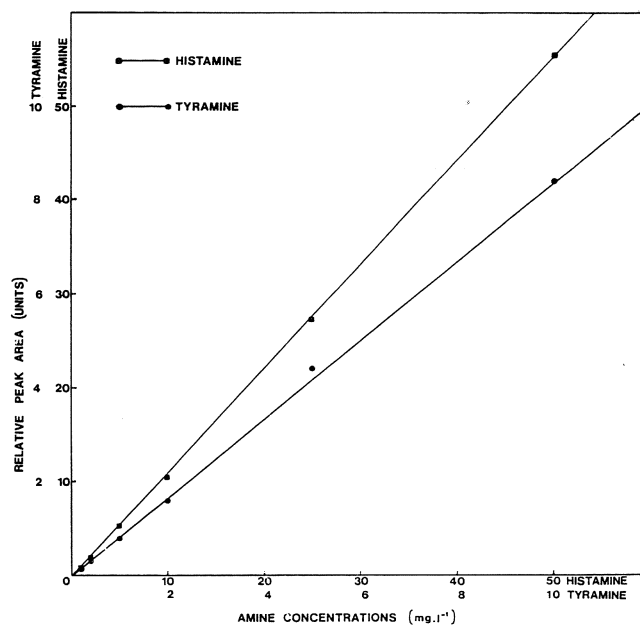


FIG. 2

Standard graph of relative peak areas of amines in relation to concentration of amines.

respectively. The standard deviation for histamine was 0,12 and for tyramine 0,08 (Table 2). The detector response was linear over the range 0 to 50 mg.l^{-1} for histamine and 0 to 10 mg.l^{-1} for tyramine (Figure 2).

The total soluble solids content of the must was measured with the aid of a hydrometer, calibrated in degrees Balling (Brix). Towards the end of fermentation the sugar content of the must was monitored by using a Clinitest kit (Ames Company) according to the procedure prescribed by the manufacturer.

The pH of must and wine samples was measured with a Beckman Model 3 500 digital pH meter and a combination pH electrode.

Total acidity of samples was measured by titrating 25 ml of must or wine with 0,33 N NaOH to an end point of pH 7.

The paper chromatographic procedure of Kunkee (1968) was used to determine the presence or absence of malic and lactic acid in wine samples. If malic acid was absent, it was assumed that the wine had undergone malolactic fermentation.

The free and total SO_2 content of samples was determined according to the Ripper method (Amerine & Ough, 1980).

Materials: Various wines produced and commercially available in South Africa were analysed.

Wines were also made from Chenin blanc and Pinotage grapes from the Stellenbosch district in the following three ways. Chenin blanc grapes were crushed and destalked in the normal way. While the pomace was thoroughly mixed, it was divided into two lots. The one lot was treated as for normal white wine production. Must was separated from skins in a static separator and left to settle overnight at 14 °C. No SO₂ was added. The other lot was treated as for red wine production according to the thermovinification method. The Pinotage grapes were also treated after crushing and destalking, and subsequently pressed, according to the thermovinification method. Each quantity of must was then divided into 14 lots of 3,5 litres. To each lot 20 mg.l⁻¹ of *l*-histidine (4 percent stock solution) and 20 mg.l⁻¹ of *l*-tyrosine (4 percent stock solution) was added.

All lots were then inoculated with 5 percent *Saccharomyces cerevisiae* (WE 500) yeast, prepared from dried yeast the previous day. Bacteria starter cultures were then added (0,5 percent) to the individual musts as summarised in Table 3. All treatments were duplicated.

TABLE 3
Bacteria starter cultures added to individual musts

Treatment No	Bacteria used
1. (a), (b), (c)	No bacteria added (control)
2. (a), (b), (c)	<i>Leuconostoc oenos</i> (PSU-1)
3. (a), (b), (c)	<i>Leuconostoc oenos</i> (France)
4. (a), (b), (c)	<i>Lactobacillus</i> (Equilait)
5. (a), (b), (c)	<i>Pediococcus cerevisiae</i>
6. (a), (b), (c)	<i>Lactobacillus plantarum</i>
7. (a), (b), (c)	<i>Lactobacillus buchnerii</i>

(a) White wine (Normal wine making procedure)

(b) White wine (thermovinification)

(c) Red wine (thermovinification)

The must and subsequent wine was kept in a waterbath at 20 °C until alcoholic as well as malo-lactic fermentation was completed. The wines were then racked, 60 mg.l⁻¹ SO₂ added and stored at 0 °C until analysed.

RESULTS

Histamine and tyramine content of South African wines:

The histamine content of 184 commercial wines averaged 3,1 mg.l⁻¹. The wines analysed include 117 red wines, 62 white wines and five rosé wines. The average histamine content of the red wines was 4,8 mg.l⁻¹. A summary of the different amounts of histamine detected in the wines is shown in Figure 3. Most of the wines (88,9 percent) had a histamine content of less than 10 mg.l⁻¹. The average histamine content of the 91 red wines in which no malic acid could be detected and had presumably undergone malo-lactic fermentation, was 5,4 mg.l⁻¹, compared to an average of 2,5 mg.l⁻¹ for the 26 red wines in which malic acid was present and which presumably had not undergone malo-lactic fermentation. The highest histamine content measured in a red wine was 49,1 mg.l⁻¹. This wine was made from Pinotage grapes, had undergone malo-lactic fermentation and was of 1974 vintage.

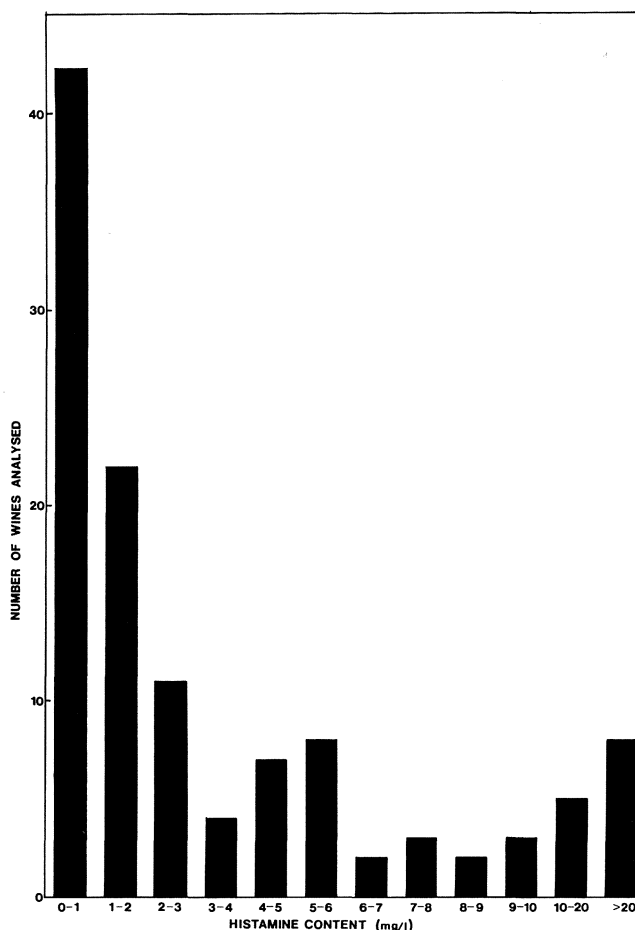


FIG. 3

Histamine content of South African commercial red wines.

The average histamine content of the red wines with high pH's was higher than the average histamine content of red wines with low pH's (Table 4). The average pH of the red wines analysed was 3,77. All the wines containing more than 10 mg.l⁻¹ of histamine had pH's above 3,7. Of the 25 red wines which contained malic acid and were accordingly classified as wines which had not undergone malo-lactic fermentation, six wines had a histamine content of more than 5 mg.l⁻¹ the highest being 13,4 mg.l⁻¹.

TABLE 4
Histamine content of South African commercial red wines per wine pH level

pH	Histamine mg.l ⁻¹				Average	Number of wines
	<1	1-5	5-10	>10		
<3,4	4				0,1	4
3,4-3,5	3	3			1,0	6
>3,5-3,6	5	8			1,1	13
>3,6-3,7	6	7	4		2,7	17
>3,7-3,8	6	6	7	1	4,7	20
>3,8-3,9	9	14	4	5	5,5	32
>3,9-4,0	5	4	3	3	4,8	15
>4,0	4	2		4	14,8	10
Total	42	44	18	13	4,8	117

The average histamine content of the white wines was 0,1 mg.l⁻¹. None of the wines had undergone malo-lactic fermentation, and all contained less than 1 mg.l⁻¹ of histamine. The average pH of the white wines was 3,45.

The average histamine content of the rosé wines was 1.03 mg.l⁻¹. None of the rosé wines had undergone malo-lactic fermentation.

The tyramine content of 156 commercial wines averaged 0,4 mg.l⁻¹. The wines include 105 red wines, 47 white wines and four rosé wines.

The average tyramine content of the 105 red wines was 0,5 mg.l⁻¹. The 82 red wines which had undergone malo-lactic fermentation had an average tyramine content of 0,6 mg.l⁻¹ compared to the 0,2 mg.l⁻¹ of the 23 wines which had not undergone malo-lactic fermentation. Most of the

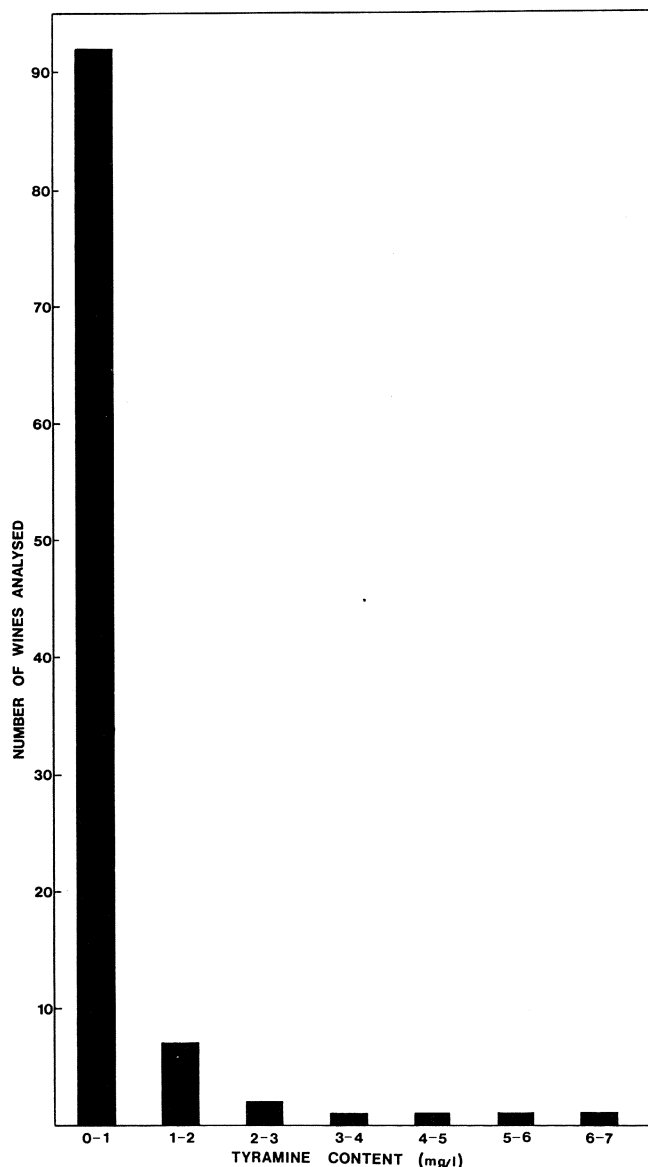


FIG. 4

Tyramine content of South African commercial red wines.

wines (87,6 percent) had a tyramine content of less than 1 mg.l⁻¹ (Figure 4). There seems to be no relationship between the pH of the red wines and their tyramine content (Table 5). The highest tyramine content measured in a red wine was 6,4 mg.l⁻¹. The wine was made from Shiraz grapes, had undergone malo-lactic fermentation and was of 1980 vintage.

TABLE 5
Tyramine content of South African commercial red wines per wine pH level

pH	Tyramine mg.l ⁻¹					Number of wines
	<1	1-3	3-5	>5	Average	
<3,4	3				0,05	3
3,4-3,5	5	1			0,63	6
>3,5-3,6	12				0,25	12
>3,6-3,7	11	2	2	1	1,31	16
>3,7-3,8	15	4			0,42	19
>3,8-3,9	29			1	0,31	30
>3,9-4,0	11				0,15	11
>4,0	6	2			0,65	8
Total	92	9	2	2	0,50	105

The average tyramine content of the 47 white wines was less than 0,1 mg.l⁻¹, the highest value obtained being 2,1 mg.l⁻¹. All the white wines analysed had not undergone malo-lactic fermentation.

The average tyramine content of the four rosé wines was 0,1 mg.l⁻¹, all the wines contained less than 0,3 mg.l⁻¹ of tyramine.

Influence of malo-lactic fermentation on the formation of amines: The histamine and tyramine content of wines made by normal and thermovinification procedures and had undergone malo-lactic fermentation by various bacterial strains is reported in Table 6. All the wines contained less than 0,5 mg.l⁻¹ of histamine. No tyramine was detected in any of them.

TABLE 6
The influence of wine type, method of production and bacterial strain on the formation of histamine and tyramine

Type of Bacteria	White wine (Normal wine making procedure)		White wine (Thermovinification)		Red Wine (Thermovinification)	
	Histamine ¹	Tyramine ¹	Histamine ¹	Tyramine ¹	Histamine ¹	Tyramine ¹
Control ³	0,2	0	0,2	0	0,3	0
PSU-1	0,5	0	0,1	0	0,2	0
L. oenos	0,2	0	0,1	0	0,2	0
Equilait	²	0	0,2	0	0,2	0
P. cerevisiae	0,3	0	0,2	0	0,2	0
L. plantarum	0,4	0	0,2	0	0,2	0
L. buchnerii	0,3	0	0,1	0	0,3	0

¹mg.l⁻¹

²Did not undergo malo-lactic fermentation.

³Was not inoculated with bacteria, only yeast.

The analyses of the different musts used to determine the effect of bacterial strain on the amount of amines formed, are summarised in Table 7. Only the Pinotage must contained traces of histamine. None of the musts contained tyramine.

TABLE 7

Analyses of musts used to determine the effect of malo-lactic bacteria on the formation of amines

Analysis	Chenin blanc	Chenin blanc (Thermovinification)	Pinotage (Thermovinification)
Free SO ₂ mg.l ⁻¹	3	7	15
Total SO ₂ mg.l ⁻¹	15	17	30
pH	3,46	3,59	3,58
Total acidity g.l ⁻¹	5,2	6,0	6,3
Sugar content (°B)	20,4	21,0	25,6
Free amino nitrogen	1 071	1 280	1 300
Histamine mg.l ⁻¹	0	0	0,1
Tyramine mg.l ⁻¹	0	0	0

DISCUSSION AND CONCLUSIONS

Histamine and tyramine content: The average histamine content of wines produced in South Africa is very similar to those produced in other countries. (Ough, 1971; Mayer & Pause, 1973; Subden *et al.*, 1979; Zee *et al.*, 1983). The average histamine content of South African red wines that had undergone malo-lactic fermentation was more than double that of red wines that had not undergone malo-lactic fermentation. It therefore appears that bacteria responsible for malo-lactic fermentation are responsible for high histamine levels. However, some wines that presumably had not undergone malo-lactic fermentation had histamine contents of more than 5 mg.l⁻¹, indicating that it is not only the malo-lactic bacteria that could be responsible for high histamine levels.

Conditions during malo-lactic fermentation, as well as during wood maturation of red wines could be favourable for the growth of other bacteria which could produce histamine. In most cases the addition of SO₂ after alcoholic fermentation is limited so as not to inhibit the bacteria responsible for malo-lactic fermentation, thus also making conditions favourable for other bacteria. Many red wines undergo malo-lactic fermentation spontaneously during wood maturation indicating that malo-lactic, as well as other bacteria, survive in the cooperage.

All the South African red wines containing more than 10 mg.l⁻¹ of histamine had pH's above 3,77. The tendency of the South African red wines with higher pH's to have a higher histamine content is in good agreement with results reported by Mayer (1976) and Aerny (1982). It would seem that many South African red wines have the potential to have high histamine contents as the average pH of the red wines analysed was 3,77. Therefore wines, especially those which have to undergo malo-lactic fermentation, should have pH's of less than 3,77 to ensure low histamine levels.

The red wines analysed contained much more histamine than the white wines.

Probable reasons for this phenomenon are:

Red wine vinification is usually carried out in the presence of grape pulp, and this causes a higher amount of histidine in the must (Zee *et al.*, 1983).

Many red wines undergo malo-lactic fermentation spontaneously, specially during wood maturation. Histamine could be produced by bacteria responsible for the malo-lactic fermentation or by contaminating bacteria developing under conditions favourable for their growth.

The practice of settling must before alcoholic fermentation, racking off the lees, adding SO₂ as soon as fermentation is completed and the filtration of the wine at an early stage during the vinification of white wines, lower the histidine as well as total nitrogen content and minimize the possibility of bacterial contamination (Amerine & Kunkee, 1968; Amerine & Joslyn, 1973).

Bentonite partially absorbs histamine (Jacob, 1968). Bentonite is used more often during white wine vinification than during red wine vinification and therefore also contributes to the lower histamine levels of the white wines.

The tendency of white wines to have lower pH values than red wines (average pH 3,45 for white wines, compared to pH 3,77 for red wines) also contributes to lower histamine levels of the white wines.

The rosé wines analysed, contained more histamine than the white wines, but less than the red wines. As rosé wines are often produced by blending white and red wines, average histamine content of such wines can be expected to be more than that of white wines, but less than that of red wines.

The average tyramine content of the South African wines is much lower than the values reported by Mayer & Pause (1973) and Zee *et al.*, (1983). Sen (1969) and Zappavigna & Cerutti (1973), however, reported tyramine contents in wines which are comparable with those of South African wines. One of the probable reasons for the relatively big differences in the average tyramine content of wines as reported, could be as a result of the varied methods used for their determination.

As was the case for histamine, differences were observed in the average tyramine content of red wines that had undergone malo-lactic fermentation compared to wines that had not undergone malo-lactic fermentation. Although the average tyramine values were much lower, and the differences smaller, the same tendencies are apparent. Wines which had undergone malo-lactic fermentation had an average tyramine content of 0,6 mg.l⁻¹ compared to the 0,2 mg.l⁻¹ of the wines which had not undergone malo-lactic fermentation.

The tyramine content of the South African red wines does not seem to be dependant on the pH of the wines. Wines with high pH's do not seem to have higher tyramine levels as found with histamine.

Although the tyramine content of the wines analysed was much lower than the histamine content, similar differences between red and white wines were observed, with the average tyramine content of red wines being 0,5 mg.l⁻¹ compared to 0,1 mg.l⁻¹ for white wines. The same reasoning for the differences in the histamine content of red and white wines would apply to the differences in the tyramine content.

Factors influencing the formation of amines during malo-lactic fermentation: Three types of wine were made to investigate the ability of bacteria, used for malo-lactic fermentation, to form histamine and tyramine. White wine was made according to the traditional method, as

well as according to the red wine method of thermovinification and a red wine was made by the thermovinification method. The six bacterial strains used did not produce histamine or tyramine. It therefore seems that the ability to produce histamine and tyramine occurs infrequently amongst the bacteria that could be used for malo-lactic fermentation. Previous investigators have also indicated that only certain specific strains of malo-lactic bacteria possess the ability to decarboxylate histidine and tyrosine (Lagerborg & Clapper, 1952; Rodwell, 1953). Of various malo-lactic bacteria isolated from wine by Lafon-Lafourcade (1975), only one strain of *Leuconostoc* had the ability to produce histamine. Weiller & Radler (1976) tested more than a hundred strains of lactic acid bacteria isolated from wine and found only a single strain of *Pediococcus* able to decarboxylate histidine. None of the bacteria used in this study, including a *Pediococcus* strain as well as two *Leuconostoc* strains produced histamine or tyramine.

The high average histamine content of the commercial red wines that had undergone malo-lactic fermentation, could also have been partly due to bacteria other than those responsible for malo-lactic fermentation. Although most studies concerning bacteria in must and wine are limited to lactic acid bacteria (Du Plessis & Van Zyl, 1963; Costello, Morrison, Lee & Fleet, 1983) the presence of other bacteria such as *Pseudomonas*, *Micrococcus* or *Bacillus* in must has been indicated (Amerine & Kunkee, 1968).

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