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THE EFFECT OF HARVEST TIME AND BENTONITE TREATMENT ON HISTAMINE AND TYRAMINE CONTENTS OF WINES

ŞARAPLARIN HİSTAMİN VE TİRAMİN İÇERİKLERİ ÜZERİNDE HASAT ZAMANI VE BENTONİT İŞLEMİNİN ETKİSİ

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ABSTRACT: Histamine and tyramine contents of 16 white wines from Colombard grapes and 4 red wines from Carignan grapes were determined by high performance liquid chromatography. Ten wines were produced from early harvested grapes and the remaining 10 wines from late harvested ones. Various amounts of bentonite were added before fermentation in the processing of 12 white wines. Histamine and tyramine contents of wines of low acidity from late harvested grapes were found greater than those for more acidic wines from early harvested grapes. Alcohol percentage of wines was not effective on histamine and tyramine contents. Bentonite treatment caused a decrease in histamine and tyramine concentrations in white wines containing high levels of these biogenic amines.

Keywords: Biogenic amines, wine, bentonite

ÖZET: Colombard üzümlerinden üretilen 16 adet beyaz şarap ve Carignan üzümlerinden üretilen 4 adet kırmızı şarabın histamin ve tiramin içerikleri Yüksek Basınç Sıvı Kromatografisiyle (HPLC) belirlendi. 10 adet şarap erken hasat edilen üzümlerden 10 adet şarap ise geç hasat edilen üzümlerden üretildi. 12 adet beyaz şaraba fermantasyon öncesi çeşitli miktarlarda bentonit ilave edildi. Geç hasat edilen düşük asitli şarapların histamin ve tiramin içerikleri erken hasat edilen üzümlerin yüksek asitli şaraplarına göre daha fazla bulundu. Şarapların alkol oranlarının histamin ve tiramin düzeylerine etkisinin olmadığı gözlendi. Bentonit ilavesi bu biyojen aminleri yüksek seviyede içeren beyaz şarapların histamin ve tiramin miktarlarında azalmaya neden oldu.

Anahtar kelimeler: Biyojen aminler, şarap, bentonit

INTRODUCTION

Biogenic amines are produced by lactic acid bacteria during the process of fermentation of foods and beverages as a result of amino acid decarboxylation, for example in cheese, sausage, fermented vegetables and wine. Large amounts of these amines have been reported in aged, fermented or spoiled products. Many bacterial genera are able to decarboxylate amino acids (1, 2). On the other hand biogenic amines may be formed and degraded in normal metabolic activity of animals, plants and microorganisms (3).

High concentrations of biogenic amines may cause undesirable physiological effects in sensitive humans. Biogenic amines may exert vasoactive effects and psychoactive effects. Symptoms that can occur after excessive oral intake of biogenic amines are nausea, respiratory distress, hot flush, sweating, heart palpitations, headaches, bright red rash, oral burning and hyper or hypotension (4).Under normal conditions, in human beings exogenous biogenic amines from foods are rapidly detoxified by some enzymes involved in

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biogenic amine metabolism, such as monoaminoxidase, diaminoxidase and histamine methyltransferase. Thus biogenic amines do not usually represent any health hazard to individuals unless large amounts are ingested or the natural mechanism for their catabolism is inhibited or genetically deficient. Biogenic amines, especially histamine, are considered to be the main substances responsible for allergic reactions towards wines.

The identification of biogenic amines in wine samples was carried out by several investigations. More than 20 amines were identified in wines and their total concentrations were reported to range from a few mg/L to about 50 mg/L depending on many factors, but in general it was related to the quality of wine (5). Histamine, tyramine and putrescine are the most significant biogenic amines encountered in wines. Of these, histamine was studied the most and several papers reported its presence in wine (6-11). According to Lonvaud-Funel (2), biogenic amines were produced by lactic acid bacteria during the malolactic fermentation following the alcohol fermentation. Indigenous bacteria population present on wine cellar equipment, resulting from the natural microflora of the grape berries, caused the formation of these amines in wines. The amino acid-decarboxylating activity was strain specific and histidine-decarboxylating bacteria were found in all the wine-producing areas. On average 80 % of the total population was potentially able to produce histamine, and histamine-containing wines also contained tyramine and putrescine. pH was a selective factor for microorganisms in wine, as pH increased, the number and variety of the microbial population increased. In addition, SO_2 added at the end of malolactic fermentation was less efficient at higher pHs and relatively high lactic acid bacteria populations were often encountered several months after vinification. Even if they were not growing, these populations survived and were still metabolically active. They still produced biogenic amines. Goni and Azpilicueta (12) studied the effect of yeast strain on biogenic amines content in wines. Depending on the yeast strain involved in the fermentation, there was a slight difference in the content of biogenic amines in wines, although high concentrations were never reached.

Bentonite is most commonly used to remove excess protein from white wines. The treatment is carried out after the termination of the biological processes. Sometimes bentonite is used before fermentation. Thus biogenic amines content of wines may be significantly reduced by bentonite treatment. Special care is needed with red wines to avoid decolourisation. It was reported that treatment with 5 g/L of bentonite was generally sufficient to reduce the histamine content of wines below the tolerance limit of 2 mg/L (13).

In most wine-producing areas, good wine is only achieved with fully ripe grapes and good vintages are related invariably with hot summers, which give grapes of optimum maturity. The state of maturity of grapes was one of the principal factors in winemaking (14). In this connection, pH value was a very important factor and affected biogenic amines content of wine. At high pHs, biogenic amines were always produced in high amounts (15). White wines which are generally more acidic contained lower biogenic amine concentrations than red wines (2). It was reported that the higher histamine concentrations were related with increasing wine pH (16).

Vinification method may also influence the final biogenic amines content in wines (15). Therefore in this work the effect of harvest time of grapes, bentonite treatment, retaining of lees on the production of histamine and tyramine in white wines and red wines was examined. Concentrations of histamine and tyramine in wines were determined after two years of ageing.

MATERIALS and METHODS

Materials

Colombard and Carignan grapes were harvested from vineyards of Sevilen Winery in İzmir. White wines and red wines were produced from the Colombard and Carignan grapes respectively.

Reagents and standards: Histamine dihydrochloride and tyramine were purchased from Sigma. Sodium hydroxide, benzoyl chloride, hydrochloric acid, sodium chloride and anhydrous sodium sulphate were obtained from Merck. Methanol HPLC grade and diethyl ether were purchased from Lab-Scan and polyvinylpyrrolidone (PVP) from ISP England. Stock biogenic amines solutions were prepared by dissolving 50 mg of histamine dihydrochloride and 25 mg of tyramine in 25 mL of 0.1 M HCl solution separately.

METHODS

Sample preparation

Grapes were harvested at two different maturities, 25 kg each (total soluble solids of musts were 18.7 brix and 21.0 brix for Colombard grapes and 17.5 brix and 23.0 brix for Carignan grapes). In white wine production the grapes, after separating from their stems, were crushed and pressed. Then the juice alone was put into 3 liters demijohns. Different amounts of bentonite (0.5, 1.0, 1.5 g/L) were added to the white grape (Colombard) juice before fermentation process. Bentonite treatment was not applied to red wines to avoid removing of red pigments. For red wines the grapes were destemmed and crushed. The resulting juice and skins were put into the demijohns for skin fermentation. Then free run and press wine were put into the demijohns for finishing fermentation to metabolize residual sugar. All the wines were sulfited (50 mg/L SO_2) before fermentation. Fermentations were realised with Fermivin (2 %, *Saccharomyces cerevisiae* 7013 INRA) at 23-25°C. After fermentation, half portions of white wines and red wines were not separated from the lees for encouraging malolactic fermentation. Wines of malolactic fermentation were racked off after 12 days. All wines were aged for 2 years. Bottled wines were stored at 15°C.

Determinations of histamine and tyramine

Histamine and tyramine content of wines was determined using HPLC technique at the end of the storage period.

Derivatization procedure: The benzoyl derivatives of histamine and tyramine were prepared following the method of Hornero-Méndez and Garrido-Fernández (17), but with modification. Before the derivatization, phenolic compounds in wine were removed with polyvinylpyrrolidone to eliminate their interference. 0.2 g of PVP was added into 5 mL of the wine sample. The resulting mixture was then stirred for 15 minutes and filtered through Whatman (41) filter paper. Filtrate was made up to the initial volume of 5 mL by distilled water and transferred to a test tube. Three mL of 5 M sodium hydroxide and 60 mL of benzoyl chloride were added. After vigorous shaking for 30 seconds the mixture was left for 45 minutes at 25°C. Following the addition of 5 mL of saturated sodium chloride solution the mixture was extracted 4 times successively with 3 mL aliquots of diethyl ether in a separator funnel. The organic phases were pooled and dried with anhydrous sodium sulphate, decanted and evaporated under a current of nitrogen. The residue was dissolved in 1 mL of methanol, filtered through a 0.50 mm pore size filter and 10 mL of the solution was injected on HPLC column. Chromatograms were obtained for two aliquots of the same wine sample. Quantifications were performed by the standard addition method.

Apparatus: Chromatographic experiments were performed using HP 1050 Liquid Chromatograph equipped with Waters 486 absorbance detector and an injection loop of 20 µL. Separation was carried out by using Phenomenex Bondclone C_{18} column (10 µm particle size, 300 x 3,9 mm) at 25°C.

Chromatographic conditions: Chromatographic separations were achieved by using a binary gradient elution with methanol and deionised water. The gradient started at 55 % (v/v) methanol and was increased to 100 % methanol in 20 minutes. The run time was 30 minutes. The flow rate was 1 mL/min and detection was performed at 254 nm. HPLC gradient profile for the separation of histamine and tyramine is reported in Table 1.

Chemical analyses

Total soluble solid contents of the musts were measured with the aid of Abbe Refractometer (IT 4T). Alcohol percentages of wines were determined by picnometer. Total acidities of wine samples were measured following the method of Ough and Amerine (18). pH values of wines were measured with a digital pH meter (Accumed Model 10 pH meter, Fischer Scientific). Alcohol percentages, total acidities and pH values of the wine samples were measured after 2 years of storage. All chemical analyses were realised as three replicates in one sample.

Table 1. HPLC gradient profile for the separation of benzoyl derivatives of histamine and tyramine

	Mobile phase composition (%, v/v)	
Time (min)	Methanol	Water
0	55	45
4	60	40
12	85	15
15	85	15
20	100	0
25	55	45
30	55	45

Statistical analysis

Paired t test was used to find the differences between amounts of biogenic amines in wine samples.

RESULTS and DISCUSSION

Histamine and tyramine concentrations, alcohol percentages, total acidities and pH values of 16 white wines from Colombard grapes and 4 red wines from Carignan grapes were determined. Description of these 20 wines is reported in Table 2. Histamine and tyramine concentrations of 8 white wines produced from early harvested Colombard grapes are shown in Table 3. Histamine and tyramine values of 8 white wines produced from late harvested Colombard grapes are presented in Table 4. Histamine and tyramine values of 4 red wines obtained from early harvested and late harvested Carignan grapes are given in Table 5. Average histamine, tyramine, alcohol, total acid and pH values of red wines and white wines are tabulated in Table 6.

Table 2. Description of 20 wine samples

White wines		
1. early harvested	with lees	0.5 g/L bentonite
2. early harvested	with lees	1.0 g/L bentonite
3. early harvested	with lees	1.5 g/L bentonite
4. early harvested	with lees	without bentonite
5. early harvested	without lees	0.5 g/L bentonite
6. early harvested	without lees	1.0 g/L bentonite
7. early harvested	without lees	1.5 g/L bentonite
8. early harvested	without lees	without bentonite
9. late harvested	with lees	0.5 g/L bentonite
10. late harvested	with lees	1.0 g/L bentonite
11. late harvested	with lees	1.5 g/L bentonite
12. late harvested	with lees	without bentonite
13. late harvested	without lees	0.5 g/L bentonite
14. late harvested	without lees	1.0 g/L bentonite
15. late harvested	without lees	1.5 g/L bentonite
16. late harvested	without lees	without bentonite
Red wines		
17. early harvested	with lees	
18. early harvested	without lees	
19. late harvested	with lees	
20. late harvested	without lees	

Table 3. Histamine and tyramine compositions of white wines produced from early harvested 18.7 brix Colombard grapes

Type of wine	With lees		Without lees	
	Histamine mg/L	Tyramine mg/L	Histamine mg/L	Tyramine mg/L
Without bentonite	0.80	0.34	0.23	0.64
0.5 g/L bentonite	1.37	0.39	1.72	0.52
1.0 g/L bentonite	0.33	0.74	0.47	0.15
1.5 g/L bentonite	1.38	0.09	0.60	0.70

Table 4. Histamine and tyramine compositions of white wines produced from late harvested 21.0 brix Colombard grapes

Type of wine	With lees		Without lees	
	Histamine mg/L	Tyramine mg/L	Histamine mg/L	Tyramine mg/L
Without bentonite	5.12	1.91	15.7	1.17
0.5 g/L bentonite	3.13	0.82	5.37	1.15
1.0 g/L bentonite	3.97	1.22	2.64	0.60
1.5 g/L bentonite	1.51	0.38	4.19	2.05

Table 5. Histamine and tyramine compositions of red wines produced from early harvested (17.5 brix) and late harvested (23.0 brix) Carignan grapes

Type of wine	With lees		Without lees	
	Histamine mg/L	Tyramine mg/L	Histamine mg/L	Tyramine mg/L
Early harvested	1.18	0.58	2.95	0.97
Late harvested	32.4	0.94	9.88	1.13

Effect of acidity and alcohol concentration

As it is seen from the Tables 3 and 4, histamine concentrations of white wines produced from late harvested Colombard grapes are significantly higher than those of white wines from early harvested Colombard grapes ($p \leq 0.05$). Tyramine values are also significantly higher in white wines from late harvested Colombard grapes than in early harvested ones ($p \leq 0.05$). The same trends are also seen in red wines. The red wines from late harvested Carignan grapes contain more histamine and tyramine than those from early harvested Carignan grapes (Table 5). It is seen from the Table 6 that, acidities of the late harvested wines are lower than early

Table 6. Average histamine, tyramine, alcohol, total acid and pH values of red and white wines (numbers in parentheses are standard deviations)

	Red wines		White wines			
			Early harvested		Late harvested	
	Early harvested	Late harvested	Without bentonite	With bentonite	Without bentonite	With bentonite
Histamine, mg/L	2.06(1.3)	21.1(16)	0.52(0.40)	0.98(0.58)	10.4(7.5)	3.47(1.3)
Tyramine, mg/L	0.78(0.28)	1.04(0.13)	0.49(0.21)	0.43(0.27)	1.54(0.52)	1.04(0.59)
Alcohol, % (v/v)	8.4(0.1)	10.5(0.1)	8.7(0.1)	8.6(0.1)	9.8(0.1)	10.2(0.1)
Total acida, g/L	6.23(0.08)	5.15(0.10)	8.22(0.08)	8.56(0.12)	6.10(0.09)	6.06(0.11)
pH	3.40(0.02)	3.74(0.01)	3.20(0.01)	3.15(0.01)	3.35(0.01)	3.40(0.01)

[a] as tartaric acid

harvested wines. It is concluded that histamine and tyramine concentrations are higher in white and red wines with low acidity. These results are in accordance with the report of Vasquez-Lasa et al. (16) who found that histamine levels of Rioja wines with high pH were greater than those in low pH wines. Cilliers and Van Wyk (19) reported that pH values of red wines with high histamine levels were above pH 3.7. Inigo-Leal and Bravo-Abad (20) observed that wine samples contained elevated levels of histamine when their pHs were greater than 3.5. Lonvaud-Funel (2) reported that indigenous lactic acid bacteria population resulting from wine cellar equipment caused the formation of biogenic amines in wines, and that pH acted as a selective factor for microorganisms in wine, as pH increased the number and variety of the microbial population increased.

From the examination of Table 6, it is seen that alcohol percentages of white wines and red wines from late harvested grapes are greater than those from early harvested grapes. High alcohol percentage is not effective to inhibit the production of histamine and tyramine by microorganisms.

Effect of bentonite treatment before fermentation

In the past bentonite has been used just before bottling to obtain protein stability, but now it is used before fermentation. This application prevents protein turbidity even if protein content is high in that year. In addition, polyphenoloxidase enzymes are eliminated significantly and tendency of browning of wine decreases, thus less SO_2 is required. Bentonite also binds amino acids and phenolic compounds and usually it is not used in red wines as it removes red pigments.

As it is seen from the Table 4, histamine contents of late harvested white wines with lees and with bentonite are 3.13 mg/L, 3.97 mg/L and 1.51 mg/L for different concentrations of bentonite. All of these values are smaller than 5.12 mg/L of histamine for the white wine with lees but without bentonite. The same trend is seen for the tyramine contents of late harvested white wines with lees. Tyramine concentrations of wines with bentonite are 0.82 mg/L, 1.22 mg/L and 0.38 mg/L for 0.5 g/L, 1.0 g/L and 1.5 g/L bentonite respectively and these tyramine values are smaller than 1.91 mg/L of tyramine for wine with lees but without bentonite. In addition, histamine content is 15.7 mg/L for white wine without lees and bentonite. This value is greater than 5.37 mg/L, 2.64 mg/L and 4.19 mg/L for bentonite containing white wines without lees. Then it was concluded that bentonite application reduced protein and amino acid contents of white wines, thereby decreased histamine and tyramine contents. On the other hand, however, the binding effect of bentonite was not observed on the tyramine contents of late harvested white wines without lees, and on the histamine and tyramine contents of early harvested white wines. As is seen from the Tables 3 and 4, histamine and tyramine contents of early harvested

white wines without bentonite, and tyramine content of late harvested white wine without lees and bentonite are very low. We consider that, in these wines microbial activity was so low that the amounts of proteins and amino acids were not a limiting factor and the addition of bentonite had no effect on biogenic amine content.

Effect of keeping in contact with yeast lees after fermentation

Many papers report that red wines contain more histamine than white wines. During the production of red wine, grape skins and must are together during mash fermentation. In this period more histidine is transferred into must and decarboxylation of histidine by microorganisms causes high histamine content in red wines (Zee et al., 1983).7.The second reason for the high histamine content of red wines is malolactic fermentation that is often applied for red wines. According to some studies, histamine is produced from microorganisms responsible for malolactic fermentation (2, 9, 19, 21). It is seen from the Tables 3-5 that, keeping the wines in contact with lees for 12 days to support the malolactic fermentation had no effect on biogenic amine content. It may be supposed that, in our study, separation of lees by decantation was not perfect and a small part of lees remained in wines.

CONCLUSIONS

Wines with low pH contained lower amounts of biogenic amines. It was observed that, when the acidity increased, the levels of histamine and tyramine decreased in white wines and red wines. High alcohol percentage of white wines and red wines from late harvested grapes was not effective to inhibit the production of histamine and tyramine by microorganisms. In addition, it was found that bentonite treatment decreased the levels of histamine and tyramine in white wines containing high levels of these biogenic amines.

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