PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 46, NO. 1–2, PP. 73–81 (2002)

DETERMINATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN HUNGARIAN WINES

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Received: March 30, 2003

Abstract

Free amino acid, biogenic amine, resveratrol and polyphenol contents of Hungarian wines were studied. Principal component analysis was used to compare data. Significant differences were found between red, white and Tokaj wines concerning free amino acid and biogenic amine contents. The wine making technology had dominant effect on resveratrol and polyphenol contents of wines.

Keywords: wine, free amino acid, biogenic amine, resveratrol, polyphenols, chromatography.

1. Introduction

Wines are known to contain many biologically active compounds. The amounts and compositions of these compounds depend on the type of grapes and their degree of ripeness, climate and soil of the viticultural area, as well as vinification techniques.

Amino acid composition is of great importance in wine production. Amino acids provide a significant part of the nutritional requirements of both yeast and malolactic bacteria during wine fermentation and also serve as substrate for aroma compounds in wines [1].

Biogenic amines are among the factors that contribute to the quality of wines. These compounds are formed by microbiological decarboxilation of free amino acids. Consumption of beverages rich in some biogenic amines (e.g. histamine, tyramine) can lead to headaches, nausea, hot flushes, skin rashes, sweating, respiratory distress, cardiac and intestinal problems [2]. The recommended upper limit for histamine in wines is 2–10 mg/dm^3 [3, 4].

Resveratrol, a prominent representative of polyphenols present in fresh grapes and wines, has a pronounced biological activity. Resveratrol has cardioprotective effect, because it reduces e.g. the susceptibility of low-density lipoproteins (LDL) to lipid peroxidation (antioxidant effect), and shows a cancer preventing activity [5, 6].

The aim of our work was to characterize and compare Hungarian wines on the basis of biologically active compounds.

2. Materials and Methods

2.1. Effect of Technology on Composition of Wines

The effect of time of fermentation with skin of berries on the composition of two types of Hungarian red wines (Blauburger and Cabernet Sauvignon) from Eger harvested in 1999 was investigated. Fermentation time with skin was the following: 0, 3, 8, 15, 22 and 30 days [7].

2.2. Principal Component Analysis of Wines

Red and white wines from the same wine making region were compared on the basis of biogenic amine, resveratrol and polyphenol contents using principal component analysis (PCA) [8]. 17 red and 8 white wines from region of Pécs, harvested in 1998 were investigated. The wine samples were as follows: R1: Cabernet Franc, R2: Cabernet Sauvignon, R3: Merlot, R4: Pinot Noir, R5: Kadarka, R6: Kékfrankos, R7: Kékoportó, R8: Zweigelt, R9: Rubintos, R10: Vranac, R11: Blauburger, R12: Medina, R13: Bíborkadarka, R14: Kármin, R15: Alicante Bouschet, R16: Turán, R17: Titán, W18: Italian Riesling, W19: Rhenish Riesling, W20: Furmint, W21: Hárslevelű, W22: Sauvignon Blanc, W23: Chardonnay, W24: Cirfandli, W25: Zenit.

2.3. Comparison of Wine Varieties

Different kinds of wine varieties were compared on the basis of free amino acids and biogenic amines [9]. Six different white wine varieties (Italian Riesling, Rhenish Riesling, Szürkebarát, Sauvignon Blanc, Pinot Blanc and Chardonnay), two Tokaj wine varieties (Szamorodni and Aszú), and three red wine varieties (Kékfrankos, Cabernet Sauvignon and Cabernet Franc) were investigated.

2.4. Determination Methods of Biologically Active Compounds

Determination of free amino acids was made by ion-exchange chromatography using amino acid analyser [10]. Biogenic amines were determined by OPLC (over-pressured layer-chromatography) method (2.1., 2.2.) [11], and by ion-exchange chromatography (2.3.) [10]. Resveratrol was determined by OPLC method [12]. Total polyphenols were measured by spectrophotometric method [13].

3. Results and Discussion

3.1. Effect of Technology on Composition of Wines

The content of free amino acids ranged from 799 mg/dm^3 to 1143 mg/dm^3 in Blauburger and from 765 mg/dm^3 to 1418 mg/dm^3 in Cabernet Sauvignon. The total free amino acid content decreased until the 8th day of fermentation with skin of berries and slightly increased after that, and reached the maximum on the 15 days and 22 days treatment in Blauburger and in Cabernet Sauvignon, respectively. The main amino acids were proline (39% – 72% in Blauburger and 58% – 86% in Cabernet Sauvignon), γ-amino-butyric acid (2% – 10% in Blauburger and 2% – 14% in Cabernet Sauvignon) and glutamic acid (2% – 8% in Blauburger and 1% – 6% in Cabernet Sauvignon).

The content of biogenic amines varied from 27 mg/dm^3 to 69 mg/dm^3 in Blauburger and from 7 mg/dm^3 to 48 mg/dm^3 in Cabernet Sauvignon without any specific trend. The main biogenic amines were agmatine (15% – 49% in Blauburger and 5% – 77% in Cabernet Sauvignon), spermine (16% – 42 % in Blauburger and 7% – 26% in Cabernet Sauvignon) and spermidine (14% – 31% in Blauburger and 6% – 27% in Cabernet Sauvignon). Histamine was not detected in wine samples and tyramine occurred only in small amounts.

The content of resveratrol ranged from 0 mg/dm^3 to 3.10 mg/dm^3 in Blauburger and from 0 mg/dm^3 to 4.48 mg/dm^3 in Cabernet Sauvignon (*Fig. 1*). The content of total polyphenols varied from 386 mg/dm^3 to 1612 mg/dm^3 in Blauburger and from 322 mg/dm^3 to 1987 mg/dm^3 in Cabernet Sauvignon (*Fig. 2*). The highest amounts of these compounds were detected in the case of 22 days of fermentation with skin of berries in both wines, only total polyphenols had the highest concentration in Blauburger in the case of the 30 days treatment.

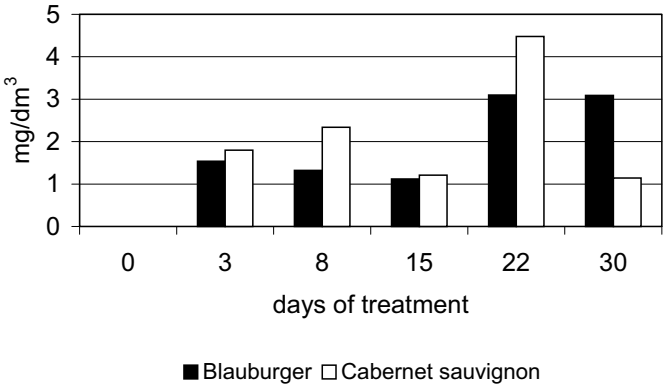

Fig. 1. The resveratrol content of wine samples

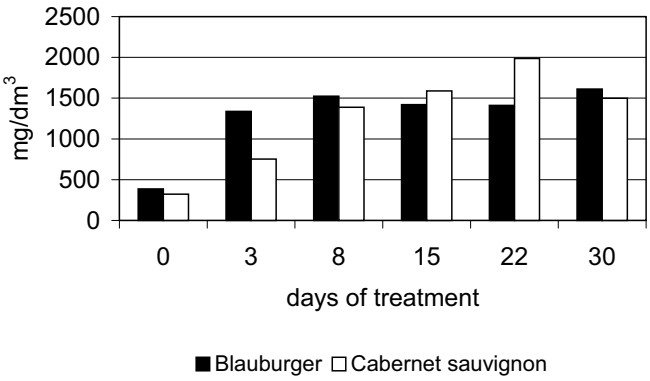

Fig. 2. The total polyphenol content of wine samples

3.2. Principal Component Analysis of Wines

PCA yields four principal components explaining more than 80% total variance in the data. The first new principal component correlates with SPD, TYM, RVR and SPPH well. TYM correlates with the new principal component negatively. The second principal component correlates with AGM and SBA, the third one with SPM and CAD, and the fourth one with HIM.

Red wines differ from white wines mainly because of their RVR and SPPH contents, which derives from the differences in the winemaking process. There is fermentation on the skin of the berries in case of red wines. This is because these compounds need more time to be solved.

Score plots show similarities amongst the different wine sorts. The first two principal components (PC) perform almost a perfect classification between red and white wines. Two distinct groups can be observed in *Fig. 3* separated with a line. The wine marked by R1 has low SPD and SPPH contents. Similarly, its RVR content is relatively low. This can justify the closeness of R1 point to those of the white wines. The next close point to the white wines is the point for R12. It is in the negative area because of its low SPPH and SBA contents. The points in anomalous position, R5 and R11 are outliers, and they have huge AGM and high SBA contents. There is no RVR and only low SPPH content in the wine marked by R5. W24 stands out amongst the white wines with its high AGM and SBA contents.

3.3. Comparison of Wine Varieties

The total content of free amino acids ranged from 386.92 mg/dm^3 to 2516.88 mg/dm^3 in white wines (*Table 1*), from 1161.40 mg/dm^3 to 3388.28 mg/dm^3 in Tokaj wines (*Table 2*), and from 644.32 mg/dm^3 to 1957.66 mg/dm^3 in red wines (*Table 3*).

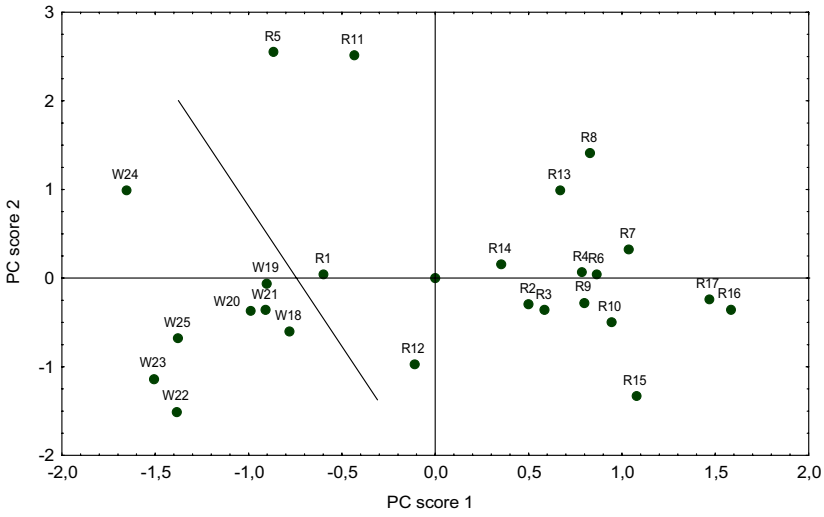

Fig. 3. Classification of red and white wines, PC score 1 vs. PC score 2

Based on the total free amino acid contents there were 2 distinct groups among white wines. Sauvignon Blanc, Rhenish Riesling and Italian Riesling belong to the first group with 794.33 mg/dm^3 average free amino acid content, and Pinot Blanc, Chardonnay, Szürkebarát belong to the second group with 1498.28 mg/dm^3 average free amino acid content.

There were significant differences among white wines, Tokaj wines and red wines concerning free amino acid compositions (*Fig. 4*). The dominant free amino acids were proline (80.7%, 54.2%, 29.7%, in red wines, in white wines, in Tokaj wines, respectively) and arginine (27.5%, 17.0%, 1.7% in Tokaj wines, in white wines, in red wines, respectively). Among the other amino acids γ-amino-butyric acid, alanine, aspartic acid, lysine, histidine, glutamic acid and ornithine were present at higher concentrations.

Proline:arginine ratio for red wines, white wines, and Tokaj wines were 46.2, 3.2 and 1.1, respectively (*Fig. 4*). Proline:arginine ratio seems to be used successfully to differentiate among wines.

There were significant differences among the different types of wines concerning biogenic amine contents and compositions. Biogenic amines were present at higher concentrations in red wine samples than in white wines. The total content of biogenic amines varied from 0.96 mg/dm^3 to 5.47 mg/dm^3 in white wines (*Table 1*), from 3.93 mg/dm^3 to 28.56 mg/dm^3 in Tokaj wines (*Table 2*), and from 1.77 mg/dm^3 to 33.85 mg/dm^3 in red wines (*Table 3*).

Fig. 5 shows the comparison of biogenic amine contents of different wines. The major biogenic amines were tyramine (77.3%, 33.9%, 12.7% in Tokaj wines, in white wines, in red wines, respectively), putrescine (56.5%, 54.5 %, 10.7 %, in

Table 1. Total free amino acid and biogenic amine contents of white wines (mg/dm^3)

Mark of sample	Geographical origin	Wine varieties	Year of vintage	Σ free amino acid	Σ biogenic amine
W-1	Somló	Italian Riesling	1998	895.23	3.25
W-2	Csopak	Italian Riesling	2000	750.47	1.35
W-3	Bogács	Italian Riesling	2000	599.48	2.64
W-4	Akasztó	Italian Riesling	2000	922.73	3.90
W-5	Badacsony	Italian Riesling	2000	1020.34	2.87
W-6	Szekszárd	Rhenish Riesling	1999	386.92	1.33
W-7	Dunavölgy	Rhenish Riesling	2000	1174.75	3.95
W-8	Badacsony	Szürkebarát	1997	1111.57	3.52
W-9	Badacsony	Szürkebarát	1998	922.62	3.88
W-10	Mátraalja	Szürkebarát	2000	2516.88	5.47
W-11	Szentantalfa	Szürkebarát	2000	1222.88	2.98
W-12	Tolna	Szürkebarát	2000	1978.81	0.96
W-13	Szőlősgyörök	Sauvignon Blanc	2000	780.70	4.49
W-14	Neszmély	Sauvignon Blanc	2000	883.74	2.65
W-15	Etyek	Sauvignon Blanc	2000	528.93	1.07
W-16	Csopak	Pinot Blanc	2000	1017.96	1.97
W-17	Dél-Dunántúl	Pinot Blanc	2000	1499.69	1.72
W-18	Lesencetomaj	Chardonnay	2000	1657.12	3.01
W-19	Dörgicse	Chardonnay	2000	1477.96	1.91
W-20	Siklós	Chardonnay	2000	991.43	2.06
W-21	Bogács	Chardonnay	2000	1288.89	2.62
W-22	Dél-Dunántúl	Chardonnay	2000	2293.52	2.73

red wines, in white wines, in Tokaj wines, respectively), histamine (23.7%, 5.6%, 3.6%, in red wines, in Tokaj wines, in white wines, respectively) and spermidine (5.5%, 4.4%, 3.3%, in white wines, in red wines, in Tokaj wines, respectively).

Based on putrescine:tyramine ratio it was possible to distinguish 2 groups of white wines. Italian Riesling, Rhenish Riesling and Szürkebarát belong to the first group, where the putrescine:tyramine ratios were 1:1 and the second group contains Sauvignon Blanc, Pinot Blanc and Chardonnay where the putrescine:tyramine ratios were 4:1, similarly to red wines. In the contrary the putrescine:tyramine ratios were 1:7 in Tokaj wines (*Fig. 5*).

4. Conclusion

Based on the results of technological experiments it seems that there is an optimal fermentation time with skin for resveratrol and polyphenol contents. The free

Table 2. Total free amino acid and biogenic amine contents of Tokaj wines (mg/dm^3)

Mark of sample	Geographical origin	Wine varieties	Year of vintage	Σ free amino acid	Σ biogenic amine
T-1	Tokaj	Sweet Szamorodni	1999	1625.95	3.93
T-2	Tokaj	Sweet Szamorodni	1999	1833.22	14.43
T-3	Tokaj	Sweet Szamorodni	1998	1161.40	12.67
T-4	Tokaj	Sweet Szamorodni	1997	2721.18	19.09
T-5	Tokaj	Sweet Szamorodni	1996	1228.94	13.86
T-6	Tokaj	5-butt Aszú	1999	1750.70	25.45
T-7	Tokaj	6-butt Aszú	1999	1589.52	15.33
T-8	Tokaj	6-butt Aszú	1999	1918.25	28.56
T-9	Tokaj	6-butt Aszú	1997	3388.28	25.14
T-10	Tokaj	6-butt Aszú	1995	2047.97	23.15
T-11	Tokaj	5-butt Aszú	1993	2677.28	28.34

Table 3. Total free amino acid and biogenic amine contents of red wines (mg/dm^3)

Mark of sample	Geographical origin	Wine varieties	Year of vintage	Σ free amino acid	Σ biogenic amine
R-1	Egerhegy	Kékfrankos	1997	974.56	3.61
R-2	Eger	Kékfrankos	1999	789.27	2.85
R-3	Kiskőrös	Kékfrankos	1994	795.12	13.96
R-4	Mőcsény	Kékfrankos	1999	1345.08	19.91
R-5	Kunság	Kékfrankos	2000	1209.72	4.69
R-6	Kunság	Kékfrankos	2000	1019.61	4.98
R-7	Sopron	Kékfrankos	2000	1344.37	33.85
R-8	Kunság	Kékfrankos	2000	644.32	1.77
R-9	Nagyréde	Kékfrankos	2000	1571.40	6.12
R-10	Villány Barrique	Cabernet Sauvignon	1999	1778.66	10.63
R-11	Sopron	Cabernet Sauvignon	1999	988.92	5.74
R-12	Eger	Cabernet Sauvignon	2000	1022.12	6.78
R-13	Szekszárd	Cabernet Sauvignon	1999	1846.60	4.79
R-14	Kéthely	Cabernet Sauvignon	2000	1162.23	4.54
R-15	Szekszárd	Cabernet Franc	1999	1957.66	6.21

amino acid and biogenic amine contents can be used to differentiate among wine varieties. Red and white wines can be distinguished according to their biogenic amine, resveratrol and polyphenol contents using principal component analysis.

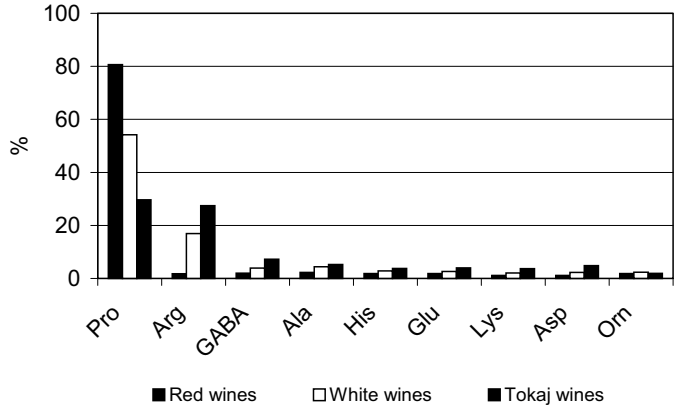

Fig. 4. Comparison of free amino acid contents of wines. Pro (proline), Arg (arginine), GABA (γ-amino-butyric acid), Ala (alanine), His (histidine), Glu (glutamic acid), Lys (lysine), Asp (aspartic acid), Orn (ornithine)

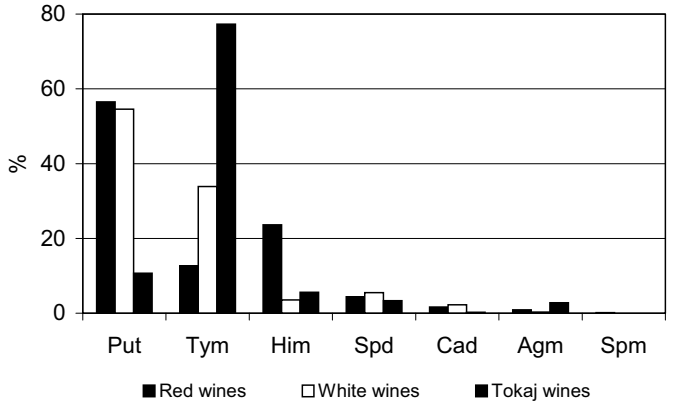

Fig. 5. Comparison of biogenic amine contents of wines. Put (putrescine), Tym (tyramine), Him (histamine), Spd (spermidine), Cad (cadaverine), Agm (agmatine), Spm (spermine)

Acknowledgement

This research was supported by a grant from Hungarian National Scientific Research Fund (OTKA T 029389).

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