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Protection of Originality of Tokaji Aszú: Amines and Organic Acids in Botrytized Wines by High-Performance Liquid Chromatography

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Amine and organic acid composition of Aszú wines from the Tokaj region of Hungary, nonbotrytized Hungarian wines from different regions, and foreign botrytized wines were analyzed by high-performance liquid chromatography. Hungarian and foreign wines (36 Hungarian and seven foreign botrytized wines) were compared in different ways by calculation of ratios of given amine compounds, analyses of variance, principal component, and discriminant analysis. In wines, putrescine and in some samples 3-methyl-butylamine and/or phenyl ethylamine were found in remarkable concentrations, while in botrytized wines four other amines were verified in high concentration. Good separation between Aszú and foreign botrytized wines was found by calculation of the amine component's ratio. The first two principal components of the principal component analysis accounted for 77 and 84% of the total variance in the data of amines and acids, respectively. The component scores of samples grouped according to Aszú, foreign botrytized, and nonbotrytized wines. Linear discriminant analysis was used for differentiation of Aszú, foreign botrytized, and normal wines. Using nine amines and two acids as variables, the correct classification was 97.6%. On the basis of results, an objective evaluation method can be elaborated for quality control in order to protect the authenticity and origin of wine specialties made from botrytized grapes.

KEYWORDS: Tokaji Aszú wines; biogenic amines; polyamines; HPLC; *Botrytis cinerea*; principal component analysis; discriminant analysis

INTRODUCTION

Botrytis cinerea can cause both a very destructive gray mold rot and in certain conditions the so-called noble rot on grape berries, which yields wines of special quality that are highly prized, sweet, smooth, and full-bodied with a pleasant bouquet (*1*). *B. cinerea*, as a noble mold, is responsible for the formation of Aszú grapes. This fungus pierces and weakens the grape skin while altering the composition of grapes by converting it to a raisin-like form (*2*). During this natural process, the water content decreases and the compounds are concentrated (sugar, acids, aroma compounds, etc.). The result is an unusually delicious and noble taste and flavor developing in the grape.

Tokaj wines are made from white grape varieties such as Furmint, Yellow Muscat, and Linden Leaf. The Tokaj region offers a full and varied range of products, all made according to time-honored traditions. To produce Aszú wines, the shriveled, raisin-like grapes affected by the noble rot of *B. cinerea* are harvested in October and November into wooden butt with a capacity of 20−25 kg. Three, four, five, or six butts of Aszú paste are added to newly fermented dry wine of the same year in a Gönci oak barrel (136 L), mixed, and soaked for 1 or 2 days in order to extract the natural sugar content and flavors. The wine is then drawn off to ferment. Eszencia is the first run juice of the Aszú grapes, which seeps from the press under the own weight of the grapes (*2*).

Methods suitable for determination of origin and identity of products for such a rare wine as Tokaji Aszú are especially important from economical and health points of view. An increasing number of papers studying specific compounds or groups of compounds for the detection of manipulation or authentity of foods and beverages can be found in the literature (*3*−*5*). Many successful works have shown that it is possible to distinguish grape variety, vintage years, or geographical zones on the basis of chemical parameters (trace elements, major organic acids, amino acids, isotopic composition) (*6*−*9*).

In this regard, the study of amines has great importance because one part of the biologically active and nonactive amines is formed in Aszú grapes during botrytization (*10*, *11*). Biogenic amines, as a group of aliphatic, alicyclic, or heterocyclic compounds of low molecular weight, naturally occur in plants and in fermented foods (*12*).

Putrescine, spermidine, and spermine are the major cellular polyamines in living organisms. These biogenic amines are involved in cellular growth, regulation of nucleic acid and protein synthesis, stabilization of lipids, brain development,

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nerve growth, and regeneration (*13*). Other biogenic amines such as histamine, tyramine, and phenylethylamine in foods and beverages can cause allergenic reactions in humans. These compounds are formed primarily from the decarboxylation of amino acids by the action of microorganisms. They can be indicator compounds of hygiene, technology, and for the activity of *B. cinerea* (*14*).

Although numerous methods have been developed for separation and determination of the biogenic amines in foods and beverages, including wine (*15*−*20*), only few authors studied the effect of botrytization on the composition of amines in grapes and wines so far (*10*, *11*, *14*, *21*).

Quantitative determination of organic acids can corroborate sensorial, microbiological quality assessment (*22*), and authentication (*6*) of wines. The most important (*23*) organic acids occurring at relatively considerable amounts in wines are tartaric, malic, citric, lactic, succinic, and acetic acids. Tartaric, malic, and citric acids originate from the fruit (*23*). Among organic acids of biological origin, the most relevant one is lactic acid, which originates from alcoholic or malolactic fermentation. When grapes become infected with *B. cinerea* under ideal conditions, water is lost from the berry and the acid constituents are concentrated. However, at the same time, the *Botrytis* fungus metabolizes these organic acids for use as an energy source (*23*).

The goal of our work was to explore whether the amine and organic acid composition is suitable to characterize botrytized wines and to determine the origin and authenticity of wines as well. We analyzed Hungarian Aszú wines, nonbotrytized wines, and foreign botrytized wines to study their differences and establish the foundations of an objective method for evaluating and classifying wines.

MATERIALS AND METHODS

Reagents. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from Merck, and ultrapure water generated by the Milli-Q System (Millipore) was used. Other reagent grade chemicals were as follows: anhydrous sodium acetate, boric acid, potassium hydroxide, acetic acid, Brij 35, and 2-mercaptoethanol from Reanal (Budapest, Hungary); *o*-phthaldialdehyde from Fluka; and sodium-octanesulfonate from Romil (Cambridge, United Kingdom). Biologically active amines such as putrescine (Put), *i*-butylamine (iBa), cadaverine (Cad), tyramine (Tyr), histamine (His), 2-methyl-butylamine (2MeBa), agmatine (Agm), 3-methyl-butylamine (3MeBa), *n*-pentyl-amine (Pa), spermidine (Spd), phenylethylamine (Phe), tryptamine (Trp), spermine (Spn), and hexylamine (internal standard, Istd) were from Sigma. Organic acids (tartaric acid, citric acid, acetic acid, and lactic acid) were purchased from Reanal, except malic acid (Schuchardt, München), shikimic acid (Sigma), and fumaric acid (Merck).

Materials. Thirty-two four-, five-, six-butt Tokaji Aszú, four Tokaji Eszencia wine specialities of different vintages and producers, and seven foreign botrytized wines (G, S, F1, F2, F3, A1, and A2) from four different countries (Germany, Slovakia, France, and Austria) were analyzed. The studied wines took part in the 6th International Wine Competition VinAgora 2002 organized in Budapest. After the bottles were opened, wine samples were taken and frozen at −20 °C until analysis. Hungarian nonbotrytized wines were purchased in local stores and opened before analysis. Wines were injected directly into the column after filtration without any further sample preparation.

HPLC Analysis and Chromatographic Conditions. *Amines.* The separation of amines and organic acids was performed with Alliance Waters 2690 HPLC chromatographic system equipped with a Waters 474 fluorimetric detector (λ_{ex} = 345 nm, λ_{em} = 455) and photodiode array detector (Waters 996), respectively.

Amines were separated and quantified with ion pair formation (octanesulfonic acid) on reverse phase column (μBondapak C18, 300 mm × 3.9 mm, 10 μm; from Waters) using postcolumn derivatization with OPA (*o*-phthaldialdehyde, 2-mercaptoethanol) (*24*) according to the method of Seiler and Knödgen (*25*). This method provides good separation of amines without possible interference of amino acids (*25*). It was verified that amino acids were eluted in the first 20 min; therefore, they did not interfere with the amines studied. For gradient elution, three solutions were used as follows: A, 0.165 M sodium acetate, pH 5.25, containing 10 mM octane sulfonate; B, 0.2 M sodium acetate, pH 4.5, containing acetonitrile:water (66:34) and 10 mM octane sulfonate; and C, 0.01 M sodium acetate, pH 5.25, containing 10 mM octane sulfonate. The gradient program is shown in **Table 1**.

Table 1. Gradient Elution Program for Separation of Biogenic Amines in Botrytized Wines

	solution		
elution time (min)	A (%)	B (%)	C (%)
4	100	0	0
5	100	0	0
13	85	15	0
14	85	15	0
25	75	25	0
48	0	40	60
49	0	52	48
63	0	52	48
75	0	70	30
80	0	100	0
90	0	100	0
92	100	0	0

The gradient program with A, B, and C solutions was necessary to separate the 14 amines in a single run. Changing ion concentration during the run, the first group of amines (Put, iBa, Cad, unknown1/Unk1/, Tyr, His, and unknown2/Unk2/) was separated with an eluent of higher ion concentration, while for separation of the other amines (2MeBa, Agm, 3MeBa, Pa, Spd, and Phe) a lower ion concentration was needed.

The flow rate of the mobile phase was 1 mL min^{-1}, and the flow rate of OPA was 0.8 mL min^{-1}. After filtration (0.42 μm) of samples, 10 μL of them was injected into the column. Identification of amines was accomplished with comparing the retention time of amine standards with compounds present in samples or by addition of standard solution to the samples.

Peak areas were recorded and calculated using the Waters Millenium Software package. Standard solutions of amines prepared in 10% perchloric acid in the concentration range of 0.1−10 mg L^{-1} were used for the assay. Calibration curves were linear in the range above. For Cad, His, and Pa, the concentration range of 0.1−1 mg L^{-1} was used, which was linear as well.

Least squares analyses produced a correlation coefficient of $r \geq 0.9990$ for Cad, Tyr, His, Agm, Spd, Phe, Trp, and Spn, $r \geq 0.9973$ for Put and iBa, $r \geq 0.9784$ for Pa and 3MeBa, and $r = 0.9041$ for 2MeBa. To study repeatability, five determinations of a wine sample were made using the same reagents and apparatus. The relative standard deviation (RSD) values were acceptable, being less than 2.8% for Put, iBa, Tyr, 2MeBa, 3MeBa, Pa, and Phe. RSD values of Cad (10%), His, Spd, and Agm (15%) were higher due to their low concentrations. The detection limit ranged from 0.02 (3MeBa) to 1 ng (His and Tyr) in a 10 μL injection volume.

A recovery study was carried out with spiking amines to the wine sample H96/5-5. The concentration of amine solutions added to the wine sample was as follows. The concentration of Put, iBa, 2MeBa, 3MeBa, and Phe (2.12, 1.27, 4.81, 6.14, and 4.27 mg L^{-1}, respectively) was about half or third part of the concentration found in the wine sample. The concentration of Cad, Tyr, His, Agm, Spd, and Pa (0.69, 1.28, 0.25, 1.67, 0.72, and 1.15 mg L^{-1}, respectively) was about twice the concentration measured in the wine sample. The recovery of amines ranged from 93.4 (Pa) to 101.1% (Tyr).

Acids. Separation of organic acids was performed with ODS-AQ (YMC European GMB) column (250 mm × 4.6 mm i.d., S-5μm) using a precolumn ODS-AQ cartridge (YMC European GMB). For elution, 0.02 M phosphate buffer (2.75 g/L KH_2PO_4, pH 2.7) was used under

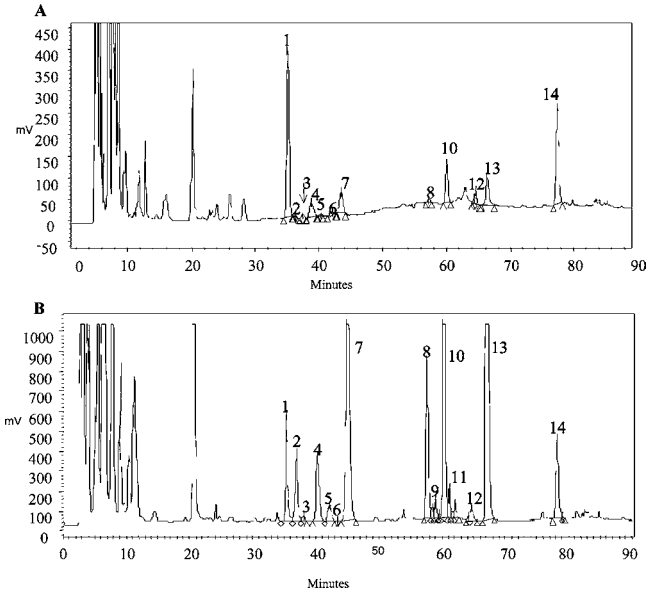

Figure 1. Chromatogram of biogenic amines. (**A**) Nonbotrytized wine from the Tokaj region of Hungary (normal wine); (**B**) botrytized Hungarian wine from Tokaj. Peak identities: Put, 1; iBa, 2; Cad, 3; Unk1, 4; Tyr, 5; His, 6; Unk2, 7; 2MeBa, 8; Agm, 9; 3MeBa, 10; Pa, 11; Spd, 12; Phe, 13; and Istd, 14.

isocratic conditions at 0.7 mL min^{-1} flow rate at room temperature. After filtration (0.42 μm) of samples, 10 μL of them was injected into the column. Peaks were identified with authentic tartaric, malic, citric, shikimic, and fumaric acids. Compounds prepared in solution of 2% *meta*-phosphoric acid were detected at 214 nm.

The detection limit of organic acids varied from 0.1 (fumaric acid) to 2.5 ng (acetic acid) in 10 μL injection volume, and the linear range for all acids investigated was between 0.04 and 1.0 g L^{-1} except for fumaric acid (0.08−2 mg L^{-1}). The correlation coefficient (r) was 0.9999 for all acids. The repeatability was determined with five parallel injections of a wine sample using the same reagents and apparatus. The RSD was less than 4.2% for citric and malic acid and less than 3.5% for shikimic, lactic, acetic, and fumaric acids.

Statistics. Analysis of variance, correlation study, principal component analysis (PCA), and linear discriminant analysis (LDA) were performed by Microsoft Excel or the MINITAB statistical program. PCA is a powerful visualization tool for data evaluation. It can represent graphically intersample and intervariable relationships and provides a way to reduce the dimensionality of the data. PCA is an unsupervised method of pattern recognition in the sense that no grouping of the data has to be known before the analyses. Using PCA, class membership is easy to assign. LDA is a method to discriminate between two or more groups of samples. LDA is a supervised pattern recognition, which means that the class membership has to be known prior to the analysis. The groups to be discriminated can be defined either naturally by the problem under investigation or by some preceding analysis, such as a cluster analysis or PCA.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of nonbotrytized (A) and botrytized wines (B). Eleven amines were identified from 13 isolated amines of botrytized wines. The names of identified compounds are listed in **Figure 1**. It was assumed that the other two nonidentified compounds are simple amines as well, considering that amines elute separately from amino acids appearing in the beginning of chromatograms. Tryptamine was not detected in wines, and detection of spermine was ambiguous. Comparing chromatograms, it can be seen that nonbotrytized wines (normal white wines) are much poorer in amines than botrytized wines as expected. There are only 2−5 compounds (Put, Tyr, Phe, 3MeBa, and Unk2) in nonbotrytized wines, whose concentrations were quantified above 1 mg L^{-1}.

Table 2 shows the concentration of biologically active and nonactive amines of normal wines. Results show clearly that Hungarian normal white wines scarcely contained biologically nonactive amines (or did not contain them). Only 3-methylbutylamine (0.1−5.0 mg L^{-1}) appeared in most cases in normal wines in high concentration. From biologically active amines, putrescine (0.7−4.0 mg L^{-1}) and phenylethylamine (0.1−3.0 mg L^{-1}) were the most relevant ones, although they were in low concentrations, as compared to those of Aszú wines.

Table 2. Biologically Active (**A**) and Nonactive Amine (**B**) Content of Hungarian Nonbotrytized Wines (Normal White Wines)[a]

A

	biologically active amines (mg L^{-1})						
wines	Put	Cad	Tyr	His	Agm	Spd	Phe
NH-2002[b]	4.01 (0.42)	0.32 (0.01)	3.24 (0.03)	0.48 (0.01)	0.09 (0.02)	0.20 (0.04)	1.41 (0.01)
EL-2002[c]	1.46 (0.01)	0.16 (0.01)	0.07 (0.01)	0.13 (0.01)	D	0.02 (0.01)	1.14 (0.01)
BC-2001[d]	1.85 (0.01)	0.29 (0.01)	0.09 (0.01)	0.10 (0.01)	0.12 (0.01)	0.03 (0.01)	0.91 (0.01)
BK-2002[e]	0.70 (0.02)	0.04 (0.01)	0.15 (0.01)		D	0.01 (0.01)	0.39 (0.02)
AO-2003[f]	2.13 (0.02)	0.24 (0.01)	2.51 (0.01)	1.43 (0.01)	D	0.01 (0.01)	2.38 (0.02)
TF-1999[g]	1.48 (0.01)	0.11 (0.01)	0.12 (0.01)	0.09 (0.01)	D	0.01 (0.01)	3.05 (0.04)
TH-2000[h]	1.33 (0.01)	0.12 (0.01)	0.08 (0.01)	0.10 (0.01)	D	0.01 (0.01)	2.44 (0.03)
SO-2003[i]	3.28 (0.03)	1.01 (0.02)	0.16 (0.01)		D	0.32 (0.02)	0.06 (0.01)

B

	nonactive amines (mg L^{-1})					
wines	iBa	2MeBa	3MeBa	Pa	Unk1	Unk2
NH-2002[b]	0.05 (0.01)	0.15 (0.01)	2.51 (0.12)	D	0.49 (0.07)	2.70 (0.45)
EL-2002[c]		0.07 (0.01)	1.61 (0.05)	0.10 (0.01)	0.13 (0.01)	0.71 (0.04)
BC-2001[d]	0.03 (0.004)	0.13 (0.01)	0.79 (0.01)	0.11 (0.01)	0.03 (0.01)	3.07 (0.27)
BK-2002[e]		0.07 (0.01)	0.12 (0.01)	D	0.04 (0.01)	0.19 (0.01)
AO-2003[f]		0.11 (0.01)	2.88 (0.13)	D	0.41 (0.06)	1.76 (0.20)
TF-1999[g]	0.04 (0.01)	0.28 (0.02)	4.97 (0.25)	D	1.29 (0.07)	2.53 (0.19)
TH-2000[h]	0.04 (0.01)	0.24 (0.01)	4.95 (0.22)	D	1.10 (0.03)	3.15 (0.23)
SO-2003[i]	0.16 (0.02)	0.13 (0.01)	0.05 (0.00)	0.31 (0.01)	0.10 (0.01)	0.44 (0.03)

[a] Standard deviations in brackets. [b] NH, Nagyrédei Hárslevelû (Linden Leaf). [c] EL, Egri Leányka. [d] BC, Balatonboglári Muskotály (Muscat) Cuvee. [e] BK, Balatonboglári Királyleányka. [f] AO, Abasári Olaszrizling (Riesling). [g] TF, Tokaji Furmint. [h] TH, Tokaji Hárslevelû (Linden Leaf). [i] SO, Szederkényi Olaszrizling (Riesling); D, detected.

Table 3. Biologically Active Amine Content of Botrytized Wines[a]

	biologically active amines (mg L^{-1})						
wines	Put	Cad	Tyr	His	Agm	Spd	Phe
H90/5-1[b]	3.33 (0.39)	0.15 (0.02)	0.89 (0.04)	0.08 (0.03)	0.12 (0.08)	0.1 (0.01)	13.7 (0.4)
H98/5-2	3.31 (0.82)	0.20 (0.01)	2.17 (0.03)	0.11 (0.02)	0.23 (0.05)	0.43 (0.19)	22.7 (4.2)
H75/5-3	2.33 (0.31)	0.13 (0.04)	2.01 (0.13)	0.08 (0.01)	0.02 (0.01)	0.07 (0.1)	17.8 (0.8)
H94/5-4	2.89 (0.63)	0.21 (0.04)	2.42 (0.24)	0.15 (0.03)	0.07 (0.03)	0.09 (0.12)	18.5 (0.4)
H96/5-5	4.12 (0.38)	0.33 (0.01)	0.6 (0.04)	0.10 (0.02)	0.89 (0.11)	0.35 (0.15)	16.6 (1.2)
H96/5-6	3.13 (0.52)	0.23 (0.01)	2.25 (0.18)	0.08 (0.03)	0.19 (0.15)	0.09 (0.03)	19.4 (1.7)
H95/5-7	6.37 (0.24)	0.3 (0.06)	0.51 (0.04)	0.11 (0.07)	0.02 (0.02)	0.06 (0.08)	14.0 (0.2)
H98/5-8	1.58 (0.24)	0.09 (0.04)	0.21 (0.03)	0.10 (0.003)		0.10 (0.01)	8.56 (0.63)
H98/5-9	2.55 (0.51)	0.16 (0.03)	1.76 (0.23)	0.09 (0.03)	0.49 (0.06)	0.95 (0.48)	16.1 (1.7)
H96/5-10	1.89 (0.14)	0.05 (0.02)	0.4 (0.07)	0.07 (0.01)	0.003 (0.002)	0.07 (0.06)	6.99 (0.11)
H96/5-11	2.75 (0.87)	0.14 (0.03)	0.65 (0.14)	0.09 (0.003)	0.07 (0.03)	0.67 (0.47)	12.9 (4.1)
H96/4-12	2.52 (0.75)	0.24 (0.08)	1.61 (0.17)	0.06 (0.01)	0.11 (0.004)	0.36 (0.12)	17.8 (3.9)
H93/4-13	1.09 (0.44)	0.07 (0.02)	0.34 (0.02)	0.08 (0.004)		0.32 (0.23)	4.56 (0.23)
H91/4-14	1.67 (0.63)	0.09 (0.03)	1.71 (0.15)	0.08 (0.001)	0.04 (0.01)	0.08 (0.07)	14.9 (2.7)
H99/5-15	1.74 (0.67)	0.14 (0.09)	0.76 (0.04)	0.10 (0.02)	0.03 (0.01)	0.1 (0.04)	10.3 (1.1)
H94/6-16	3.69 (1.00)	0.14 (0.04)	2.03 (0.22)	0.12 (0.001)	0.24 (0.1)	0.05 (0.05)	16.5 (3.0)
H96/6-17	2.33 (0.55)	0.11 (0.02)	1.77 (0.22)	0.08 (0.01)	0.26 (0.1)	0.02 (0.03)	14.9 (2.9)
H2000-18	3.80 (0.96)	0.31 (0.08)	2.44 (0.20)	0.10 (0.01)	0.13 (0.12)	0.09 (0.08)	15.7 (2.8)
H99/6-19	2.64 (0.27)	0.21 (0.004)	0.75 (0.12)	0.08 (0.02)	0.19 (0.25)	0.29 (0.12)	17.7 (0.1)
H94/6-20	2.85 (0.30)	0.13 (0.01)	1.06 (0.18)	0.09 (0.03)	0.04 (0.01)	0.04 (0.003)	19.5 (0.5)
H96/6-21	3.81 (0.20)	0.28 (0.07)	0.97 (0.01)	0.09 (0.01)	0.04 (0.02)	0.08 (0.09)	18.1 (0.0)
H97/6-22	4.82 (0.03)	0.29 (0.09)	2.24 (0.25)	0.08 (0.001)	0.24 (0.09)	1.59 (0.77)	21.6 (1.4)
H2000-23	5.01 (0.06)	0.34 (0.06)	0.53 (0.10)	0.10 (0.04)	0.24 (0.05)	0.18 (0.12)	9.14 (0.37)
H95/6-24	3.54 (0.61)	0.13 (0.05)	1.40 (0.26)	0.07 (0.01)	0.02 (0.02)	0.07 (0.05)	16.6 (1.4)
H97/6-25	2.16 (0.18)	0.16 (0.01)	2.03 (0.23)	0.08 (0.02)	0.09 (0.06)	0.05 (0.07)	17.3 (1.5)
H2000-26	8.72 (1.72)	0.4 (0.06)	1.03 (0.06)	0.14 (0.09)	20.2 (0.8)	0.45 (0.62)	21.3 (0.1)
H99/6-27	4.74 (0.65)	0.13 (0.02)	1.74 (0.09)	0.08 (0.03)	0.25 (0.1)	0.06 (0.03)	20.0 (1.0)
H97/6-28	5.39 (0.83)	0.19 (0.01)	0.58 (0.19)	0.21 (0.06)	0.57 (0.23)	2.22 (0.33)	23.6 (1.4)
H72/6-29	2.27 (0.34)	0.10 (0.07)	2.27 (0.11)	0.10 (0.06)	0.21 (0.18)	0.20 (0.01)	17.6 (0.5)
H93/6-30	1.67 (0.32)	0.04 (0.02)	0.69 (0.07)	0.07 (0.01)	0.07 (0.02)	0.01 (0.001)	7.73 (0.08)
H93/6-31	3.54 (0.65)	0.13 (0.02)	2.72 (0.03)	0.07 (0.04)	0.29 (0.22)	0.03 (0.02)	21.1 (0.6)
H86/6-32	2.40 (0.07)	0.08 (0.03)	1.27 (0.06)	0.08 (0.03)	0.03 (0.02)	0.12 (0.03)	14.3 (0.2)
HE93-33[c]	2.24 (0.53)	0.12 (0.02)	1.05 (0.02)	0.07 (0.03)	0.23 (0.05)	0.03 (0.02)	15.6 (0.6)
HE93-34[c]	2.06 (0.33)	0.07 (0.03)	1.57 (0.09)	0.07 (0.02)	0.29 (0.09)	0.02 (0.01)	19.4 (0.9)
HE98-35[c]	4.29 (0.35)	0.16 (0.05)	2.71 (0.05)	0.16 (0.05)	0.30 (0.16)	0.10 (0.13)	19.9 (1.9)
HE93-36[c]	2.31 (0.34)	0.08 (0.04)	2.01 (0.08)	0.07 (0.04)	0.42 (0.15)	0.04 (0.03)	17.3 (1.5)
A1-37[d]	3.09 (0.45)	0.29 (0.13)	0.65 (0.19)	0.09 (0.01)	1.04 (0.20)	1.43 (0.99)	16.8 (2.6)
A2-38[d]	3.05 (0.02)	0.27 (0.01)	0.27 (0.07)	0.17 (0.005)	0.98 (0.24)	1.97 (0.86)	20.3 (1.4)
G-39[d]	0.23 (0.16)	0.04 (0.03)	0.12 (0.02)			0.03 (0.02)	14.8 (3.0)
F1-40[d]	4.24 (0.19)	0.09 (0.06)	5.91 (0.27)	3.49 (0.63)	0.02 (0.01)	0.05 (0.06)	2.25 (0.23)
F2-41[d]	1.73 (0.13)	0.05 (0.03)	0.07 (0.03)	0.09 (0.04)	0.01 (0.005)	0.26 (0.13)	5.47 (0.37)
F3-42[d]	3.4 (1.20)	0.06 (0.02)	0.06 (0.04)	0.08 (0.03)	0.15 (0.07)	0.52 (0.35)	11.0 (0.6)
S-43[d]	1.52 (0.43)	0.03 (0.03)	0.22 (0.02)	0.11 (0.03)	0.04 (0.02)	0.08 (0.04)	2.86 (0.05)

[a] Standard deviations in brackets. [b] H (Hungarian) 90/5 (wine of 1990 vintage/butt number) 1...43 (numbering). [c] HE, H (Hungarian); E (Eszencia). [d] Foreign wines.

Tables 3 and **4** show the concentration of biologically active and nonactive amines in botrytized wine samples. From biogenic amines, phenylethylamine (7.0−23.6 mg L^{-1}) was in the highest concentration followed by putrescine (1.1−8.7 mg L^{-1}) and tyramine (0.2−2.7 mg L^{-1}). The concentration of other amines was below 1 mg L^{-1} in Hungarian botrytized wines. These tendencies were observed for foreign botrytized wines as well, but the average tyramine content was below 1 mg L^{-1}.

Among biologically nonactive amines, 3-methyl-butylamine (8.1−26.7 mg L^{-1}) and unknown 2 (6.9−22.4 mg L^{-1}) were found in the highest concentrations, similarly to foreign wines. The concentration of unknown 1 and unknown 2 was calculated on the basis of the calibration curve of *i*-butylamine. Aside from *n*-pentylamine, the concentrations of all biologically nonactive amines were above 1 mg L^{-1} except for one or two samples in Hungarian botrytized wines. In foreign wines, only the concentrations of 3-methyl-butylamine and unknown 2 increased above 1 mg L^{-1} in all wine samples.

In our previous work (*10*, *11*), we established that in grape berries the content of nonbiogenic amines increases during infection of *B. cinerea*. During the wine-making process, the increase of content of these amines continues. Parallel with the raising of amines above the tyramine content increases as well. The amine composition of Aszú wines gets a specific character on the basis that botrytized wines can be distinguished from normal wines. In other words, it can be established from amine composition that a wine was produced from grapes infected by *B. cinerea* or not.

Comparing the averaged total amine content of Aszú wines of various butt numbers (different producers and vintages), it was found that the total amine content increased with the butt number of wines, but the differences were not significant among them. The standard deviation of Aszú samples increased with decreasing butt number, which means the variability of total amine content due to the different technology of producers. The similarity of amine composition of Aszú and their differences between Aszú and foreign wines were demonstrated in different ways.

Figure 2 shows the ratio of total concentration of normal amines to that of tyramine. Studying several Tokaji Aszú in an earlier work, it was established that the ratio of nonbiologically active amines to tyramine gives nearly the same value (*26*). In this study investigating some foreign botrytized wines with Hungarian Aszú of high numbers, we wanted to know whether

Table 4. Nonactive Amine Content of Botrytized Wines[a]

	nonactive amines (mg L^{-1})					
wines	iBa	2MeBa	3MeBa	Pa	Unk1	Unk2
H90/5-1[b]	1.89 (0.15)	4.34 (0.04)	18.1 (1.9)	0.55 (0.17)	2.69 (0.003)	17.0 (1.0)
H98/5-2	3.91 (0.4)	9.72 (0.03)	23.9 (6.0)	0.60 (0.04)	4.50 (0.63)	20.6 (0.0)
H75/5-3	3.32 (1.25)	8.62 (0.29)	22.8 (4.7)	0.28 (0.004)	2.17 (0.4)	15.6 (2.4)
H94/5-4	4.17 (1.36)	10.16 (0.8)	21.6 (1.9)	0.60 (0.15)	2.87 (0.42)	19.1 (0.8)
H96/5-5	2.29 (0.05)	8.66 (0.88)	20.9 (3.6)	0.53 (0.21)	5.88 (0.1)	18.1 (1.3)
H96/5-6	4.25 (0.13)	11.19 (0.14)	21.6 (2.2)	0.70 (0.09)	5.16 (0.37)	16.8 (0.6)
H95/5-7	2.2 (0.35)	3.58 (0.24)	17.4 (0.5)	0.14 (0.02)	2.13 (0.12)	7.93 (1.37)
H98/5-8	0.35 (0.09)	1.26 (0.12)	15.8 (3.2)	0.11 (0.02)	0.75 (0.02)	7.03 (0.5)
H98/5-9	5.32 (0.51)	11.84 (0.16)	21.4 (1.3)	0.47 (0.13)	4.74 (0.42)	16.2 (0.8)
H96/5-10	0.45 (0.15)	1.62 (0.07)	14.4 (0.2)	0.14 (0.01)	0.57 (0.09)	6.88 (1.03)
H96/5-11	1.82 (0.51)	5.18 (1.03)	17.0 (3.1)	0.23 (0.05)	2.36 (0.65)	12.9 (4.1)
H96/4-12	4.74 (0.51)	15.8 (2.04)	22.9 (1.7)	0.47 (0.01)	5.40 (0.65)	11.7 (2.6)
H93/4-13	0.15 (0.04)	0.79 (0.01)	8.1 (0.6)	0.26 (0.01)	0.26 (0.05)	2.28 (0.68)
H91/4-14	3.47 (0.44)	8.45 (0.45)	20.2 (0.9)	0.36 (0.03)	2.60 (0.28)	14.1 (1.5)
H99/5-15	1.47 (0.29)	3.37 (0.17)	17.1 (1.2)	0.12 (0.13)	1.35 (0.19)	7.37 (2.2)
H94/6-16	3.06 (0.34)	9.04 (0.56)	20.7 (0.7)	0.70 (0.06)	4.10 (0.32)	16.5 (2.8)
H96/6-17	3.35 (0.46)	7.65 (0.86)	18.7 (0.9)	0.41 (0.1)	2.51 (0.35)	16.1 (3.6)
H2000-18	5.26 (0.51)	7.07 (0.55)	20.4 (1.2)	0.30 (0.11)	5.68 (0.61)	15.2 (1.3)
H99/6-19	1.08 (0.15)	3.24 (0.20)	21.3 (2.9)	0.30 (0.01)	2.42 (0.02)	12.6 (0.5)
H94/6-20	2.19 (0.64)	7.35 (0.05)	23.7 (2.3)	0.57 (0.07)	2.53 (0.48)	11.2 (1.2)
H96/6-21	2.10 (0.02)	5.71 (0.17)	24.0 (2.9)	0.31 (0.05)	2.26 (0.08)	11.9 (0.4)
H97/6-22	6.34 (0.32)	7.95 (0.54)	24.4 (4.6)	0.76 (0.11)	3.51 (0.22)	22.0 (0.1)
H2000-23	2.02 (0.52)	3.11 (0.05)	16.7 (1.1)	0.18 (0.04)	1.12 (0.07)	8.15 (2.98)
H95/6-24	1.98 (0.05)	4.76 (0.12)	18.6 (1.1)	0.58 (0.01)	3.22 (0.21)	14.8 (1.30)
H97/6-25	3.58 (0.08)	8.50 (0.38)	25.0 (5.5)	0.60 (0.17)	4.30 (0.28)	13.1 (1.1)
H2000-26	2.36 (1.02)	5.43 (0.61)	24.8 (2.6)	0.36 (0.18)	2.88 (0.21)	18.5 (1.5)
H99/6-27	3.42 (0.51)	7.88 (0.05)	25.4 (4.0)	0.36 (0.07)	3.46 (0.19)	14.6 (1.8)
H97/6-28	3.18 (1.03)	11.26 (0.64)	24.8 (1.5)	0.61 (0.05)	1.72 (0.34)	22.4 (1.9)
H72/6-29	3.58 (0.39)	8.20 (0.59)	21.1 (1.4)	0.67 (0.01)	1.80 (0.18)	18.7 (1.7)
H93/6-30	1.07 (0.08)	2.38 (0.02)	14.2 (0.3)	0.23 (0.03)	0.72 (0.03)	10.4 (0.3)
H93/6-31	3.15 (0.19)	9.69 (0.38)	26.7 (4.9)	0.83 (0.20)	4.30 (0.13)	18.3 (0.5)
H86/6-32	2.11 (0.07)	5.01 (0.21)	18.9 (0.1)	0.31 (0.08)	2.03 (0.03)	16.4 (0.4)
HE93-33[c]	0.83 (0.28)	3.05 (0.24)	22.2 (4.7)	0.81 (0.19)	1.53 (0.08)	10.6 (0.4)
HE93-34[c]	2.64 (0.67)	8.03 (0.20)	25.7 (1.5)	0.26 (0.07)	2.74 (0.45)	13.4 (1.1)
HE98-35[c]	2.92 (0.17)	8.66 (0.35)	26.0 (6.4)	0.60 (0.26)	3.29 (0.08)	18.3 (1.3)
HE93-36[c]	4.15 (0.05)	9.59 (0.53)	24.1 (7.3)	0.57 (0.17)	2.52 (0.01)	16.5 (0.1)
A1-37[d]	2.55 (0.42)	12.78 (0.37)	21.0 (1.9)	0.18 (0.08)	4.51 (0.24)	15.4 (0.6)
A2-38[d]	0.79 (0.03)	6.42 (0.28)	22.0 (4.0)	0.17 (0.02)	2.89 (0.01)	11.1 (0.9)
G-39[d]	0.29 (0.04)	4.49 (0.02)	18.7 (0.9)	0.09 (0.07)	1.73 (0.37)	5.46 (0.41)
F1-40[d]	0.08 (0.07)	0.41 (0.04)	8.4 (0.2)	0.27 (0.01)	0.64 (0.0002)	8.43 (0.02)
F2-41[d]	0.09 (0.09)	0.54 (0.04)	9.2 (0.2)	0.17 (0.08)	0.83 (0.07)	2.06 (0.11)
F3-42[d]	0.46 (0.36)	2.5 (0.10)	20.4 (2.8)	0.11 (0.01)	0.89 (0.35)	6.57 (3.35)
S-43[d]	0.26 (0.28)	0.87 (0.02)	6.9 (0.3)	0.06 (0.02)	0.17 (0.14)	4.73 (3.65)

[a] Standard deviations in brackets. [b] H (Hungarian) 90/5 (wine of 1990 vintage/butt number) 1...43 (numbering). [c] HE, H (Hungarian); E (Eszencia). [d] Foreign wines.

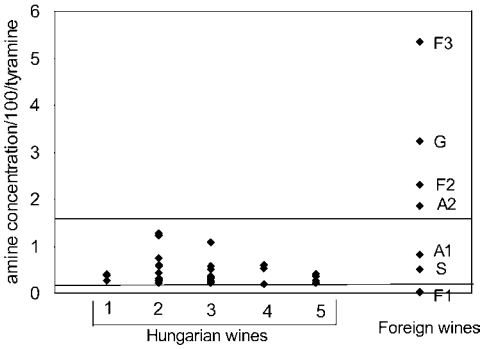

Figure 2. Ratio of total amine to tyramine concentration. 1–4-butt Aszú wines; 2–5-butt Aszú wines; 3–6-butt Aszú wines; 4, other botrytized wines (late harvest); 5, "Tokaji Eszencia".

foreign botrytized wines could be distinguished from Hungarian ones on the basis of this value above. In **Figure 2**, the ratio of the concentration of the total nonbiologically active amines and phenylethylamine to that of tyramine of all botrytized wines studied was plotted. As it can be seen, the calculated value of Hungarian wines falls in a definite range of ratio (0.23−1.27), while foreign botrytized wine samples fall out of the range. Only two foreign wine samples (S and A1) appeared in this range.

Figure 3 shows the spider web diagrams (*10*) of the logarithm of amine concentrations representing six-butt Aszú wines and several foreign wines (S, A1, F1, and F2). The foreign wine samples are marked with the black, thick line, distinguishing it from six-butt Aszú wines with thin, pale lines. Logarithms of concentrations were calculated because concentrations in samples varied by 1 or 2 orders of magnitude. In accordance with a previous study, it has been found that the shape of diagrams is similar in the case of all Hungarian Aszú wines, independently of butt number (three-, four-, five-, or six-butt). It indicates the characteristic amine composition of Aszú wines of different vintages and producers. Fairly high differences were found only in the case of agmatine and spermidine. Because the shape of the spider web diagram was similar in all Aszú wines, we showed only the six-butt Aszú wines in **Figure 3**. S wine, which is the most similar in ratio to the above Aszú wines, fell into the range in **Figure 2**, but it could be distinguished from Hungarian wines on the basis of the spider web diagram. The other foreign wines were all discriminated in this way as well, except A1 (**Figure 3B**).

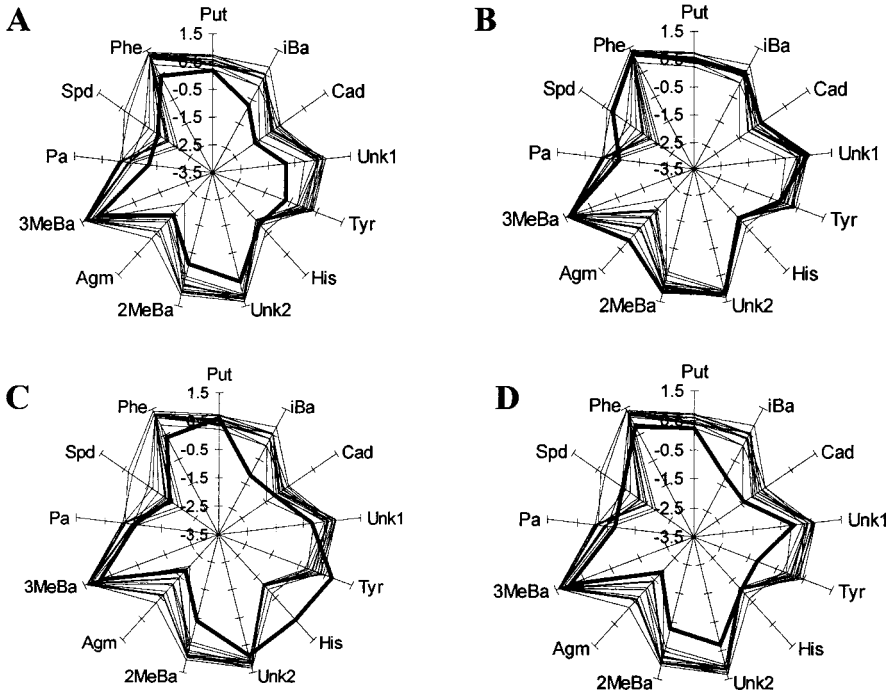

Figure 3. Spider web diagram of 6-butt Aszú wines and foreign botrytized wines (concentrations of logarithms). 6-Butt Aszú wines, foreign botrytized wines (S, A1, F1, and F2). (**A**) 6-Butt Aszú wines and the Slovakian wine (S). (**B**) 6-Butt Aszú wines and the Austrian wine (A1). (**C**) 6-Butt Aszú wines and the French wine (F1). (**D**) 6-Butt Aszú wines and the French wine (F2).

The differences in amine composition of wine samples illustrated in **Figures 2** and **3** were analyzed statistically. The first step of data analysis was carried out using one-way analysis of variance. Significant differences were found between Aszú and foreign botrytized wines (F_{cal} 5.038 > F_{crit} 3.874; $p < 0.05$) and between Aszú and normal wines (F_{cal} 51.108 > F_{crit} 3.872; $p < 0.001$).

Using correlation analysis, linear correlation was found between pairs of amines. From normal amines, iBa, 2MeBa, 3MeBa, Unk1, Unk2, and Pa ($0.628 < r < 0.968$, $p < 0.001$) and from biogenic amines Agm and Phe showed significant correlation to other amines ($0.443 < r < 0.968$, $p < 0.003$). These results confirm our earlier observation that *B. cinerea* affects the amount of above amines. This effect is shown as a proportional increase in amine concentration. The other important amine of Aszú wine, tyramine, did not show correlation to other amines. Tyramine is primarily produced or its concentration increased during the wine-making process.

PCA was performed for amines of all wines studied. The score plot of PCA is shown in **Figure 4**. The first two principal components accounted for more than 77% of the total variance in the data. All nonbiogenic amines and agmatine, phenylethylamine as biogenic ones, were used as variables in PCA. Principal component (PC) scores of Hungarian botrytized and normal wines show a good separation. Scores of Hungarian botrytized wines fall in a narrow range along the second PC enclosed by a rectangle, except for two samples. One of them is a late-harvested wine that is not actually Aszú. The wine with the name of H93/4-13 in this figure is an Aszú (four-butt). Its PC scores fall near those of normal wines. Taking into account its amine composition (**Tables 3** and **4**), it was observed that this wine sample had very low amine content similarly to normal wines. PC scores in all foreign wines including S and A1 samples fall out of the rectangle in contrast with the ratio of total amine and tyramine concentration (**Figure 2**).

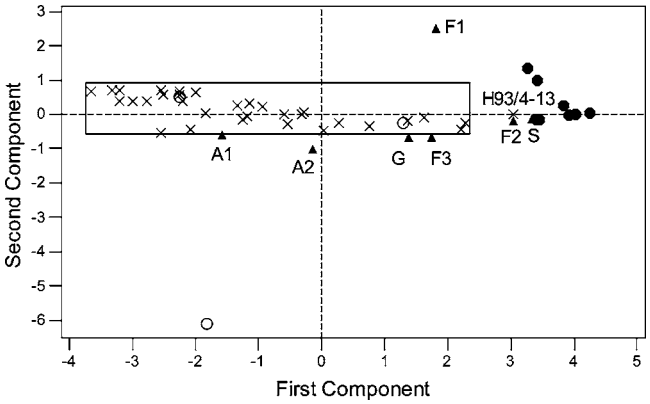

Figure 4. Principal component analysis of amines. Variables: iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, and Phe. ▲, Foreign botrytized wines; ×, Hungarian botrytized wines; ●, Hungarian nonbotrytized (white) wines; and ○, late harvested (botrytized) Hungarian wines from Tokaj.

Table 5. Linear Discriminant Analysis of Amines—Classification with Cross-Validation[a]

	original groups		
groups	Aszú wines	foreign wines	normal wines
Aszú wines	46	2	0
foreign wines	4	12	0
normal wines	2	0	16
total number	52	14	16
correctly classified	46	12	16
proportion	0.885	0.857	1.000
total number: 82	correctly classified: 74	proportion correct: 0.902	

[a] Variables: iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, and Phe.

LDA was used for the differentiation of Aszú, foreign botrytized, and normal wines. **Table 5** shows the classification of wines, which was achieved by the following amines as

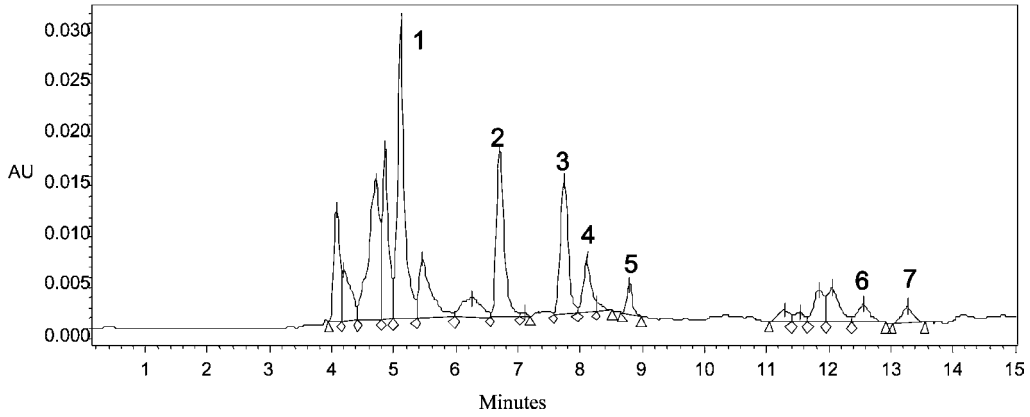

Figure 5. Chromatogram of organic acids (sample H94/5-4). Peak identities: tartaric acid, 1; malic acid, 2; shikimic acid, 3; lactic acid, 4; acetic acid, 5; citric acid, 6; and fumaric acid, 7.

Table 6. Organic Acid Content of Hungarian Nonbotrytized Wines (Normal White Wines)

	organic acids				
wines	malic (g L^{-1})	shikimic (mg L^{-1})	acetic (g L^{-1})	citric (g L^{-1})	fumaric (mg L^{-1})
DC-2001[a]	1.81	33.8	0.27	0.47	0.88
TF-2001[b]	1.34	44.3	0.52	0.19	0.56
TH-2000[c]	0.95	31.0	0.77	0.36	0.54
TF-2000	0.42	31.4	0.58	0.46	0.42
NH-2002[d]	2.24	20.7	0.85	0.31	0.77
EL-2002[e]	2.26	32.6	0.57	0.25	1.81
AO-2003[f]	1.19	38.3	1.13	0.22	0.80
BC-2001[g]	2.18	21.1	0.50	0.23	1.22
BK-2002[h]	2.18	19.4	0.28	0.27	0.52
SO-2003[i]	0.78	7.6	0.52	0.39	0.28

[a] Dél-Dunántúli Chardonnay. [b] TF, Tokaji Furmint. [c] TH, Tokaji Hárslevelû (Linden Leaf). [d] NH, Nagyrédei Hárslevelû (Linden Leaf). [e] EL, Egri Leányka. [f] AO, Abasári Olaszrizling (Riesling). [g] BC, Balatonboglári Muskotály (Muscat) Cuvee. [h] BK, Balatonboglári Királyleányka. [i] SO, Szederkényi Olaszrizling (Riesling).

variables: iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, and Phe. The percentage of correctly classified wines with these variables is 90.2%. The classification of normal wines is correct (100%), but worse classification was obtained in the case of Aszú and foreign wines, 88.5 and 85.7%, respectively. We also studied the acids to obtain information whether this group of compounds is specific enough for the investigation of authenticity and origin of botrytized wines. The chromatogram of acid composition of a five-butt Aszú wine can be seen in **Figure 5**. The concentration of acids in Hungarian normal wines is listed in **Table 6**, and that of botrytized wines is given in **Table 7**.

Normal white wines contain organic acids in lower concentrations on average than botrytized wines. Malic acid was in the range of 0.4−2.3 g L^{-1} in normal wines and 2.0−5.7 g L^{-1} in Aszú wines, and citric acid was between 0.2 and 0.5 g L^{-1} and 0.8 and 9.8 g L^{-1}, respectively. In most samples, acetic acid was below 1 g L^{-1} both in Hungarian normal wines and in botrytized wines, except for just a few ones. The higher acid content of Aszú samples can be explained by the technology, since Aszú paste is added to newly fermented dry wine, soaking them for 1 or 2 days in order to extract the natural sugar, acid, and flavors, etc.

The acid composition was relatively similar to each other in Hungarian botrytized wines. Foreign wines show higher variability, especially for malic (0.8−9.8 g L^{-1}) and citric (0.17−2.4 g L^{-1}) acid than Hungarian wines. It is not surprising because Hungarian wines originate from one viticulture region with similar technology while foreign wines originate from different countries with different technology.

Table 7. Organic Acid Content of Botrytized Wines[a]

	organic acids				
wines	malic (g L^{-1})	shikimic (mg L^{-1})	acetic (g L^{-1})	citric (g L^{-1})	fumaric (mg L^{-1})
H90/5-1[b]	3.23 (0.01)	44.2 (1.7)	0.69 (0.09)	0.40 (0.09)	0.46 (0.003)
H98/5-2	4.47 (0.12)	42.4 (0.8)	0.69 (0.17)	0.43 (0.06)	1.13 (0.078)
H75/5-3	3.30 (0.12)	34.3 (0.7)	0.82 (0.17)	0.38 (0.03)	0.78 (0.228)
H94/5-4	2.44 (0.03)	31.4 (1.2)	0.82 (0.22)	0.33 (0.08)	0.73 (0.013)
H96/5-5	3.97 (0.08)	19.1 (0.2)	1.17 (0.09)	0.54 (0.16)	2.29 (0.226)
H96/5-6	3.30 (0.18)	31.2 (1.9)	1.20 (0.16)	0.53 (0.23)	1.64 (0.107)
H95/5-7	2.16 (0.12)	29.1 (0.1)	0.51 (0.02)	0.43 (0.04)	1.57 (0.029)
H96/5-11	3.96 (0.02)	26.9 (0.7)	0.87 (0.18)	0.42 (0.05)	1.47 (0.084)
H96/4-12	2.97 (0.02)	22.7 (2.3)	0.74 (0.26)	0.56 (0.01)	2.30 (0.050)
H93/4-13	2.64 (0.01)	55.2 (0.1)	0.69 (0.01)	0.82 (0.01)	0.47 (0.006)
H91/4-14	2.15 (0.01)	34.1 (0.2)	0.95 (0.04)	0.44 (0.004)	0.68 (0.006)
H99/5-15	3.18 (0.03)	37.1 (0.8)	0.94 (0.12)	0.52 (0.01)	1.21 (0.017)
H94/6-16	3.43 (0.09)	34.5 (0.5)	0.43 (0.02)	0.41 (0.06)	1.41 (0.468)
H99/6-19	5.66 (0.09)	21.0 (0.0)	0.67 (0.37)	0.61 (0.10)	4.40 (0.010)
H94/6-20	1.97 (0.03)	21.4 (0.4)	0.56 (0.23)	0.39 (0.11)	0.91 (0.043)
H96/6-21	3.43 (0.03)	25.3 (0.8)	0.58 (0.26)	0.41 (0.09)	1.80 (0.078)
H97/6-22	5.70 (0.26)	38.5 (2.0)	0.77 (0.12)	0.59 (0.05)	1.47 (0.164)
H2000-23	2.32 (0.01)	18.1 (0.1)	0.82 (0.02)	0.48 (0.07)	1.27 (0.008)
H95/6-24	2.88 (0.01)	39.4 (0.5)	0.47 (0.08)	0.42 (0.03)	0.75 (0.303)
H2000-26	3.72 (0.01)	22.6 (0.8)	1.03 (0.01)	0.48 (0.02)	2.07 (0.115)
H99/6-27	4.28 (0.04)	34.0 (0.5)	0.83 (0.06)	0.62 (0.06)	1.23 (0.288)
H97/6-28	5.44 (0.14)	35.6 (0.3)	1.39 (0.08)	0.57 (0.08)	0.86 (0.004)
H72/6-29	3.18 (0.002)	44.5 (0.1)	0.84 (0.09)	0.36 (0.01)	0.32 (0.001)
H93/6-31	3.25 (0.06)	32.3 (0.6)	0.62 (0.02)	0.47 (0.04)	1.08 (0.283)
HE93-33[c]	3.54 (0.17)	35.2 (1.2)	0.43 (0.02)	0.70 (0.37)	1.65 (0.041)
HE93-34[c]	2.56 (0.22)	28.4 (3.4)	0.58 (0.06)	0.55 (0.16)	0.48 (0.139)
HE98-35[c]	4.17 (0.02)	43.6 (2.6)	0.68 (0.03)	0.50 (0.07)	1.22 (0.014)
HE93-36[c]	3.17 (0.01)	29.6 (0.5)	0.90 (0.001)	0.71 (0.10)	1.00 (0.004)
A1-37[d]	9.81 (2.00)	5.0 (2.9)	2.12 (0.17)	2.42 (1.41)	11.20 (2.552)
A2-38[d]	4.69 (0.04)	25.5 (1.1)	1.38 (0.02)	0.76 (0.17)	2.94 (0.171)
G-39[d]	2.42 (0.03)	8.5 (0.7)	0.95 (0.05)	0.83 (0.29)	3.73 (0.119)
F1-40[d]	0.84 (0.04)	14.3 (0.0)	0.79 (0.04)	0.17 (0.04)	0.22 (0.178)
F2-41[d]	2.27 (0.01)	15.0 (1.2)	1.09 (0.06)	0.50 (0.14)	2.32 (0.002)
F3-42[d]	2.59 (0.06)	22.0 (0.7)	1.57 (0.02)	0.79 (0.23)	1.96 (0.098)
S-43[d]	4.13 (1.19)	54.7 (4.1)	0.76 (0.16)	0.53 (0.11)	0.46 (0.648)

[a] Standard deviations in brackets. [b] H (Hungarian) 90/5 (wine of 1990 vintage/butt number) 1...43 (numbering). [c] HE, H (Hungarian); E (Eszencia). [d] Foreign wines.

For organic acids, the composition or the ratio of acids does not show clear differences between foreign and Hungarian wines. Using one-way variance analysis, no significant differences were found between Aszú and foreign botrytized wines. However, a significant difference was found between Aszú and normal wines (F_{cal} 4.032 > F_{crit} 3.897; $p < 0.05$).

To assess suitable information embraced by acids, PCA was used. The results can be seen in **Figure 6**. PCA allowed more

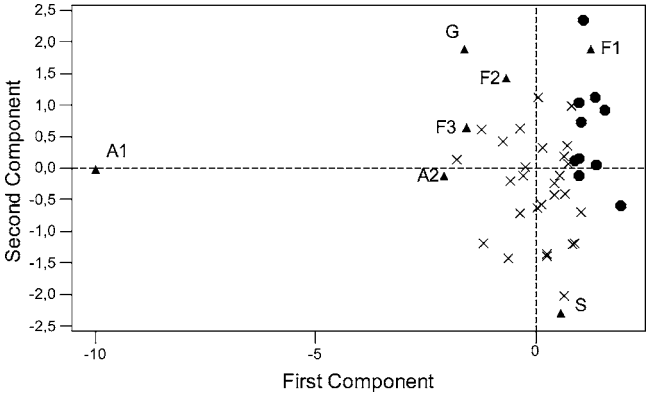

Figure 6. Principal component analysis of organic acids. Variables: malic acid, shikimic acid, acetic acid, citric acid, and fumaric acid. ▲, Foreign botrytized wines; ×, Hungarian botrytized wines; and ●, Hungarian nonbotrytized (white) wines.

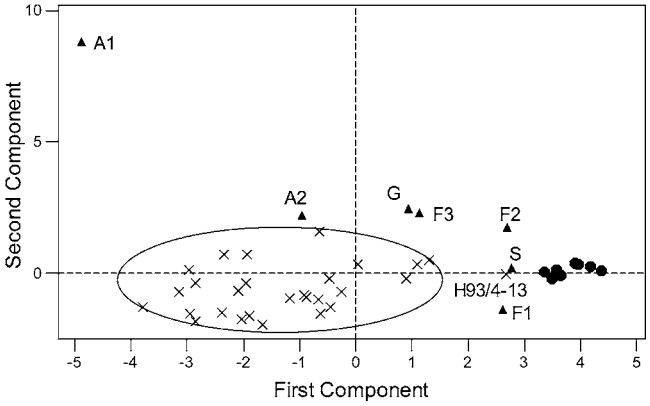

Figure 7. Principal component analysis of amines and organic acids. Variables: iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, Phe, malic acid, shikimic acid, acetic acid, citric acid, and fumaric acid. ▲, Foreign botrytized wines; ×, Hungarian botrytized wines; and ●, Hungarian nonbotrytized (white) wines.

than 84% of the total variance to be explained by the first two principal components computed from the correlation matrix of the acid compounds as variables.

A score plot of acids shows separation between foreign and Hungarian wines. Principal component scores of Hungarian botrytized wines fall in a definite range, and those of foreign botrytized wines are located somewhat apart from it. Wine sample A1 appeared far from the other wines, as its acid composition is quite different from them in contrast to that of others' found for amines. The scores of Hungarian normal wines form a group next to the botrytized wines, but their separation is not perfect.

Using PCA, amines and organic acids were combined to achieve better separation of different types of wines studied. The results can be seen in **Figure 7**. Comparing it with **Figure 4**, better separation of foreign botrytized wines from Aszú was found, although in this case PCA allowed 68% of the total variance to be explained by the first two principal components. The separation of scores of normal wines from Aszú and foreign botrytized wines was significant.

For better differentiation of Aszú and foreign botrytized wines, more variables were used for LDA, which is shown in **Table 8**. Using the above nine amines mentioned complemented with malic and citric acid as variables showed higher discriminant power. This way, the correct classification is 97.6%. The classification of normal and foreign botrytized wines is correct

Table 8. Linear Discriminant Analysis of Amines and Organic Acids—Classification with Cross-Validation[a]

	original groups		
groups	Aszú wines	foreign wines	normal wines
Aszú wines	50	0	0
foreign wines	2	14	0
normal wines	0	0	16
total number	52	14	16
correctly classified	50	14	16
proportion	0.962	1.000	1.000
total number: 82	correctly classified: 80	proportion correct: 0.976	

[a] Variables: malic acid, citric acid, iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, and Phe.

(100%), and for Aszú wines, 96.2% was obtained. Only one Aszú wine falls into the group of foreign botrytized wines (H93/4-13), similarly to PCA of amines and organic acids (see **Figure 7**).

Our results shows that the amines are suitable for classification of botrytized wines while the organic acids alone are less proper for categorization. The content of nine amines (iBa, Unk1, Tyr, Unk2, 2MeBa, Agm, 3MeBa, Pa, and Phe) and that of the main acids (malic and citric acids) are closely related to the characteristics of wines.

The characteristic composition of Aszú wines originates from the presence of amines, which indicates the action of *B. cinerea* on grapes. The similarities (among Tokaji Aszú wines) and differences (between Aszú and foreign wines) of amine and acid content of wines depend on winemaking technology determined by the origin and the tradition of region. On the basis of results, an objective evaluation method can be elaborated for quality control in order to protect the authenticity and origin of wine specialties from botrytized grapes.

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