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Study of Amine Composition of Botrytized Grape Berries

JUDIT KISS,[†] MARGIT KORBÁSZ,[‡] AND AGNES SASS-KISS*,[†]

Departments of Analytics and Microbiology, Central Food Research Institute, Herman Ottó út 15, 1022 Budapest, Hungary

Aliphatic primary and biogenic amines of grape varieties from two vintages were studied. We established that appearance and/or increase of both primary aliphatic and biogenic amines is due to microbiota living in/on grape berries. Aszú, gray rotten grapes infected mainly with *Botrytis cinerea*, grape berries infected mainly with *Penicillium* species, and intact grape berries were compared on the basis of amine composition using *t*-test, analysis of variance, and multivariate statistical analysis (principal component analysis and linear discriminant analysis). The amine composition and amine content of Aszú grapes were significantly different ($p < 0.05$) from those of intact grapes despite the effect of the vineyards and the vintages. Grape samples coming from the same growing location, intact, Aszú grapes, and grape berries infected mainly with *Penicillium* species could be separated from each other with multivariate statistical analysis. We distinguished intact, Aszú (noble rotten), and gray rotten berries from each other as well. Evaluating amine values of grape samples independently of the place of origin, the Aszú and green rotten berries could not be differentiated. The varieties and vineyards have affected the amine composition of Aszú grapes, while these effects on intact grapes appeared only slightly.

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

INTRODUCTION

The Tokaj wine region is one of the most famous wine districts in Hungary, where the well-known botrytized Tokaji Aszú wines are produced (*1*, *2*). The excellence of the wines of Tokaj is due to several factors such as microclimate, volcanic, mostly clay soil, and last but not least the grape varieties, which are the most suitable ones to the formation of Aszú grapes during noble rot caused by *Botrytis cinerea*. As the result of noble rot, Aszú grapes form the most important raw material of Tokaj wine specialities (*3*).

Four grape varieties are authorized for the production of Tokaj wines, which are late-ripening in order to make good use of the sunshine and warmth of the long autumn and are susceptible to noble rot. The most widespread variety is Furmint, which accounts for 70% of the wine region and is by far the most important grape variety in the production of Aszú wines. Other varieties are Linden Leaf (Hárslevelü), Yellow Muscat (Muscat Blanc, Muscat de Lunel), and a new variety, Zéta (a cross-bred variety between Furmint and Bouvier). Old local varieties such as White Grape (Kövérszölö) are thought to play a larger role in the near future among the main varieties (*1*). The last four varieties are sensitive cultivars, which restrict their planting in larger numbers. Zéta and White Grape received state recognition only in 1990 and 1998, respectively. The importance of new varieties may lie in that they reach maturity and are botrytized a few weeks earlier than the others (*4*).

Although numerous studies deal with the determination of biogenic amines in grape juices and wines (*7*−*13*), only few authors studied amines in grapes (*14*, *15*) and investigated the effect of botrytization of grapes on amine composition (*7*−*9*).

From biogenic amines, the major polyamines (putrescine, spermidine, and spermine) in living organisms take part in cellular growth, regulation of nucleic acid and protein synthesis, stabilization of lipids, brain development, nerve growth, and regeneration (*5*). 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 the activity of *B. cinerea* (*6*).

In our previous studies, it was established that Tokaji Aszú wines possess a characteristic amine composition coming from Aszú grapes and from traditional wine-making processes of the region. The study of amines has great importance in the aspect of detection of manipulation or authenticity of Aszú wines. The typical amine components forming in berries by the effect of *B. cinerea* are primarily primary aliphatic amines, whose contents depend on vineyards and vintages (*7*−*10*).

In the present work, our aims were to continue the study of the amine composition of traditional grape varieties, while keeping our interest in new varieties as well. We resumed our

* To whom correspondence should be addressed. Tel: 361-355-8838. Fax 361-214-2247. E-mail: a.sass@cfri.hu.
[†] Department of Analytics.
[‡] Department of Microbiology.

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Table 1. Microbial Counts of Berries and Distribution of Molds (*Penicillium* and *Botrytis*) in Vintage 2004[a]

	bacteria	yeast	mold	
		vineyard K (lg CFU g^{-1})		
Aszú grape	3.344 (0.561)	3.185 (0.799)	6.344 (0.469)	
			Penicillium	*Botrytis*
			1.434 (1.362)	4.909 (1.703)
gray rotten grape	3.611 (0.263)	3.719 (0.684)	4.987 (0.164)	
			Penicillium	*Botrytis*
			1.133 (0.393)	3.854 (0.368)
		vineyard V (lg CFU g^{-1})		
Aszú grape	2.204 (0.763)	4.568 (0.984)	6.204 (0.464)	
			Penicillium	*Botrytis*
			1.117 (0.562)	5.087 (1.703)
green rotten grape	4.342 (0.363)	4.255 (0.684)	7.176 (0.364)	
			Penicillium	*Botrytis*
			6.458 (0.197)	0.718 (0.540)

[a] Standard deviations are shown in parentheses.

previous investigations on the observation of the effect of vineyards. Furthermore, we studied the effect of different mold biota (mainly *B. cinerea* or *Penicillium* species) on the amine composition of grape berries.

MATERIALS AND METHODS

Reagents and Chemicals. All reagents and authentic compounds were of analytical reagent grade or high-performance liquid chromatography (HPLC) grade as required. Acetonitrile and methanol (HPLC grade) were obtained from Merck, and ultrapure water generated by the Milli-Q System (Millipore) was used. Anhydrous sodium acetate, boric acid, potassium hydroxide, acetic acid, Brij 35, and 2-mercaptoethanol were from Reanal (Budapest, Hungary); *o*-phthaldialdehyde was from Fluka, and sodium octanesulfonate was from Romil (Cambridge, United Kingdom). Biologically active amines—putrescine (Put), *i*-butyl amine (iBa), cadaverine (Cad), tyramine (Tyr), histamine (His), 2-methyl-butyl amine (2MeBa), agmatine (Agm), 3-methyl-butyl amine (3MeBa), *n*-pentyl-amine (Pa), spermidine (Spd), phenylethylamine (Phe), and hexyl amine (internal standard, Istd)—were purchased from Sigma.

Grape Samples. Two varieties (Furmint, Hárslevelü, and mixture of these two varieties) of intact, sun-dried, and Aszú grapes of 2003 grown in Tokaj region of Hungary were collected from five vineyards (Z, M1, M2, S, and P).

In 2004, five varieties (Furmint, Hárslevelü, Yellow Muscat, Zéta, and White Grape) of intact, dried (visibly without molds), raisin-like grapes infected mainly with *B. cinerea* (from here onward Aszú), grapes infected mainly with green molds (*Penicillium* spp., from here onward green rotten grapes), and gray rotten grape berries were obtained from three vineyards (K, G, and V) of Tokaj.

Sample Preparation. Intact and rotten grapes (25 g) were homogenized with Ultra-turrax homogenizer and centrifuged at 10000 rpm for 10 min. The supernatant was filtered through a membrane with a pore size of 0.45 μm. Because of high dry weight of Aszú, dried grapes, and green rotten grapes, these samples (12 g) were extracted with 20 mL of 10% perchloric acid. After homogenization, centrifugation, and filtration, the supernatant was analyzed by HPLC. Three replicate extractions from the grape samples of each variety were performed.

The concentration of amine compounds in grapes was calculated on a dry matter basis of samples. The dry weight was gravimetrically determined (*16*) from 15 g of grapes. Three replicate samples were analyzed.

Grape seeds (1.5 g) were grained and extracted with 5 mL of 10% perchloric acid. After filtration, it was analyzed by HPLC.

HPLC Analysis and Chromatographic Conditions. Chromatography was performed with Alliance Waters 2690 HPLC chromatograph equipped with a Waters 474 fluorimetric detector (λ_{ex} = 345 nm and λ_{em} = 455 nm). Separation of amines was performed as reported in our previous work (*10*) with ion pair formation (octanesulfonic acid) on a reverse phase column (μBondapak C18, 300 mm × 3.9 mm, 10

Table 2. Amine Content of Intact Grapes in 2003 and 2004[a]

Vintage 2003

grape variety	Furmint			Hárslevelü	
vineyard	Z	S	P	Z	S
Put	4.94 (0.37)	2.89 (0.38)	2.47 (0.29)	3.16 (0.40)	5.45 (0.21)
iBa					
Cad	0.50 (0.10)	0.37 (0.02)	0.55 (0.12)	0.22 (0.06)	1.02 (0.03)
Unk1					
Tyr					
His	0.12 (0.02)	0.22 (0.06)	0.10 (0.01)	0.34 (0.02)	0.19 (0.02)
2MeBa					
Agm					
3MeBa					
Spd	4.97 (0.34)	5.33 (1.21)	3.69 (0.39)	6.33 (0.20)	6.37 (0.53)
Phe		0.15 (0.04)		0.10 (0.004)	0.08 (0.002)

Vintage 2004

grape variety	Furmint			Hárslevelü			Yellow Muscat	Zéta	White Grape
vineyard	K	V	G	K	V	G	K	V	G
Put	7.24 (0.64)	4.42 (0.69)	4.36 (0.49)	1.67 (0.27)	1.13 (0.32)	0.70 (0.24)	2.44 (0.47)	2.00 (0.21)	4.26 (0.26)
iBa			0.14 (0.01)				0.07 (0.06)		
Cad	0.32 (0.08)	1.33 (0.40)	1.13 (0.37)	0.01 (0.001)	0.04 (0.03)	0.01 (0.001)	0.10 (0.09)	0.45 (0.19)	0.01 (0.001)
Unk1									
Tyr									
His	0.46 (0.03)	0.46 (0.01)	0.49 (0.02)	0.86 (0.03)	0.63 (0.07)	0.78 (0.04)	0.55 (0.01)	0.54 (0.06)	0.31 (0.05)
2MeBa							0.04 (0.01)		
Agm		0.10 (0.07)					0.08 (0.01)		
3MeBa			0.55 (0.03)				0.13 (0.02)		
Spd	12.25 (0.54)	10.08 (1.48)	10.93 (0.72)	9.19 (0.77)	8.51 (0.13)	8.46 (1.64)	8.22 (0.56)	7.48 (0.33)	9.29 (0.57)
Phe	0.16 (0.05)	0.16 (0.08)	0.23 (0.09)	0.11 (0.03)	0.12 (0.02)	0.14 (0.09)	0.17 (0.07)	0.45 (0.28)	0.11 (0.02)

[a] Standard deviations are shown in parentheses. Results are calculated in mg kg^{-1} on a dry matter basis.

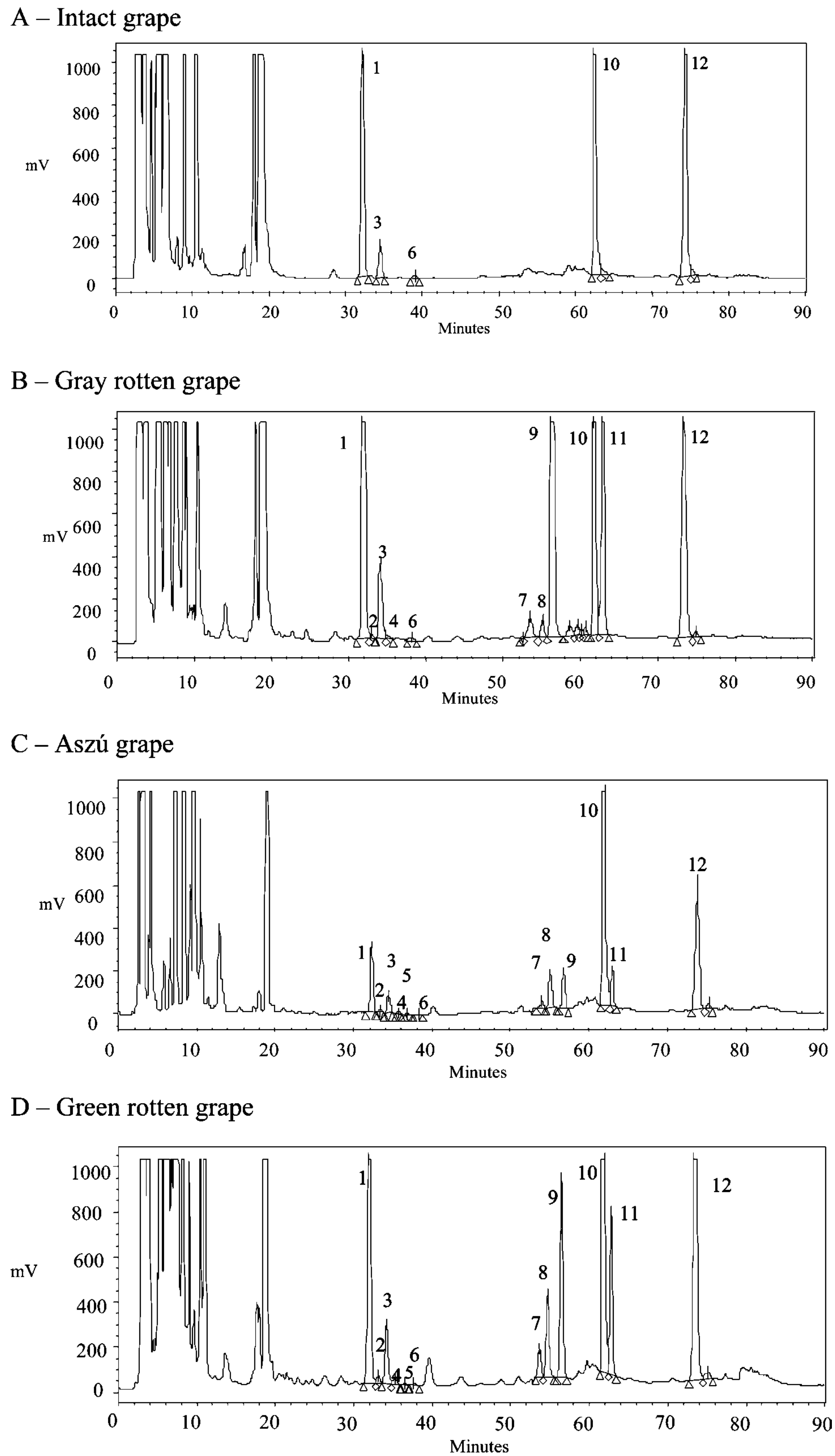

Figure 1. Chromatogram of biogenic amines (vintage 2004, Furmint varieties). The injected volumes were 30 μL, except for Aszú grapes from which 10 μL was injected into the column. (**A**) Intact grape from vineyard K, (**B**) gray rotten grape from vineyard K, (**C**) Aszú grape from vineyard V, and (**D**) green rotten grape from vineyard V. Peaks identified: Put, 1; iBa, 2; Cad, 3; Unk1, 4; Tyr, 5; His, 6; 2MeBa, 7; Agm, 8; 3MeBa, 9; Spd, 10; Phe, 11; and Istd, 12.

μm; from Waters) using postcolumn derivatization with OPA (*o*-phtaldialdehyde and 2-mercaptoethanol). Compounds were eluted with a gradient prepared from three solutions 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 flow rate of the mobile phase was 1 mL min^{-1}, and the flow rate of OPA was 0.8 mL min^{-1}.

Table 3. Amine Content of Aszú Grapes in 2003 and 2004[a]

Vintage 2003

grape variety	Furmint			Hárslevelü		mixed		
vineyard	Z	S	P	Z	S	S	M1	M2
Put	7.67 (1.10)	3.91 (1.3)	2.42 (0.33)	5.72 (1.59)	7.94 (0.99)	7.19 (0.15)	15.77 (1.85)	11.38 (3.28)
iBa	2.29 (1.36)	1.81 (0.90)	0.60 (0.23)	0.56 (0.26)	0.82 (0.11)	1.61 (0.48)	14.22 (9.03)	7.29 (1.23)
Cad	0.84 (0.07)	1.16 (0.30)	1.08 (0.04)	0.35 (0.17)	1.35 (0.23)	1.52 (0.18)	2.54 (0.40)	1.44 (0.23)
Unk1	0.10 (0.04)	0.31 (0.21)		0.11 (0.06)	0.17 (0.09)	0.29 (0.09)	5.42 (1.37)	1.08 (0.42)
Tyr	0.98 (0.32)	0.53 (0.39)	0.16 (0.05)	0.11 (0.05)	0.53 (0.09)	0.85 (0.34)	7.58 (3.75)	2.09 (0.52)
His	0.30 (0.02)	0.20 (0.13)	0.14 (0.02)	0.33 (0.11)	0.42 (0.03)	0.28 (0.08)	0.64 (0.10)	0.76 (0.44)
2MeBa	6.33 (1.67)	3.45 (1.91)	2.48 (1.35)	1.69 (0.42)	1.73 (0.8)	5.03 (0.93)	20.66 (6.16)	11.47 (1.53)
Agm	1.12 (0.57)	1.01 (0.41)	0.32 (0.07)	1.34 (0.49)	1.46 (0.40)	1.35 (0.48)	7.33 (1.24)	7.71 (3.1)
3MeBa	11.13 (0.63)	7.79 (3.21)	8.02 (1.75)	10.55 (1.26)	9.14 (0.66)	9.96 (0.78)	34.23 (3.55)	25.51 (1.30)
Spd	10.28 (0.45)	10.94 (0.65)	8.49 (0.36)	11.48 (1.29)	10.54 (1.09)	9.21 (0.60)	24.60 (1.28)	21.90 (0.40)
Phe	9.28 (0.82)	7.38 (2.62)	7.33 (0.92)	8.40 (0.38)	8.47 (0.05)	9.31 (1.14)	28.23 (2.71)	22.32 (0.92)

Vintage 2004

grape variety	Furmint			Hárslevelü			Yellow Muscat	Zéta	White Grape
vineyard	K	V	G	K	V	G	K	V	G
Put	14.27 (3.42)	2.94 (0.001)	4.95 (0.80)	7.53 (1.87)	1.67 (0.38)	2.82 (0.57)	15.31 (7.05)	1.52 (0.26)	4.25 (0.73)
iBa	0.54 (0.18)	0.13 (0.03)	0.35 (0.15)	0.16 (0.08)	0.05 (0.01)	0.18 (0.04)	0.42 (0.13)	0.06 (0.02)	0.27 (0.02)
Cad	0.39 (0.21)	0.46 (0.04)	0.44 (0.19)	0.07 (0.01)	0.07 (0.03)	0.07 (0.03)	0.61 (0.45)	0.36 (0.05)	0.09 (0.02)
Unk1	0.13 (0.04)	0.07 (0.008)	0.18 (0.07)	0.15 (0.05)	0.13 (0.01)	0.27 (0.09)	0.33 (0.20)		0.13 (0.02)
Tyr	0.87 (0.14)	0.42 (0.04)	0.77 (0.21)	0.49 (0.03)		0.47 (0.02)	0.64 (0.08)	0.38 (0.04)	0.46 (0.004)
His	0.40 (0,03)	0.31 (0.02)	0.30 (0.01)	0.43 (0.01)	0.34 (0.05)	0.35 (0.03)	0.31 (0.06)	0.28 (0.03)	0.37 (0.02)
2MeBa	1.69 (0.35)	0.44 (0.03)						0.42 (0.13)	1.23 (0.1)
Agm	4.93 (0.44)	2.71 (0.04)	4.41 (1.03)	3.02 (0.31)	2.61 (0.12)	3.07 (1.08)	3.33 (0.85)	1.08 (0.15)	3.03 (0.34)
3MeBa	8.76 (1.32)	1.98 (0.12)	7.39 (1.58)	6.10 (0.79)	1.72 (0.58)	3.36 (1.17)	10.49 (0.60)	0.66 (0.18)	4.35 (0.54)
Spd	24.23 (6.65)	22.11 (1.37)	32.13 (1.82)	25.02 (3.02)	26.43 (2.90)	29.80 (3.93)	26.30 (2.44)	20.27 (5.06)	10.62 (0.91)
Phe	12.40 (2.77)	2.87 (0.13)	9.43 (1.94)	8.84 (1.13)	2.46 (0.72)	4.93 (1.2)	9.70 (1.98)	1.76 (0.35)	4.46 (0.34)

[a] Standard deviations are shown in parentheses. Results are calculated in mg kg^{-1} on a dry matter basis.

Standard solutions of amines in the concentration range of 0.1−10 mg L^{-1} were used for the calibration. Peak areas were recorded and calculated using the Waters Millenium Software package. The gradient elution program and the validation of the present method can be found in our previous study (*10*). Unknown1 (Unk1) was calculated using the iBa calibration.

Microbiology. For microbiological investigations, the plate count agar (Merck) was used for total bacterial count. The total yeast and mold counts were determined in chloramphenicol glucose agar (Biolab) with bengal-rose (Fluka).

Ten grams from each sample was blended in 90 mL of solution (1 g of bacteriological peptone, 9 g of NaCl, and 1 L of distilled water) for 90 s. Serial dilutions of the homogenate were plated in plate count agar (5 g of peptone from casein, 2.5 g of yeast extract, 1 g of D-(+)-glucose, 14 g of agar−agar, and 1 L of distilled water) plates. The plates were incubated for 5 days at 30 °C to determine the total mesophilic bacterial count.

For mold and yeast counts, the same serial dilutions were used and were plated in chloramphenicol glucose agar (5 g of peptone, 20 g of glucose, 0.2 g of chloramphenicol, 14.8 g of agar−agar, and 1 L of distilled water) containing 0.025 g/L bengal-rose. Plates were incubated for 5 days at room temperature. Counted colonies were expressed as colony forming units (CFU) per gram berry.

Statistics. Analysis of variance, *t*-test, correlation analysis, principal component analysis (PCA), and linear discriminant analysis (LDA) were performed by Microsoft Excel and the Minitab statistical program to study differences in amine composition of grapes.

RESULTS AND DISCUSSION

Composition of Microorganisms on the Surface of Infected Grape Berries. Because one of the objectives of our study was to compare the amine composition of Aszú grapes (noble rotten) with that of gray rotten berries and of berries infected with other local mold biota, we measured the total microbial count of grape samples, which originated from the same growing location.

The mean values of the microbial population of grape varieties and distribution of molds (*Penicillium* and *Botrytis*) on grape samples growing in vineyards K and V are summarized in **Table 1**.

On the berries, six morphologically different colonies were detected. The microscopic investigations revealed that they were *Penicillium* spp. (two morphologically different colonies) and *Botrytis* spp. (four morphologically different colonies). In vineyard K, both Aszú and gray rotten berries were mainly infected with *B. cinerea*. In vineyard V, *Penicillium* spp. were present dominantly on grapes infected with green molds, and on Aszú grapes, *B. cinerea* was mostly settled.

Studying yeast and mold biota of botrytized grapes, Bene and Magyar (*3*) concluded that high quality vintages resulted in low yeast populations with low mold conidia counts. Besides *Botrytis*, other mold species such as *Penicillium* and *Aspergillus* commonly appeared in varying populations.

Effect of Microbiota on Amine Content of Grapes. Figure 1 shows the chromatograms of intact (**A**), gray rotten (**B**), Aszú (**C**), and green rotten grapes (**D**). Ten amines were identified from 11 isolated amines of grapes. Intact grapes contain less number of amines (putrescine, cadaverine, spermidine, and minor histamine) than grapes infected with molds. The chromatograms show well that primary aliphatic amines, phenylethylamine, and agmatine appeared in all grape berries infected.

In seeds of berries, Put, Cad, and Spd were found in high concentrations. Put and Spd are among the major cellular polyamines in living organisms; their occurrence was natural in grape seeds. However, the presence of Cad in high concentrations was unexpected. The relatively high concentration of cadaverine in grape samples of 2003 probably originates from the seed's cadaverine.

Table 4. Amine Content of Dried, Gray, and Green Rotten Grapes in 2003 and 2004[a]

Vintage 2003

grape type	dried grapes		
grape variety	Furmint		Hárs-levelü
vineyard	Z	P	Z
Put	9.42 (0.21)	6.69 (1.00)	7.56 (0.49)
iBa		0.05 (0.03)	0.05 (0.004)
Cad	0.55 (0.03)	0.52 (0.12)	0.06 (0.02)
Unk1			
Tyr			0.07 (0.01)
His	0.30 (0.05)	0.57 (0.29)	0.85 (0.01)
2MeBa		0.04 (0.02)	0.06 (0.003)
Agm	0.23 (0.04)	1.10 (0.68)	1.06 (0.03)
3MeBa		0.24 (0.16)	1.17 (0.17)
Spd	6.46 (0.08)	5.37 (0.91)	8.96 (0.37)
Phe	0.07 (0.02)	0.40 (0.17)	0.93 (0.12)

Vintage 2004

grape type	dried grapes			gray rotten grapes			green rotten grapes		
vineyard	K			K			V		
grape variety	Furmint	Hárs-levelü	YellowMuscat	Furmint	Hárs-levelü	YellowMuscat	Furmint	Hárs-levelü	Zéta
Put	6.15 (0.27)	4.50 (0.21)	5.48 (0.56)	7.74 (0.18)	6.45 (0.30)	6.76 (0.11)	9.64 (0.19)	9.21 (0.30)	7.22 (0.91)
iBa	0.06 (0.01)	0.06 (0.03)	0.16 (0.02)	0.12 (0.03)	0.09 (0.01)	0.16 (0.03)	0.29 (0.04)	0.28 (0.02)	0.26 (0.11)
Cad	0.74 (0.14)		0.06 (0.04)	1.57 (0.19)	0.05 (0.008)	0.79 (0.18)	1.56 (0.19)	0.13 (0.01)	0.51 (0.29)
Unk1				0.10 (0.02)	0.11 (0.04)	0.13 (0.02)	0.21 (0.03)	0.19 (0.04)	0.14 (0.08)
Tyr		0.34 (0.03)	0.42 (0.06)	0.22 (0.04)	0.32 (0.02)		0.71 (0.03)	0.71 (0.01)	0.38 (0.08)
His	0.42 (0.02)	0.88 (0.02)	0.65 (0.078)	0.34 (0.01)	0.60 (0.03)	0.44 (0.03)	0.57 (0.04)	0.62 (0.06)	0.31 (0.26)
2MeBa	0.04 (0.004)	0.05 (0.005)	0.04 (0.003)	0.83 (0.03)	0.44 (0.11)	0.58 (0.09)	1.67 (0.15)	0.34 (0.04)	0.48 (0.15)
Agm		0.07 (0.03)	0.08 (0.04)	0.72 (0.17)	0.44 (0.18)	0.33 (0.09)	4.77 (0.46)	4.00 (0.60)	2.19 (0.70)
3MeBa	0.38 (0.07)	0.39 (0.11)	0.26 (0.06)	11.55 (0.4)	9.71 (0.91)	9.68 (0.46)	9.46 (0.34)	2.58 (0.55)	1.40 (0.61)
Spd	7.96 (0.24)	7.15 (0.34)	7.60 (0.89)	8.33 (0.06)	8.39 (0.36)	7.25 (0.05)	15.75 (0.28)	17.50 (0.26)	11.16 (0.54)
Phe	0.11 (0.06)	0.20 (0.07)	0.15 (0.04)	8.73 (0.56)	5.14 (1.4)	4.62 (0.59)	8.54 (0.94)	5.16 (2.00)	3.20 (1.50)

[a] Standard deviations are shown in parentheses. Results are calculated in mg kg^{-1} on a dry matter basis.

The amine content of different varieties of intact, dried, gray rotten grapes, Aszú grapes, and green rotten grapes from different vineyards of Tokaj is shown in **Tables 2−4**, respectively.

Similarly to our earlier results, intact grapes (**Table 2**) contain mainly Put (0.7−7.24 mg kg^{-1}) and Spd (3.69−12.25 mg kg^{-1}). Other amines (Cad, His, and Phe) can occur only in small quantities (below 1 and 1.3 mg kg^{-1}), or they are not found in intact grapes. During botrytization, new compounds appeared in Aszú grapes (**Table 3**) such as iBa (0.05−14.22 mg kg^{-1}), Unk1 (0−5.42 mg kg^{-1}), Tyr (0−7.58 mg kg^{-1}), Agm (0.32−7.71 mg kg^{-1}), 2MeBa (0−20.66 mg kg^{-1}), and 3MeBa (0.66−34.23 mg kg^{-1}). The concentrations of Spd (8.49−32.13 mg kg^{-1}) and Phe (1.76−28.23 mg kg^{-1}) increased as compared to intact grapes.

In gray rotten grapes (**Table 4**), Put (6.45−7.74 mg kg^{-1}), 3MeBa (9.68−11.55 mg kg^{-1}), Spd (7.25−8.39 mg kg^{-1}), and Phe (4.62−8.73 mg kg^{-1}) occur in the highest concentrations. The Agm content (0.33−0.72 mg kg^{-1}) of gray rotten grapes remains at a low level as compared to Aszú grapes. In green rotten grapes, Put (7.22−9.64 mg kg^{-1}), Agm (2.19−4.77 mg kg^{-1}), 3MeBa (1.4−9.46 mg kg^{-1}), Spd (11.16−17.5 mg kg^{-1}), and Phe (3.2−8.54 mg kg^{-1}) are the most important amines as well.

Figure 2 shows the total amine content of intact and infected grapes studied in vintages 2003 and 2004. Put and Spd contents of grapes are plotted separately from the total content of primary aliphatic amines. The total amine contents of intact and dried grapes did not reach 21 mg kg^{-1}, while those of Aszú, rotten grapes, and grapes with green molds usually ranged from 26 to 69 mg kg^{-1}.

The appearance of primary aliphatic amines in high concentrations in grapes shows the activity of microorganisms. In vitro, Agm, 3MeBa, Put, Spd, and Phe could be detected by HPLC in large quantities in *B. cinerea* grown on nutrient solution (data not yet published). We suppose that molds and bacteria growing on grape berries can further synthesize primary aliphatic amines (iBa, 2MeBa, and supposedly Unk1) in high concentrations (**Figure 1**). Studying bacterial strains in a complex medium, Bast (*17*, *18*) found that volatile primary amines could be produced from simple aldehydes and amino acids in bacteria. Butlyamines, pentylamines, and phenylethylamine appeared most frequently in the largest quantities. According to the author, enzymatic decarboxylation and/or aldehyde (trans)amination can be the pathways of biogenesis.

In wines produced from botrytized grapes, the concentration of the aliphatic primary amines (*7*−*9*) increases, indicating the action of added yeasts used during fermentation. Studying must and wines produced from intact and rotten grape varieties, Eder et al. (*19*) established that the amount of total amines was significantly higher in products made from rotten grapes than in those made from intact grape berries. The authors ascribed the higher total amine contents mainly to *i*-pentyl-amine (2-methyl-butyl amine or 3-methyl-butyl amine) and phenylethylamine originating from rotten grapes. Other publications report about *i*-pentyl-amine in wines as well (*13*, *20*).

Using correlation analysis, we investigated the relationship between amines in grapes infected with *B. cinerea*. Studying all Aszú grapes, iBa, 3MeBa, Phe, and Tyr correlated significantly to each other and to other biogenic amines such as Put and Agm ($0.68 < r < 0.94$, $p < 0.04$). The appearance and increase of amines in grapes are due to the effect of microbiota

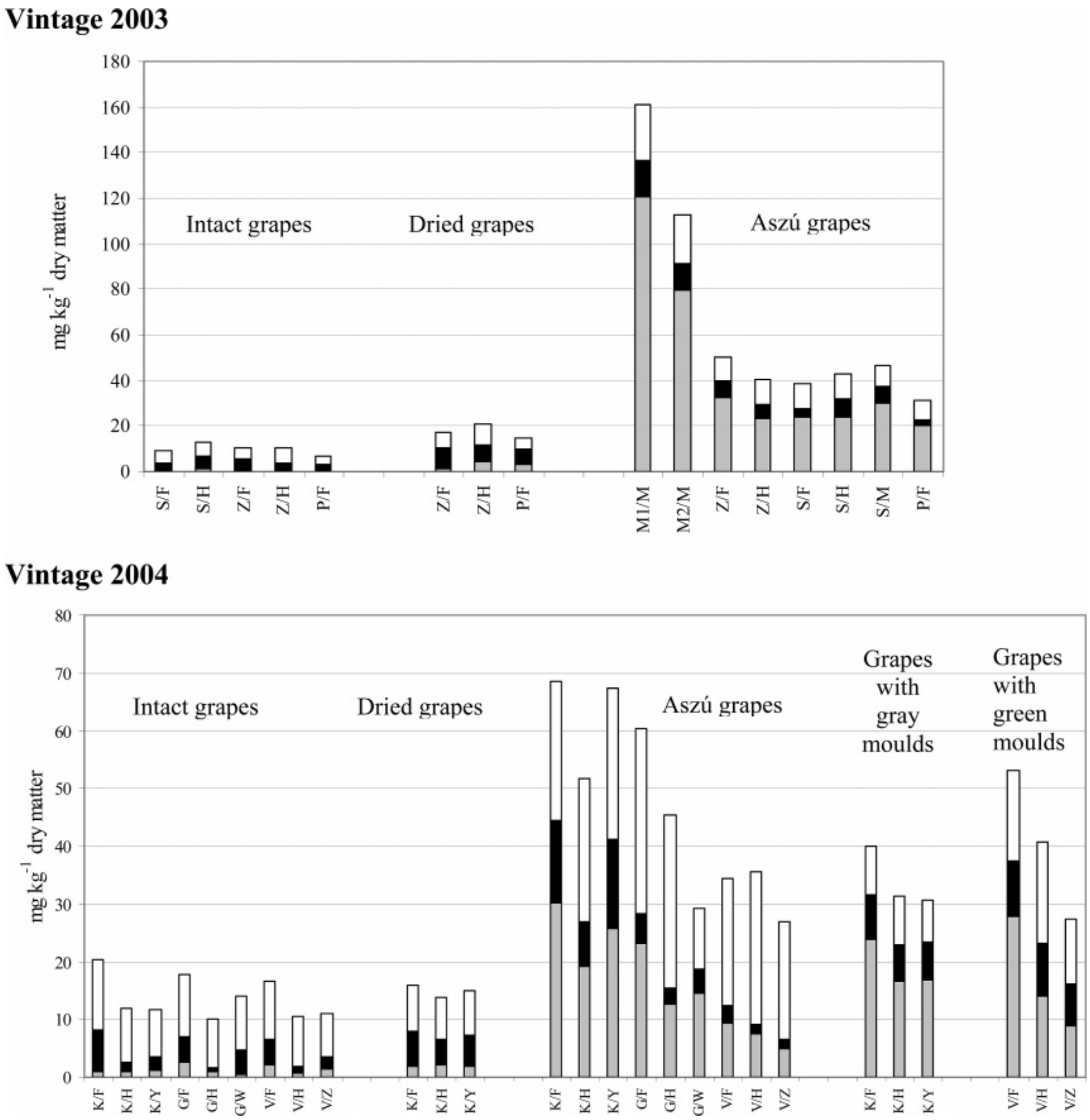

Figure 2. Total amine content of grapes in vintages 2003 and 2004. Spermidine, white bars; putrescine, black bars; and other amines, gray bars. Other amines: sum of iBa, Cad, Unk1, Tyr, His, 2MeBa, Agm, 3MeBa, and Phe. Vineyards: S, Z, P, M1, M2, K, G, and V. Grape varieties: F, Furmint; H, Hárslevelü; M, mixed varieties of Furmint and Hárslevelü; Y, Yellow Muscat; W, White Grape; and Z, Zéta.

(predominantly *B. cinerea*), which was verified with mathematical statistical methods. This observation was similar to an earlier investigation (*10*), when wines produced from botrytized grapes were analyzed. The correlation found between amines in botrytized wines also suggests that the effect of yeast during fermentation beyond the effect of Aszú grapes contributes to the specific amine composition of botrytized wines.

Evaluating green rotten grapes, only Agm, Unk1, and iBa correlated significantly to each other ($0.99 < r < 1.00$, $p < 0.03$), while no significant correlation was found between pairs of amines in gray rotten grapes. This observation is probably due to the few samples (only from one vineyard) investigated.

The amine content of Aszú, intact, dried, and gray rotten grapes grown in vineyard K from 2004 vintage can be seen in **Figure 3**. The amine contents of different varieties, such as Furmint, Hárslevelü, and Yellow Muscat, are averaged and plotted in the figure. Using *t*-test, significant differences $[0.001_{(\mathrm{Spd})} < p < 0.02_{(\mathrm{Agm})}]$ could be found between Aszú and gray rotten grapes in Tyr, Agm, and Spd contents. The average content of these compounds was 3−7-fold higher in Aszú grapes than in rotten berries.

PCA was performed to visually recognize the different groups of grapes in vineyard K (**Figure 4**). The first two principal components (PCs) accounted for more than 90% of the total variance in the data. Six amines, listed in the figure legend, were used as variables in PCA. PC scores of Aszú, intact, dried, and gray rotten grapes show good separation. Only one of the noninfected dried grape varieties was projected near the intact grape varieties.

The classification of grape samples above was studied with LDA using three variables (Agm, Spd, and Unk1). The classification of Aszú, intact, and gray rotten grapes was correct (100%), and for dried grapes, 67% was obtained in the case of grape berries from the same vineyard. One dried grape sample (Furmint) was classified into the class of intact berries.

Figure 5 shows the amine content of intact, Aszú, and green rotten grapes that came from vineyard V. The average amine content of different varieties (Furmint, Hárslevelü, and Zéta) is plotted. In earlier publications (*8*, *9*), iBa, Unk1, Tyr, Agm, Spd, and Phe were proven to be the most relevant amines in distinguishing intact grapes from Aszú. Green rotten grapes contained Put, iBa, Unk1, Agm, and Phe as well; furthermore,

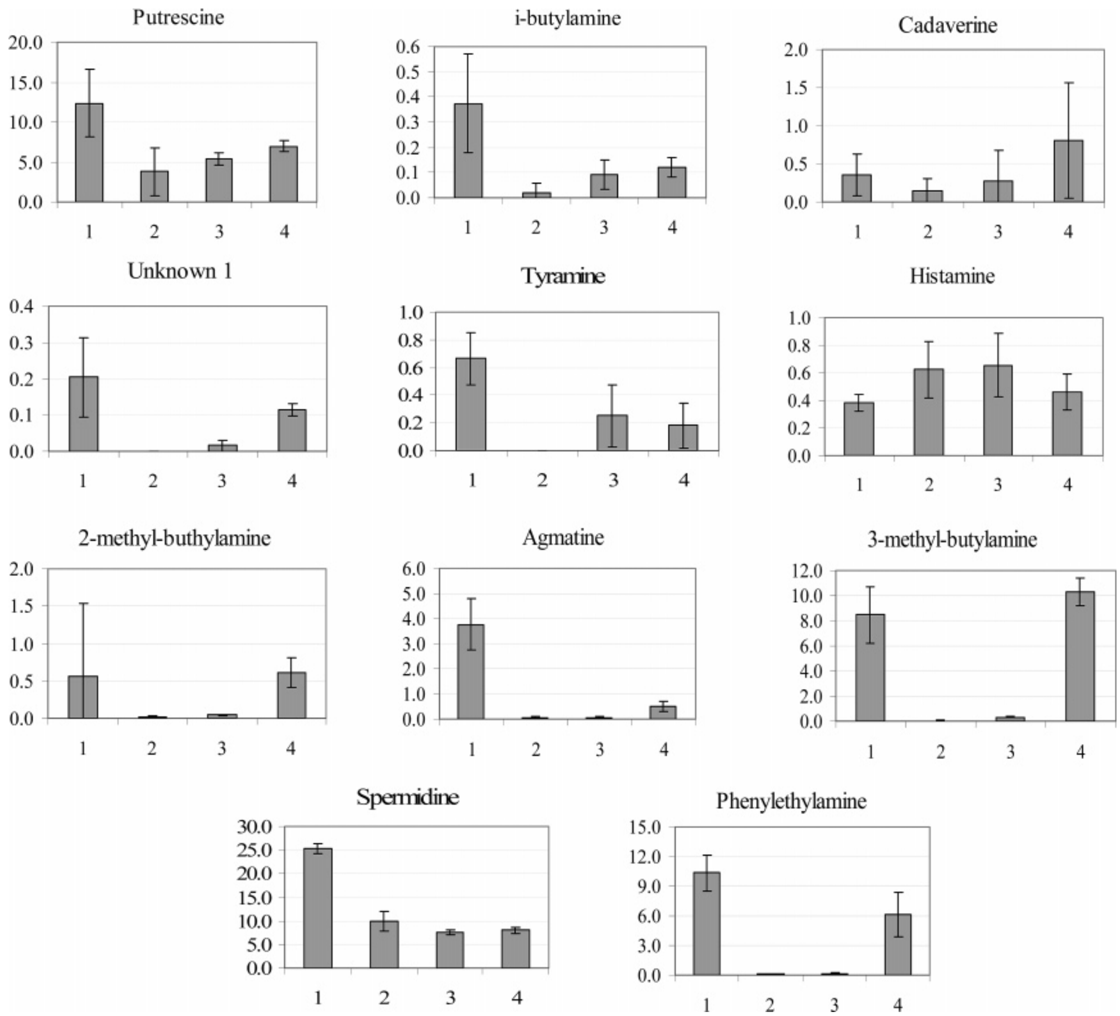

Figure 3. Amine content of grapes from vineyard K. Key: 1, Aszú grapes; 2, intact grapes; 3, dried grapes; and 4, gray rotten grapes. Results are calculated to mg kg^{-1} on a dry matter basis. The standard deviation indicates the variability of varieties.

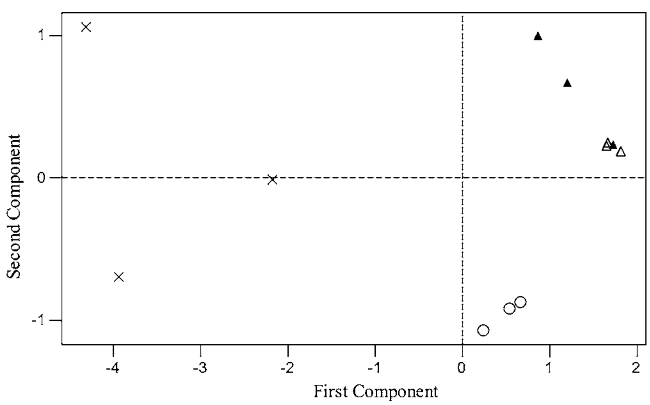

Figure 4. PCA of amines. Discriminating groups of Aszú, intact, dried, and gray rotten grapes from vineyard K (2004). Variables: iBa, Unk1, Tyr, Agm, Spd, and Phe. Aszú grapes, ×; intact grapes, △; dried grapes, ▲; and gray rotten grapes, ○.

the concentrations of Put and iBa were significantly higher (using *t*-test, $p_{(\mathrm{Put,iBa})} = 0.005$) than in Aszú grapes. Spd is the only amine found in lower concentration in green rotten grapes than in Aszú grapes ($p = 0.04$).

PCA was used (**Figure 6**) to distinguish Aszú, green rotten, and intact grapes. PCA allowed more than 93% of the total variance to be explained by the first two PCs using six variables. The score plot of amines shows a very good separation between groups of intact and infected grapes. LDA also showed a good classification for Aszú, intact, and green rotten grapes (100%) using the following three variables: iBa, Agm, and Spd.

From vineyard G, only Aszú and intact grape samples were investigated. Using PCA (**Figure 7**), the scores of Aszú and intact grapes are well-separated and more than 95% of the total variance is explained by the first two PCs. The classification of Aszú and intact grape samples was correct (100%) using LDA with two variables (iBa and Unk1).

When we evaluated amine values of grape samples independently of the place of origin (**Figure 8**), we could separate intact (normal and dried), gray rotten grapes from Aszú and green rotten grapes using PCA. The Aszú grapes and green rotten grapes were not differentiated on the score plot of the first two PCs. More than 72% of the total variance is explained by the first two PCs. To confirm that Aszú grapes can be distinguished from gray rotten grapes on the basis of amine composition, further investigations are needed.

Effect of Varieties and Vineyards on Amine Composition of Grapes. Two varieties (Furmint and Hárslevelü) in 2003 and an additional three varieties (Yellow Muscat, White Grape, and Zéta) in 2004 were studied. Comparing (**Figure 2**) varieties infected with different mold biota (Aszú, green, and gray rotten grapes) from the same vineyard, the total amine content was higher in Furmint except in two cases. In vineyard S in 2003 and in vineyard V in 2004, the total amine contents of Furmint were quite similar to those of Hárslevelü. Comparing with other

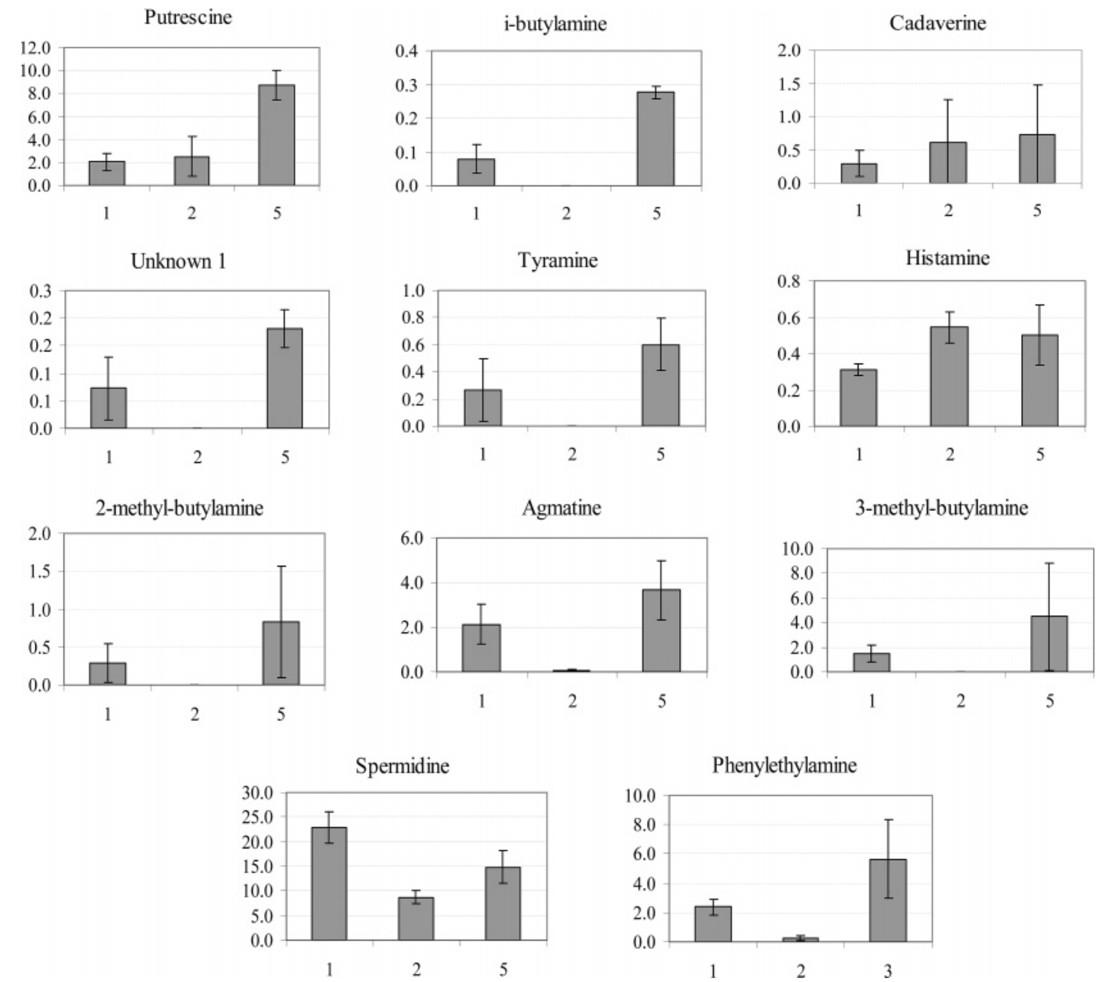

Figure 5. Amine content of grapes from vineyard V. Key: 1, Aszú grapes; 2, intact grapes; and 5, green rotten grapes. Results are calculated to mg kg^{-1} on a dry matter basis. The standard deviation indicates the variability of varieties.

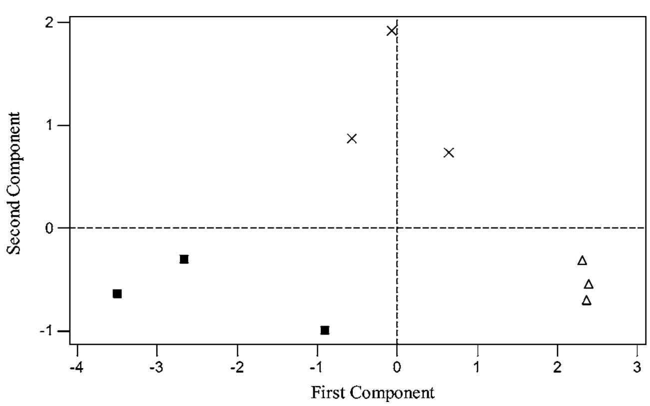

Figure 6. PCA of amines. Discriminating groups of Aszú, intact, green, rotten grapes from vineyard V (2004). Variables: iBa, Unk1, Tyr, Agm, Spd, and Phe. Aszú grapes, ×; intact grapes, △; and green rotten grapes, ■.

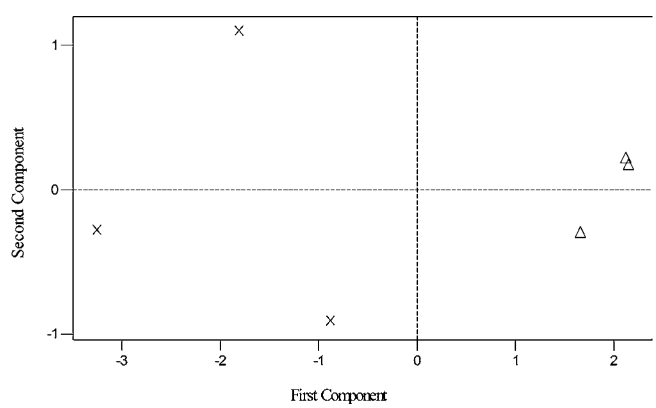

Figure 7. PCA of amines. Discriminating groups of Aszú and intact from vineyard G (2004). Variables: iBa, Unk1, Tyr, Agm, Spd, and Phe. Aszú grapes, ×; and intact grapes, △.

grape varieties in all vineyards with one-way analysis of variance, the higher total amine content of Furmint was statistically significant (F_{cal} 6.84 > F_{crit} 4.45, p < 0.05). This observation was similar to our earlier results measured for Aszú grapes (*7*).

The total amine content of Zéta from vineyard V was the smallest (**Figure 2**) among the three investigated varieties (Furmint, Hárslevelü, and Zéta) both in Aszú and green rotten berries. In vineyard K, the total amine content of Yellow Muscat was higher than that of Hárslevelü only in Aszú grapes, while in green rotten grapes no difference was found between them. In vineyard G, the total amine content of White Grape was smaller than that of Hárslevelü. To establish a possible tendency in total amine content of Aszú grapes in regard to varieties (Hárslevelü, Yellow Muscat, White Grape, or Zéta), further studies are needed.

Figure 2 also shows that the total amine content of Aszú grapes is different depending on vineyards. At the same time, in intact grapes, the total amine content practically did not vary

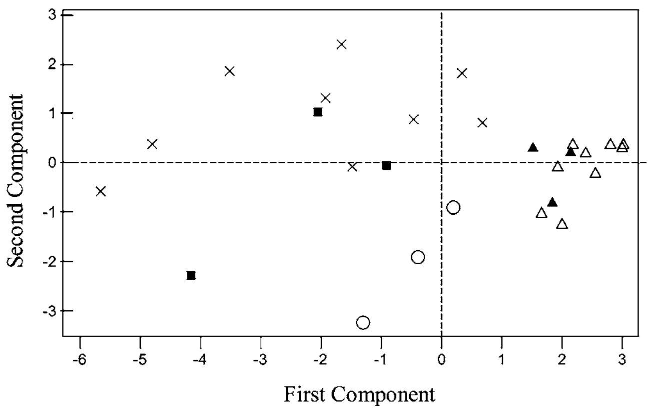

Figure 8. PCA of amines of vintage 2004. Variables: Put, iBa, Cad, Unk1, Tyr, His, 2MeBa, Agm, 3MeBa, Spd, and Phe. Aszú grapes, ×; intact grapes, △; dried grapes, ▲; green rotten grapes, ■; and gray rotten grapes, ○.

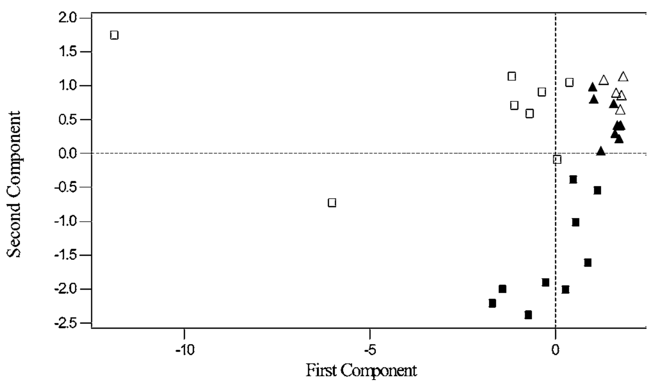

Figure 9. PCA of Aszú and intact grapes from vintages 2003 and 2004. Variables: Put, iBa, Cad, Unk1, Tyr, His, 2MeBa, Agm, 3MeBa, Spd, and Phe. Aszú grapes from vintage 2003, □; intact grapes from vintage 2003, △; Aszú grapes from vintage 2004, ■; and intact grapes from vintage 2004, ▲.

depending on the place of origin. In vintage 2003, the total amine contents of vineyards M1 (161 mg kg^{-1}) and M2 (112 mg kg^{-1}) were extremely high, while those of other vineyards ranged from 31 to 50 mg kg^{-1} in vintage 2003 and from 26 to 69 mg kg^{-1} in vintage 2004.

To recognize groups from the two studied vintages, PCA was performed (**Figure 9**). PCA allows more than 80.4% of the total variance to be explained by the first two PCs. Certain separation of scores could be observed between vintages concerning Aszú grapes, while the scores of intact grapes got closer to each other. Considering that the vineyards were not the same in the two investigated years, the separation of groups could not be due solely to the effect of vintages but to the different origin of the samples. The results of our previous works (*7*, *8*) showed that the effect of vintages had a great importance for the amine content of Aszú grapes.

In spite of various effects on grapes during botrytization discussed above, the difference of the amine composition between Aszú and intact grapes was significant for all grape samples from different origins of the two investigated years. It was confirmed by one-way analysis of variance using all compounds as variables (F_{cal} 40.85 > F_{crit} 3.86, $p < 0.0001$).

Summarizing the results, microbiota living in/on berries have a great effect on the composition and concentration of amines in grape berries. Depending on circumstances, the rotting process caused by *B. cinerea* can change into noble rot resulting in sweet, delicious, tasty, raisin-like Aszú berries or develop into gray rot, resulting in rotten, completely worthless grapes. Our results show that the composition of amines of grapes depends on the species of microbiota and on the condition of their growth on/in berries. Furthermore, the results supported our former statement that the characteristic amine composition of Aszú wines indicates the action of *B. cinerea* on grapes (*10*).

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