WAC 2022

Fermentation products, degradation parameters, (poly)phenols and potassium content in Tokaji Aszú winemaking

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Keywords: aszú; botrytization; noble rot; Tokaj

1. INTRODUCTION

The historical Tokaj Wine Region covering 5,500 ha vineyards in northeast Hungary has been a UNESCO World Heritage region since 2002. Produced from "noble rotted" grapes, Tokaji Aszú is known all over the world as one of the oldest botrytized wines. Special microclimatic conditions (due to the Bodrog and Tisza rivers, Indian summer), soil conditions (clay, loess on volcanic bedrock) and grape varieties (Furmint, Hárslevelű) of the Tokaj region offer favourable parameters to the formation of noble rot caused by *Botrytis cinerea*. The special metabolic activity of *Botrytis* results in noble rot grape called "aszú" berries (Figure 1.).

Figure 1. The formation of noble rot [1]

The three main conditions needed to occur at the same time are:
1. *Botrytis cinerea* invades the grapes in full maturity
2. Intact and undamaged (healthy) berries
3. Special climatic conditions: sunny days after the morning mists

Botrytis cinerea is an optional parasitic mould. It is generally a serious nuisance (grey rot) but in special circumstances it causes noble rot. Asexual reproduction takes place with blastoconidia, which are freely arranged at the ends of the conidia in spherical groups which look similar to be a grape bunch (Figure 2.).

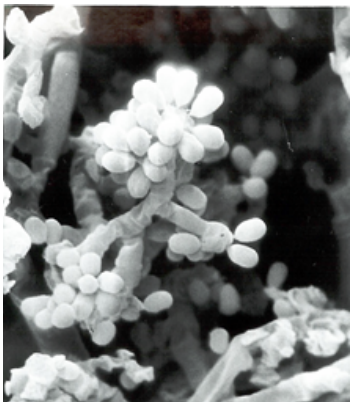

Figure 2. Electron microscopic view of blastoconidias [2]

The grapes undergo complex chemical modifications as the joint result of the enzymatic activity of *Botrytis* and the physical process of concentration.

The main chemical changes:

1. Sugar content: As a result of evaporation the sugar content of the grape increases very significantly: 350-700 g/l. As the polysaccharides and pectin substances are broken down the quantity of other hexoses (rhamnose, galactose, mannose), pentoses and galacturonic acid increases [3].
2. Polyol: Three main polyols are responsible for the increased viscosity and "body" of Botrytis wines: butanediol, glycerin and sorbitol. Must of healthy grapes contains only traces of glycerin but the glycerin content usually exceeds a value of 5 g/l in the must of noble rot grapes. Glycerin content continues to increase during fermentation, so values for wines made from botrytized aszú berries are usually above 10 g/l, while in Tokaji Aszú it is often over 20-30 g/l [4].
3. Acid composition: The effects of *B. cinerea* activity increases the quantity of citric acid and succinic acid in the must, which results in the development of an unusual kind of acid composition in the wine. The titratable acidity in Tokaji wines is higher than the average but it is not tartaric acid that dominates; the *Botrytis* uses it up during its activity. *Botrytis* produces various acids during enzyme activity (pectinase cellulose complex, protease phospholipase) that were not present in the original grape (gluconic acid, glucuronic acid, galactaric acid) [5]. Generally, a concentration of gluconic acid above 0.5 g/l is caused by bacterial or fungal infections [6], therefore it can be considered as a potential parameter for monitoring the infestation of *Botrytis cinerea* in berries [7,8]. The gluconic acid content really does not change during fermentation because it is not fermented by yeasts so it can therefore be detected in wines. An increase in galacturonic acid content has also been observed in *Botrytis*-infected grapes. Ketonic acids (pyruvic acid, 2-ketoglutaric acid) are present in elevated concentrations due to noble rot.
4. Aroma compounds and other constituents: significant roles are played by furfural, benzaldehyde, phenylacetaldehyde and benzaldehyde cyanohydrin (*other name*: prunasin-bitter almond flavour), as well as the so-called mushroom alcohol (1-octen-3-ol). Sotolon (3-Hydroxy-4,5-dimethylfuran-2(5H)-furanone) 3-hydroxy-4,5-dimethyl-2 (5H) furanone) produced by *Botrytis cinerea* is one of the most important components that gives the distinctive "Aszú" aroma (caramel flavour) [5]. *Botrytis* increases the quantity of polyphenol oxidases that increase the tendency of the wine to browning, while polysaccharides (β-glucan) synthesis causes poor filterability of wines. The phenolic materials (caffeic acid and p-coumaric acid) are converted into quinones which tend to polymerize with the formation of brown compounds [2]. *Botrytis* produces various oxidase and hydrolase enzymes that convert certain grape components, e.g. polyphenols, flavonoids and nitrogen-containing materials [4].
5. Polyphenols: During botrytization the three main hydroxycinnamic acids, p-coumaric acid, caffeic acid and ferulic acid, accumulate in the berries [9]. During the infection the polyphenol oxidases (tyrosinase and laccase) oxidize these hydroxycinnamic acids together with their esters (e.g. caftaric acid and coutaric acid). The quantity of catechins, epicatechins and galloyl-epicatechins also increases during botrytization. Bene examined the polyphenol content of Tokaji white wines in 3 vintages (2019, 2020, 2021); higher polyphenol concentrations were recorded in botrytized Tokaji wines than non-botrytized white wines [10].
6. Free amino acids: The quantity of free amino acids depends to a large extent on the winemaking technology. Generally, proline and arginine dominate; the proportion of proline and arginine is very similar in Tokaji wine specialties (30 and 28 %), with noticeable alanine and 4-amino-butanoic acid.
7. Biogenic amine: During Aszú winemaking there is an increase in the concentration of biogenic amines (tyramine, agmatine, phenylethylamine) and other aminos (primary aliphatic amines: i-butylamine, 2-methylbutylamine) [11].

During the course of my research, I examined Aszú base wines (Aszú wines after the second fermentation before maturing) made with similar winemaking philosophy from two different vintages to determine the extent to which the chemical composition carries the characteristics of noble rot, whether the characteristics of *Botrytis* activity remain, how much they differ from traditional sweet wine when young and pre-aging and the extent to which the presence of increased mineral material attributed to effects of climate change can be discerned.

2. MATERIALS AND METHODS

2.1. Aszú wine samples

The origin and winemaking procedure are shown in Table 1.

Table 1. The origin of Aszú samples

Samples	1DE20	2H20	3R20	5D20	6D20	1R21	2R21	3R21	4R21	5R21	6R21	7R21	8R21	9H21	10D21	11D21	12DE21
Winery	Dereszla	Hétszőlő	Royal	Disznókő	Disznókő	Royal	Royal	Royal	Royal	Royal	Royal	Royal	Royal	Royal	Disznókő	Disznókő	Dereszla
Vintage	2020					2021											
Aszú berries grape variety	Furmint	Furmint	Furmint&Hárslevelű	Furmint	Furmint	Sárga muskotály	Kabar	Furmint	Furmint	Furmint	Furmint	Hárslevelű	Furmint	Furmint	Furmint	Furmint	Furmint
Basic wine/must	Furmint	Furmint	Furmint&Hárslevelű	Furmint	Furmint	Sárga musk.&Furm &Hárs	Furmint&Hárslevelű	Furmint	Furmint	Furmint	Furmint	Hárslevelű	Furmint	Furmint	Furmint	Furmint	Furmint
Aszú berries processing	Storage 1-3 weeks					Storage 1-3 weeks											
Soaking time	16 h	16 h	48 h	12 h	30 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	48 h	12 h	16 h	16 h
Pressing method	pneumatic press (<2bar, 8-10 h)					pneumatic press (<2bar, 8-10 h)											
Specified yeast	yes					yes											
Fermentation	18-20°C, 20 days, stainless steel tank					18-20°C, 20 days, stainless steel tank											
Wine treatments after fermentation	Sulphuring, heating, filtering and racking into barrel					Sulphuring, heating, filtering and racking into barrel											

2.2. Wine profiling

Examination of chemical composition was carried out with analysis (NMR - Nuclear Magnetic Resonance) in Diagnosticum Zrt. laboratory, Szerencs. H NMR Technique [12]: ^{1}H NMR (proton nuclear magnetic resonance) spectra acquisitions were performed on a 400 MHz spectrometer (Bruker Avance) and a magnet Bruker AscendTM 400 MHz equipment with 2H "lock" channel and z gradient at a frequency of 400.13 MHz and 26.85 °C.
The data analysis is performed at Bruker BioSpin GmbH (Rheinstetten, Germany) according to testing method AA-72-02-06 (Wine-Profiling 4.0.4), released on 06-Nov-2020 (DIN EN ISO/IEC 17025:2018 Accreditation Certificate D-PL-19229-01-00 of Bruker BioSpin GmbH).

2.3. Measurement of mineral components and total phenols

Potassium: Turbidimetric method, the increase of the absorbance is directly proportional to the concentration of potassium in the sample according to REF984307 published by Thermo Fisher Gallery method and procedures [15]. Calcium: colorimetric method, according to REF984361 published by Thermo Fisher Gallery method and procedures [15]. Total polyphenol: Colorimetric Folin-Ciocalteu method by chromogenic complex according to REF984346 published by Thermo Fisher Gallery method and procedures [13].

I used STATA v17.0 software for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Analytical measurements

3.1.1. Standard parameters (alc., sugar, tartaric acid, malic acid, citric acid)

The quantity of citric acid is higher than normal sweet wines in every sample (300–800 mg/l). Tartaric acid proved not to be vintage-dependent and showed lower values but not in every case (5D20, 8R21, 10D21, 11D21 >3.0 g/l).

3.1.2. Fermentation products and higher alcohols (2,3 butanediol, pyruvic acid, galacturonic acid, succinic acid, glycerol)

Every sample showed significantly higher glycerin content than normal sweet white wines (14.0–31.0 g/l). The succinic acid results (with the exception of 12DE21) were similar. No samples contained raised levels of pyruvic acid (<20 mg/l). All samples were shown to have higher levels of galacturonic acid (600-800 mg/l). The quantity of 2,3-butanediol was higher (0.3-1.9 g/l) regardless of vintage and place of production.

3.1.3. Degradation parameters (acetic acid, acetoin, fumaric acid, gluconic acid)

Acetic acid levels were high in all samples (1.1–1.3 g/l). The acetoin value is similar to that of normal white wines. In all cases the fumaric acid value is higher than 4.0 mg/l in normal sweet wines; in 2020 values above 10.0 mg/l were recorded. It could not be proved in every case that gluconic acid was above 500 mg/l; it was only higher in 2 samples (2H20,3R20).

3.1.4. Polyphenols and amino acids (total polyphenols, arginine, proline, caftaric acid, epicatechin, trigonelline)

All results for polyphenol content in all samples in both vintages prove professional results (440–490 mg/l). In the case of the free amino acids (arginine, proline) a similar proportion was only proved in sample 12DE21, in the others the proline was 1.5–2.5 times the arginine content, and in 2 samples (4D20, 10D21) less proline was measured than arginine. I could not show higher quantities of epicatechin. Measurable trigonelline values were higher in both vintages compared to normal white sweet wine. In 3 samples caftaric acid was under <20 mg/l, while in the others values exceeded 45.0 mg/l, particularly in the 2021 samples.

3.1.5. Mineral components (potassium, calcium)

Both components were higher values than in previous vintages. The potassium content rose above 1000 mg/l and the calcium also had values over 100 mg/l.

3.2. Statistical examination

Based on results of the Shapiro-Wilk normality test it can be said of the examined variables that all variables except succinic acid and glycerin follow the normal distribution. Using the Manova analysis per vintage I found significant difference in the 2020–2021 vintages: measured concentration values of citric acid (F(1;15)=5.91; p=0.028), galacturonic acid (F(1;15)=6.15; p=0.0255) and glycerin (F(1;15)=13.41; p=0.0023). In the raw Aszú samples with various terroir attributes there was significant variation in concentration values of arginine (F(3;7) =7.29; p=0.0147), malic acid (F(3;13)=9.34; p=0.0015), succinic acid (F(3;13)=6.70; p=0.0057), 2,3-butanediol (F(3;13)=5.83; p=0.0094).

In conclusion, it can be stated that the chemical composition of wines containing botrytized raw materials differs from that of normal sweet white wines. Particular attention should be paid to glycerin, succinic acid, galacturonic acid, citric acid, 2,3-butanediol, arginine, proline, caftaric acid and epicatechin and to test their proportions in different vintages. Global warming makes activity of *Botrytis cinerea* more difficult too, polyphenols and mineral material content increase, the grape skins thicken while the new wine stability difficulties that arise must be taken into account.

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