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TRACEABILITY STUDY OF TOKAJI ASZÚ BY FINE-ANALYTICAL METHODS

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2 BACKGROUND AND THE OBJECTIVES OF THE RESEARCH

One of the most important products of Hungary and widely known in the world is Tokaji aszú. The basis of this specialty of wine is the aszú grain, the condition of which is the condition of the noble rot of the grapes of technological maturity under the influence of Botrytis cinerea. The process of infection changes the composition of the grapes, allowing the development of special organoleptic properties.

Knowing the technology of Tokaj aszú making, the aszú grains are soaked in a suitable proportion in the must or fermented wine from the Tokaj wine region for 12-48 hours, after which the aszú pasta are pressed and fermented at low temperatures, followed by oak barrel aging.

The aim of my research is to examine the chemical composition of the aszú grains used as raw material, the liquid samples used for extraction (Furmint and Hárslevelű wines) and the finished product made from them (Tokaji aszú wines) based on analytical parameters. My further goal is to compare the applied measurement techniques with the help of the measurement results for the chemical parameters, which I also measured in parallel.

For my research, I collected berry samples from the Tokaj foothills in the 2020 vintage. Berry samples include healthy grains 1st and 2nd class aszú grains, crushed grains and grapes intended for the preparation of szamorodni. In addition, I measured dry Furmint and Hárslevelű wines from eight vintages, which are used for aszú making during aszú making. Furthermore, I examined finished aszú wine from 19 vintages (2000-2017; 2020). I measured my samples with NMR technique, HPLC device, photometric analyzer, and I also performed fine analytical measurements on them.

In my research, I was curious about whether the grouping of the mentioned samples could be done based on the measured parameters. These categories are for berry samples: healthy grains, 1st and 2nd class aszú grains, stale grains, grains dedicated for creating szamorodni wines; in case of liquid samples: separation of Furmint and Hárslevelű varieties; and in the case of finished aszú wines, classifying by vintage.

Among other things, I used the most up-to-date technique used in today's oenological analysis, Nuclear Magnetic Resonance Spectroscopy, which is an innovative way for the analysis of wines. A single NMR spectrum that can be considered a unique, spectroscopic "fingerprint" of the sample. The technique can be successfully applied to the general chemical characterization of wines and products made from many grapes (Fotakis et al., 2013), to study the effects of wine vintage (Lee et al., 2009), geographical origins (Son et al., 2009), and to monitor alcoholic and malolactic fermentation processes (López-Rituerto et al., 2012).

The aszú grains used as the raw material for Tokaj aszú wines may contain chemical components (tartaric acid, gluconic acid, galactaric acid (mucous acid)) that are of major importance due to the activity of Botrytis cinerea. These parameters may be suitable for the subsequent classification of aszú grains as an indicator of botrytis activity, i.e. the chemical composition of aszú grains, which depends on the degree of noble rot, may be characteristic of the finished product.

In my research, it is a new, state-of-the-art technique with which no similar measurements have been made so far. This dissertation presents and processes the results of several years of research and data collection work.

3 MATERIALS AND METHODS

3.1.1 Aszú wines

In my research work, after the 2000 vintage, we collected a total of 156 Tokaji aszú wines (156 pieces) from 19 vintages (2000-2017; 2020) and then analyzed them by NMR technique.

3.1.2 Berry samples

In the 2020 vintage, we collected a total of 64 berry samples from the plantations in Tokaj-Hegyalja, and then analyzed them using various measurement methods. They were among the grapes

• 1st class aszú grains (13 pieces),

• 2nd class aszú grains (16 pieces),

• shrunken grapes (4 pieces),

• healthy grapes (4 pcs) and

• grapes dedicated for creating szamorodni wines (27 pieces).

3.1.3 Liquid samples for extraction

The extracting wine samples come from 8 vintages (total: 78 pieces), among which:

• 63 pieces of dry Furmint and

• There are 15 dry Hárslevelű varieties.

3.2 Research methods

Of the finished aszú wines, 53 chemical parameters were analyzed by NMR.
The berry and wine samples used for the extraction were analyzed by HPLC (separation method), NMR (spectroscopy), photometric analyzer (Thermo Fischer Scientific Gallery) (spectroscopy) and fine analytical methods.
Determination of the acid composition (tartaric acid, malic acid, citric acid, lactic acid, fumaric acid, sicimic acid, succinic acid, fumaric acid, gluconic acid, galactaric acid) was performed by HPLC, while L-malic acid and D-gluconic acid contents were measured by photometric analyzer. The sugar content as well as all polyphenols. Soft analytical methods were used to determine catechin and leukoanthocyanin contents.
The grapes had to be put in a state suitable for measurement, so sample preparation was performed in advance.

Sample preparation of grapes

Sample preparation of the grapes was performed by ethanol extraction. At the beginning of the process, a 12% aqueous ethanol solution is prepared, allowed to cool, and then 100 g ± 0.1 g of grapes are weighed. Then 100 ml of a 12% aqueous ethanol solution (150 ml of a 12% aqueous ethanol solution if necessary) were added. This alcoholic mixture was blended in a blender for 1.5 minutes, allowed to stand for half an hour and finally centrifuged. The filtrate was taken up in a 200 mL volumetric flask and made up to volume with 12% aqueous ethanol. The sample thus prepared was stored in a sample container at -20˚C until analysis. The supernatant was stored in a sample container at -20 ° C until analysis.

Examining the polyphenol content in samples

- Total polyphenol content was defined using the Folin-Ciocalteu reagent, calibrated for gallic acid, in accordance with the Hungarian standard nr. MSZ-9474-80,
- Leucoanthocyanin content was examined after warming butanol-hydrochloric acid containing iron (II) sulphate, with the method of spectrophotometry. Ratio 40:60. (Flanzy, 1970 Modified),
- To measure the catechin concentration the Rebelein (1965) method was used based on vanillin color reaction, and measured spectrophotometrically at 500 nm.

The sugar content of our samples was determined by the Rebelein method according to the MSZ 9479-1980 standard.

^{1}H-NMR measurement methode

The samples were examined with an NMR device in the winemaking laboratory of DIAGNOSTICUM Zrt. The laboratory has two AVANCE III 400 NMR devices manufactured by Bruker BioSpin GmbH (Rheinstetten, Germany). With a single measurement, 53 chemical parameters can be determined from the sample.

HPLC determination method

The combined determination of organic acids (citric acid, tartaric acid, malic acid, shicimic acid, lactic acid, fumaric acid) was performed by high-performance liquid chromatography (HPLC) in the research laboratory of the Department of Oenology of the Hungarian University of Agricultural and Life

Sciences. All reagents required to the measurements were obtained from Sigma Aldrich (Merck KGaA, Darmstadt, Germany).

Photometric analyzer /Thermo Fisher Scientific Gallery device/

The concentrations of L-malic acid and D-gluconic acid in the samples were measured in the winemaking laboratory of DIAGNOSTICUM Zrt. with a photometric analyzer (Thermo Scientific Gallery device) according to the methods provided by the manufacturer. L-malic acid was measured according to the manufacturer's D04670_08 method and D-gluconic acid according to the manufacturer's D11542_02 method. The L-malic acid method is an enzymatic test based on L-Malate dehydrogenase (L-MDH) and Glutamate Oxalacetate-Transaminase (GOT). The D-gluconic acid method is an enzymatic test based on 6PGDH (6-β-gluconate dehydrogenase) and GK (gluconate kinase). The measurement was performed for both components at 37 ° C and 340 nm.

3.3. Data analysis, statistical methods

The measurement results were entered into Microsoft Excel (version number: 18.2106.12410.0, license: Microsoft Corporation) and the statistical calculations were analyzed in R (R Core Team, 2021). The Kruskal-Wallis test was used to study the correlations between the chemical parameters measured by NMR and the vintages studied. In case needed, a Dunn test was performed as a post hoc test. If there was a difference between the groups according to the Kruskal-Wallis test, Dunn test was used to establish exactly which groups differ from each other. Cluster analysis was used to group the samples based on chemical parameters (vintage, sample type). To compare the measurement methods, a paired Wilcoxon test was used to examine the difference between the two groups. The measurement results of the different measurement

techniques are shown on bar graphs and the so-called boxplot illustrations. The general differences between the groups can be read from the boxplot diagram. In the middle of the “box” is the median, which can be considered a kind of average. The vertical lines at the top and bottom of the “box” show the standard deviation, while the protruding data are indicated by the dots.

4 RESULTS AND DISCUSSION

4.1 Main meteorological data and characterization of the study years

I conducted my research from finished aszú wines from vintages between 2000 and 2020. The presentation of the weather conditions of the study area and the characterization of the vintages were made on the basis of meteorological data obtained from István Leskó, a viticulturist. The vintages are classified as good, medium and poor in terms of assimilation, and some of the years studied were of a Mediterranean nature. It can be observed that the triple rule according to which good, medium and weak vintages were characteristic was still characteristic in the early 2000s, while after 2009 the Mediterranean-type vintages became more common, of which the year 2018 was of a remarkably Mediterranean character. The 2000s and 2017s were excellent vintage for aszú development. Based on the practical experience of the last 150 years, the general finding is that there are usually 3 excellent aszú vintages every 10 years. We had data on the development of precipitation from 2009 to 2020. Looking at the data, the vintages of 2010 and 2018 show outstanding values compared to the other years examined. The year 2018 is a very weak aszú vintage. Interesting is the observation that in the period 2000-2010, three vintages were a good vintage in terms of assimilation, while in the next 10 years (2010-2020) this number was 1. The other years were rather only moderate. This is also due to the effects of climate change.

4.2 Determination of some analytical parameters

4.2.1 Results for finished aszú wines

After the 2000 vintage, I performed my measurements from 156 Tokaj aszú

wines from 19 vintages (between 2000-2017 and 2020).

4.2.1.1 Assessing the differences between 2000 and 2020 vintage aszú wines

My aim was to see if there is a difference in the variables measured by the NMR technique between the aszú wines from the examined vintages (2000-2020). Concentrations of cadaverine, putrescine, and epicatechin were measured below the detection limit of the NMR instrument, so these components could not be evaluated. Based on the Kruskal-Wallis test, significant differences between the vintages for citric acid, gluconic acid, alanine, arginine, galacturonic acid, malic acid, and proline, formic acid, fumaric acid, and sorbic acid can be detected. Of these, only the result obtained for shikiminic acid (see Figure 1) suggests that it could even be considered a Botrytis index. In the case of gluconic acid and galacturonic acid, comparing our measurement results with the vintage characteristics did not confirm our hypothesis that the indicators of association were.

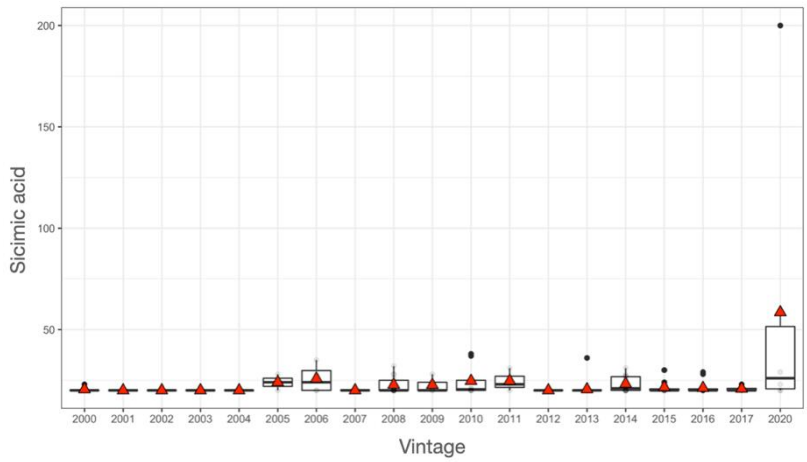

Figure 1. Boxplot diagram of shikiminic acid concentrations in samples between vintages 2000-2020

4.2.1.2 Categorization of aszú wines based on chemical parameters

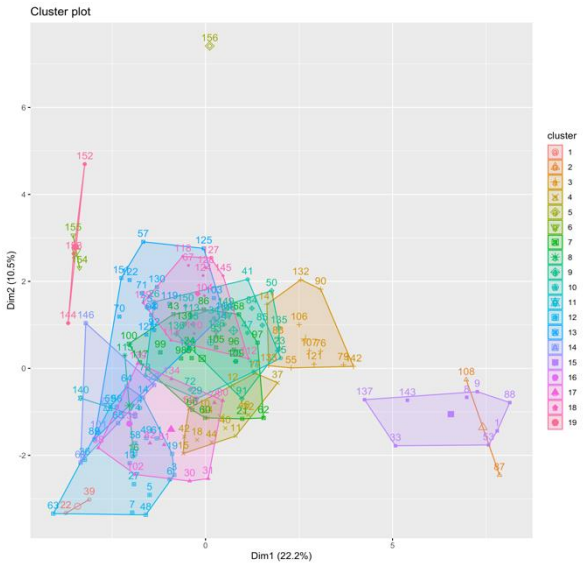

Figure 2. Results of cluster analysis for categories by vintage plotted on a cluster plot

One of the aims of my research is to answer the question of whether it is possible to classify a given aszú wine into a vintage category based on the parameters measured using the NMR technique. The groups obtained by the cluster analysis made from our measurement results are illustrated in Figure 2. The colored borders surround the wines belonging to one group. The ID of the samples can be read from the figure. It can be seen that most of the groups are not sharply separated from each other. The new groups do not overlap with the original categories (vintages), i.e. it can be stated that the vintages cannot be clearly determined on the basis of the parameters measured by NMR.

4.3 Examination of grape samples

4.3.1 Possibility of categorization grape samples

Based on the sample types, we calculated five groups (healthy grains, 1st class aszú, 2nd class aszú, grapes dedicated for creating szamorodni wines), so we examined whether the grape samples can be divided into five groups using the K-mean cluster analysis method. The groups obtained from our measurement results are shown in Figure 3.

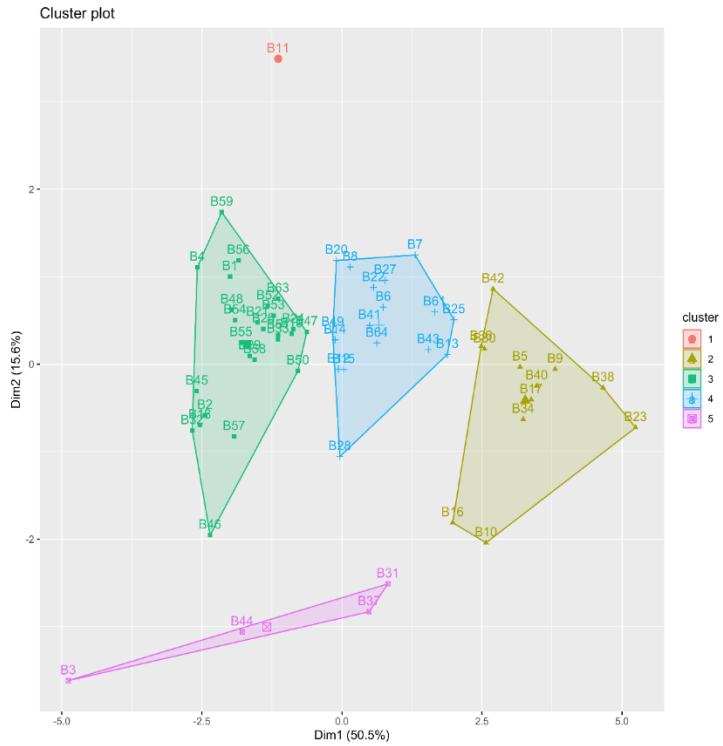

Figure 3. Results of cluster analysis for grouping berries plotted on a cluster plot

From my studies, it can be concluded that the new groups do not overlap with the real categories, so it is not possible to clearly determine the type of samples

based on the measured chemical parameters.

4.4 Examination of wine samples used for extraction

4.4.1.1 Examination of the differences between Furmint and Hárslevelű varieties

I investigated which of the components measured differed between Furmint and Hárslevelű varieties. To answer this question, I used Mann-Whintey's U-test, based on which there was a significant difference between Furmint and Hárslevelű varieties in the case of fumaric acid, shikiminic acid and acetoin measured by NMR. I illustrate these components in the diagrams below.

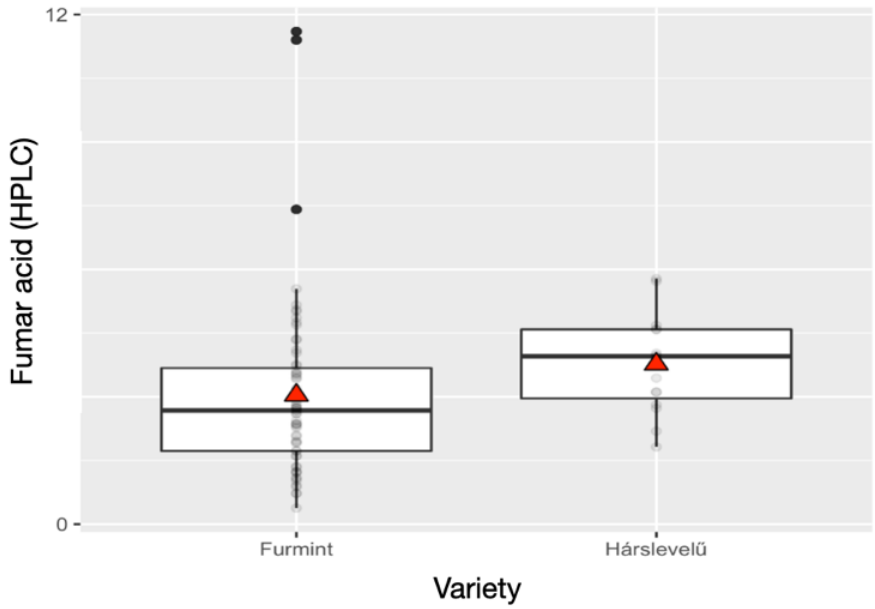

Figure 4. Fumaric acid contents of Furmint and Hárslevelű wine samples measured by HPLC on a boxplot

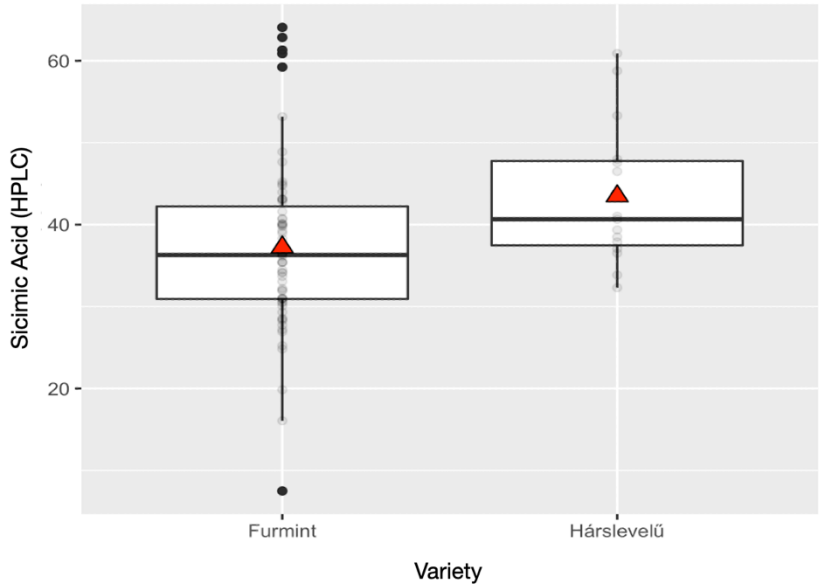

Figure 5: Shikimic acid contents of Furmint and Hárslevelű wine samples measured by HPLC on a boxplot

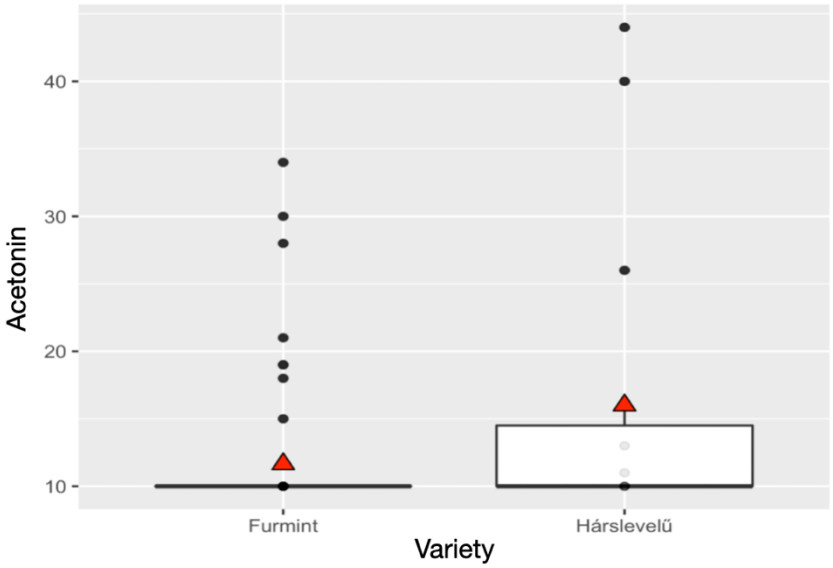

Figure 6: Acetoin contents of Furmint and Hárslevelű wine samples illustrated on a boxplot

Hárslevelű wines had higher fumaric acid, shicimic acid and acetoin contents than Furmint wines.

It can be seen that fumaric acid measured by HPLC (Fig. 4) and shicimic acid (Fig. 5) and acetoin measured by NMR (Fig. 6) show a difference between Furmin and Hárslevelű samples.

4.4.1.2 Possibility of grouping wine samples used for extraction

The grouping of the extracting wines (Furmint and Hárslevelű) was performed according to our measurement results, using the K-mean cluster analysis method. The groups formed by the method are shown in Figure 7. The colored borders surround the wines belonging to one group. The ID of the samples can be read from the figure. It can be seen that the two groups do not separate sharply. There are samples that would be difficult to tell which group they belong to. Thus, it can be concluded from the analysis that the groups do not have a distinctive characteristic.

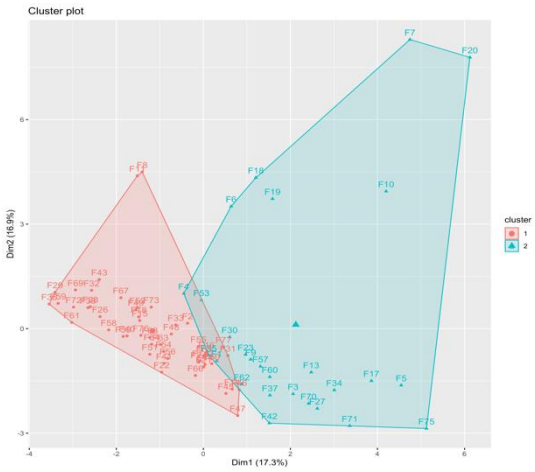

Figure 7: Results of cluster analysis for the grouping of Furmint and Hárslevelű liquid samples plotted on a cluster plot

4.4.2 Comparisons of measurement methods

4.4.2.1 Comparison of NMR and HPLC techniques for grape samples

The following components were measured with NMR and HPLC: tartaric acid, lactic acid, citric acid, fumaric acid, galacturonic acid, succinic acid, sicimic acid. Lactic acid, succinic acid, and shikimic acid were measured below the detection limit of the NMR instrument, so these components cannot be considered in the comparison. It can be seen from the table that significant results were obtained for galacturonic acid (Figure 8) and fumaric acid (Figure 9).

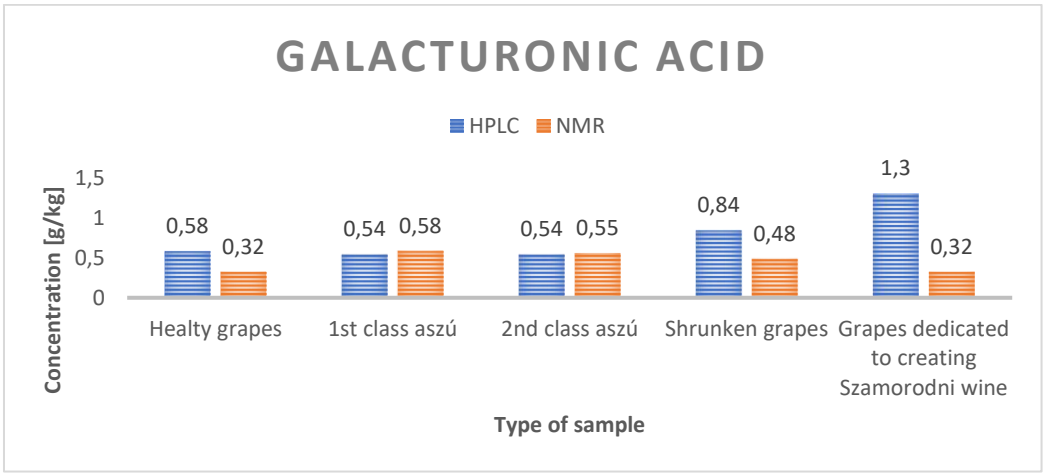

Figure 8: Development of galacturonic acid concentrations measured by HPLC and NMR techniques in different sample types

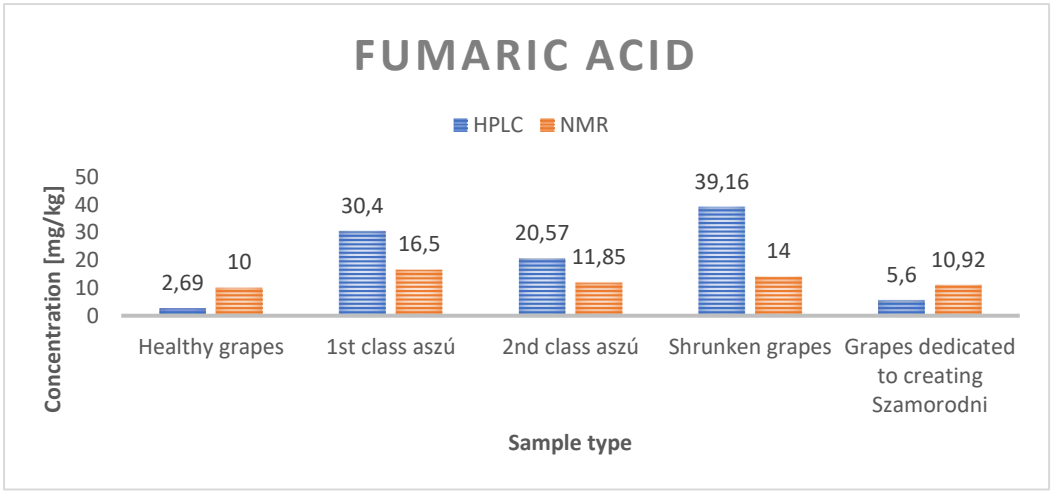

Figure 9: Development of fumaric acid concentrations measured by HPLC and NMR techniques in different sample types

When measuring galacturonic acid and fumaric acid contents, the results measured with the HPLC device were higher. This means that for these components there is a difference between the two measurement techniques used (NMR and HPLC). Overall, the two measurement methods did not show the same results for the components tested.

4.4.3 Comparison of NMR and HPLC techniques for wine samples used for extraction

It can be seen that for tartaric acid, citric acid, galacturonic acid and sikiminic acid, the measurements with the two techniques did not give the same results. In the case of tartaric acid and sicimic acid, the values measured by HPLC were higher, while in the case of citric acid and galacturonic acid, the results obtained by NMR. Among their test results, it was not possible to compare the two techniques for fumaric acid and gluconic acid concentrations, as these components are present in the Hárslevelű and Furmint samples below the detection limit of the NMR instrument.

4.4.4 Comparison of NMR technique and photometric analyzer for berry samples

L-malic acid and gluconic acid were also measured in parallel using an NMR device and a photometric analyzer. From the results obtained, it appears that there is no significant difference between the values measured by the two techniques. This means that the two techniques measure similarly relative to each other.

5 CONCLUSIONS

In my current research activity, during the collection of raw materials I accepted the information that was provided for me regarding the classes of aszú grain and the technology used to make the wines. In the future, it should be possible for the same researcher to collect a sample from the same production area and to take part in processing technology, because in the future this can only be the basis for an objective value judgment.

In the future, I consider it important to carry out research that consistently aim to examine the relationships between the raw materials and the chemical compositions of the finished products.

To study this, I would suggest designing an experiment that performs these measurements on grape samples, musts made from the same grape samples, and mature, finished aszú wines. Based on these results, the raw material would be comparable to the finished product, in addition to being able to track changes over time.

Knowledge of the examined parameters would allow the development of an objective qualification system that could be used for the quality differentiation of botrytis wines and would serve as a basis for the development of a quality assurance system.

This research is an innovation, as there is currently no objective rating system for the classification of aszú grains and aszú wines. Nowaday it is decided based on the % of refraction if an aszú grain belongs to 1st or 2nd class.

6 NEW SCIENTIFIC RESULTS

1. Based on the NMR measurements from Tokaji aszú wines originating from 19 vintages, I searched for the correlations between the 53 parameters that can be measured with the technique. My main question was whether a difference could be observed between the aszú wines from the studied vintages (2000-2017 and 2020) based on the chemical parameters measured by the NMR technique. *Based on our studies, it can be concluded that alanine, arginine, proline citric acid, formic acid, fumaric acid, galacturonic acid, sorbic acid, gluconic acid, shikimic acid and malic acid showed significant differences between each vintage.*

2. In my dissertation, I was looking for the answer to the question whether it is possible to categorize the finished aszú wines examined by NMR technique according to the vintage based on the 53 chemical components. *Using the K-middle cluster analysis method, I grouped the 153 aszú wines examined, but these newly created categories do not overlap with the vintages of the aszú wines. Based on this, it is not possible to determine the vintages of aszú wines with the knowledge of the parameters measured by the NMR technique.*

3. In my dissertation I examined grape samples from the Tokaj wine region, which included 1st and 2nd class aszú grains, grapes for making szamorodni wines and shrunked grains. These samples were measured by several measurement methods. At this stage of my research, I

conducted an investigation into whether berries could be grouped based on the values obtained for the measured parameters. *From the cluster analysis performed, it can be stated that the samples cannot be reliably categorized on the basis of the measured components, as the groups obtained from the results do not overlap with the original categories according to the sample type.*

4. In the rest of my research, I looked at whether the Furmint and Hárslevelű liquid samples can be grouped based on the measured analytical parameters. *It can be seen from the cluster analysis that the Furmint and Hárslevelű varieties cannot be clearly distinguished from each other on the basis of the measured components.*

5. From the liquid samples intended for extraction - from different vintages - we examined which parameters of the Furmint and Hárslevelű varieties differ from all the measured values. *Based on the results, the contents of fumaric acid, shicimic acid and acetoin of the two varieties differed significantly. Fumaric acid, shicimic acid and acetoin were measured in higher concentrations in Hárslevelű wines than in Furmint wines. It can therefore be concluded that there is a difference in the chemical composition of the two varieties.*

6. For my investigations we used several measurement techniques, with the help of which we also measured certain chemical parameters in parallel. The purpose of these measurements was to compare the individual measurement techniques. The following parameters were

used to compare NMR and HPLC techniques: tartaric acid, lactic acid, citric acid, fumaric acid, galacturonic acid, succinic acid, shikimic acid. Malic acid and gluconic acid from berry samples were also measured in parallel with NMR and photometric analyzers. *It can be seen that the NMR and HPLC techniques gave different values, i.e. for lactic acid, fumaric acid, galacturonic acid, succinic acid and shikimic acid there is a significant difference between the analyzes obtained by the two methods. It can be seen from the findings that the differences in analytical methods give different results, a fact that is particularly important during official controls. In contrast, the NMR technique and the photometric analyzer showed relatively reliable results.*

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Conference summaries

Glicerin és glükonsav koncentrációk vizsgálata NMR technikával Tokaji aszúszemekben és Tokaji borokban Matolcsi Réka, Antal Eszter, , Matolcsi, Réka ; Antal, Eszter ; Kállay, Miklós ; Nyitrainé, Sárdy Diána Ágnes Glicerin és glükonsav koncentrációk vizsgálata NMR technikával Tokaji aszúszemekben és Tokaji borokban

In: Fodor, Marietta; Bodor-Pesti, Péter; Deák, Tamás (szerk.) Lippay János – Ormos Imre – Vas Károly (LOV) Tudományos Ülésszak : Összefoglalók Budapest, Magyarország : Magyar Agrár- és Élettudományi Egyetem, Budai Campus (2021) p. 86 , 1 p. Közlemény:32530903 Admin láttamozott Forrás Könyvrészlet (Absztrakt / Kivonat) Tudományos