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DOI: https://doi.org./10.52091/EVIK-2022/3-1-ENG

Received: May 2022 – Accepted: July 2022

# Examination of skin-fermented natural wines

**Keywords:** amphora, qvevri, ceramic egg, organic production, antioxidants, NMR analysis, quercetin, procyanidins, catechins, caffeic acid, p-coumaric acid, galacturonic acid, succinic acid, caftaric acid, tartaric acid, malic acid, hydroxycinnamic acid

## 1. SUMMARY

**The ancient white wine making technology, the “qvevri”, is gaining more and more attention among consumers, not only because it is unique and special, but also because sustainability and closeness to nature are fundamental characteristics of this winemaking process. All of this is demonstrated by the fact that this ancient Georgian process using traditional clay vessels was added to the UNESCO List of Intangible Cultural Heritage of Humanity in 2013, and in 2020, The International Organisation of Vine and Wine (OIV) included skin-fermented white wine in the category of special wines. This wave is also present in Hungary, since “natural” wine and “orange wine” have already appeared in a 2021 law as „Other restricted terms”. The essence of the winemaking process is skin-contact fermentation and microoxidation, for which a number of vessels can be used: amphoras or qvevris, ceramic eggs or spin barrels, as a function of which the chemical composition of the wines may vary, as well as the formation of the precursor compounds of the aroma components. In this study, natural wines produced in the Tokaj wine region, using amphoras and ceramic egg vessels were examined.**

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## 2. Introduction

Today, the philosophy of natural winemaking has grown into a movement, finding producers and consumers in many countries. According to their philosophy, winemaking society had never before used so many pesticides to protect grapes, so many winemaking aids and preservatives, as today, which is extremely harmful to both wildlife and flora, and this is not sustainable farming. It is necessary to return to the roots, to the winemaking practice of ancient times, when winemaking was an art and the wines produced this way had a soul, combining the spirit of the place of production with the artistic world of the winemaker. This is especially true for the world of amphora wines made in the South Caucasus **[1]**.

The counterargument that often arises against these products is that, on the one hand, they are not microbiologically stable, since no technological operations are carried out that would reduce the amount of microorganisms entering from the grapes and proliferating in the must and wine and, on the other hand, there is no adequate plant protection activity in the grapes against pathogens (e.g., black rot) that lead to a changed chemical composition and may produce mycotoxins. Another factor of concern that only a limited amount of test results is available on the migration properties of the various storage vessels.

## 3. Literature review

### *3.1. The concept of natural wine, the peculiarities of its production*

The roots of the natural winemaking movement can be traced back to 1978, when Marcel Lapierre and Julet Chauvet first made wines free of sulfur and additives in Beaujolais, France **[2]**.

Commonly used names for natural wines include low-intervention wine, naked wine and raw wine, which refer to the rules used during their production.

In March 2020, a Charter formulating the regulation for natural wines and  the official name „vin méthode nature" were adopted by the French ministry of Agriculture, the INAO (Institut national de l'origine et de la qualité, the National Institute of Origin and Quality) and the DGCCRF (Direction générale de la concurrence, de la consommation et de la répression des fraudes, the General Directorate for Competition Policy, Consumer Affairs and Fraud Control), together with the Association of Natural Wines.

3.1.1. Most important characteristics of natural wines:

1. Must be produced from grapes that are certified organic (EU or Nature&Progrés) or come from a vineyard that is at least in the second year of transition;
2. The grapes intended for winemaking may only be harvested by hand;
3. Only spontaneous fermentation processes may be used;
4. The use of additives is prohibited;
5. No modification of the composition of the grapes (increase in acid or alcohol content) is allowed;
6. No procedures classified as "rough" are allowed (e.g., filtration, tangential filtration, flash pasteurization, heat treatment, reverse osmosis);
7. Addition of sulfur before or during fermentation is prohibited;
8. Depending on the use of sulfur, producers can use two types of logos on the labels: „without added sulfur" or „less than 30 mg/l sulfur added";
9. Lots that are not considered natural wines must be clearly distinguishable (differentiated labeling), thus avoiding consumer deception **[2]**.

### *3.2. Skin-fermented white wines*

A special category of natural wines is skin-fermented white wines, often called qvevri, amphora, amber or orange wines. As a result of changing trends, older, traditional styles are starting to appear among winemakers as well. The popularity of skin-fermented white wines is constantly increasing, similarly to the ever-increasing demand for natural wines. Additionally, orange wines represent a special category because, due to skin-contact fermentation, they simultaneously carry the flavors typical of white wines and the texture and tannins characteristic of red wines **[3]**. Consumers especially like it when the flavor of the wine is enriched with a special aroma range by the storage vessel, so more and more winemakers use ceramic eggs and amphoras. This technology has many followers in France, Portugal, the USA, Italy, Slovenia and Austria **[4, 5, 6, 7, 8]**. The most important distinguishing features are different color (from deep yellow to amber), increased polyphenol content **[9, 10, 11]**, the formation of volatile compounds (vanilla, roasted peanuts, walnuts) **[12, 7]** and the appearance of mineral notes **[13, 14]**.

The duration of contact with the skin plays a particularly important role not only during fermentation, but also during the subsequent maturation. A long contact time with the skin promotes the dissolution of both phenolic and mineral substances. Procyanidins and catechins, which are important from an oenological point of view, occur in the skin, the seeds and the stem, while simple phenols (caffeic acid, p-coumaric acid) are found in the highest concentration in the berry flesh. As a result of skin-contact soaking for as long as possible, increasing alcohol concentration and the continuously increasing temperature during fermentation, the proportion of tannins in the wine from the seeds also increases. This process may be related to the improved permeability and/or rupture of the cells that contain the phenolic substances. If the fermented new wine is kept on the skin for a longer time after the completion of the fermentation, tannins from the seeds become dominant in its composition and the proportion of polymeric pigments increases **[15, 16]**. Several research have been published on the effect of the place of production **[17]**, the grape variety **[18]** and the grapevine load **[19]** on the phenolic composition of musts and wines.

Among polyphenols, quercetin and shikimic acid are of outstanding importance. Quercetin is found in amounts of 10 to 20 mg/l and shikimic acid in amounts of 30 to 50 mg/l in white wines. The head of the Wine and Health Committee of the International Organisation of Vine and Wine drew attention to this after it had become known that these two compounds are the main active ingredients of the drug Tamiflu, which is used as an antidote to avian flu and is made from Chinese star anise extract. This was another argument for the beneficial effect of white wine consumption **[20]**.

### *3.3. Special storage vessels*

#### 3.3.1. Amphora

They are made in many places all around the world, each master potter uses a unique process and raw material, and the shapes are often different. In Hungary, the works of a domestic potter are the most widespread, the raw material of his amphoras is a fire-resistant material, which is made thinner with chamotte made from its own material. They are solid, with a shell-like fracture surface, the raw materials are fire-resistant clays that burn to color and which, after firing at 1,200 to 1,250 °C, turn into pots resistant to acids and alkalis, with a water absorption of less than 4% (***Figure 1***).

*Figure 1. Plain amphora [21]*

Most important characteristics of amphora use:

- As opposed to metal containers, microoxidation takes place in the amphora;
- While wooden barrels leave a strong mark on the aroma and taste of the wines, the character of the grape variety and the terroir prevails in the amphoras;
- In the amphoras, the specific characteristics of the grape variety which are otherwise covered by conventional winemaking processes (e.g., the herbal flavor of the Furmint grape variety) become more prominent;

- Terracotta amphoras are made from minerals that are similar in composition to that of the vine soil, and which are absorbed by the vines during their life, which means that during fermentation and maturation the grapes end up in a chemical environment similar to the one they were in while on the vine; making wine in amphoras thus enhances the mineral notes in the wines;
- The effective thermal insulation of the amphora continuously ensures that the fermentation process takes place under balanced temperature conditions.

3.3.2. Ceramic egg

The ceramic egg is an egg-shaped vessel based on a cement material that is widespread in Australia. An Australian company that sells its products for wine fermentation and storage worldwide has gained a good reputation among ceramic egg manufacturers. The Australian vessels have a wall thickness of 11-12 mm, a volume of 675 liters and a tare weight of 180 kg. They are fired at 1,285 °C for 42 hours, which ensures the special microporous structure of the vessel's wall. The shape of the inverted egg ensures a special material flow, which guarantees the beneficial mixing of the fermenting must stored in it (***Figure 2***).

*Figure 2. Ceramic egg in a winery in Tállya*
*(Source: own photo)*

## 4. Materials and methods

### *4.1. Comparative analysis of natural wines of the same vintage when using ceramic eggs and clay amphoras*

***Table 1*** contains the data on the origin of the examined wines. In the winery operating in Tállya, natural winemaking technology is used for the preparation of the wines. The grape growing areas are located on the border of Tállya and Mád in eight vineyards, with Furmint and Hárslevelű varieties cultivated in integrated farming. They strive to use as little pesticides as possible and use no absorbable active ingredients at all. Their wines undergo spontaneous fermentation, no wine processing agents are used, and the wines are made and bottles without sulfur. For fermentation, the Australian ceramic eggs described above are used.

Furmint wine was made from organic grapes in a winery in Bodrogkeresztúr. Fermentation was carried out in a black clay amphora from Hungary (***Figure 3***).

*Figure 3. Anthracite amphora in a Tokaj winery*
*(Source: own photo)*

One of the characteristic white grape varieties of the Savoie wine region in France is Roussette de Savoie (named after the French word for „rust")[1], which shows many similarities with the Furmint grape variety in terms of its ampelographic properties. Genetic tests have not confirmed the familial relationship, but in recent years the Altesse variety has appeared all over Europe in various wine regions famous for their sweet wines. The raw material which was processed at the Tokaj Wine Region's Research Institute for Viticulture and Oenology and fermented in a clay amphora comes from the Lencsés vineyard in Tokaj.

*Table 1. Origin of the wine samples used in the analysis*

| Name | Hetény | Sipos | Furmint | Altesse |
|---|---|---|---|---|
| Origin | Hetény vineyard, Tállya | Sipos vineyard, Tállya | Lapis vineyard, Bodrogkeresztúr | Lencsés vineyard, Tokaj |
| Grape variety | 100% Furmint | 85% Furmint – 15% Hárslevelű | 100% Furmint | 100% Altesse |
| Vintage | 2020 | 2020 | 2020 | 2020 |
| Hungarian must grade | 20.0 mM° | 20.9 mM° | 21.0 mM° | 18.0 mM° |
| Viticulture | Biodynamic | Biodynamic | Organic | Organic |
| Processing method | Skin-contact soaking and whole bunch pressing | Skin-contact soaking and whole bunch pressing | Skin-contact soaking and whole bunch pressing | Skin-contact soaking and whole bunch pressing |
| Fermentation and storage | Ceramic egg | Ceramic egg | Clay amphora | Clay amphora |
| Yeast use | Spontaneous | Spontaneous | Spontaneous | Spontaneous |
| Wine processing agent use | Free of processing agents and sulfur | Free of processing agents and sulfur | 20 mg/l sulfurous acid | 20 mg/l sulfurous acid |

The chemical composition was examined with large instrument analysis (NMR - Nuclear Magnetic Resonance) in the Szerencs laboratory of Diagnosticum Zrt.

H NMR technique **[22]**: H NMR spectra were recorded at 26.85 °C with a Bruker AVANCE 400 spectrometer and a 400'54 ASCEND magnet system (Bruker, Karlsruhe, Germany) in proton NMR mode at a frequency of 400.13 MHz. For targeted analysis, sample preparation and analytical parameters were as follows: pH adjustment to pH 3.1 with an automatic BTPH system, addition of deuterium and tetramethylsilane, relaxation delay 4 s, sampling time 3.98 s, spectral width: 8223.68 Hz.

For the statistical analysis of the data, MANOVA and independence tests and IBM Corp. 2016 SPSS Statistics for Windows, Version 23.0. Armonk, NY (USA) software were used.

1 Altesse is the synonymous variety name – author's note

## 5. Analytical results

### *5.1. NMR analysis of natural wines made in ceramic eggs and amphoras*

The results are shown in ***Table 2***.

*Table 2. Chemical composition of the wine samples and the data of the relevant NMR reference database compared to white wines produced in a conventional way*

| Name of wine | Furmint amphora wine | Altesse amphora wine | Sipos ceramic egg | Hetény ceramic egg | Normal white wine |
|---|---|---|---|---|---|
| | Measured values | | | | NMR reference database |
| **Basic analysis** | | | | | |
| Alcohol (%vol) | 13.6 | 10.8 | 13.6 | 13.1 | 10.8 - 16.3 |
| Sugar (g/l) | <1.0 | <1.0 | <1.0 | <1.0 | 1.1 - 80.7 |
| Tartaric acid (g/l) | 2.20 | 1.2 | 3.02 | 3.7 | 3.0 - 4.1 |
| Malic acid (g/l) | 2.30 | 4 | <0.2 | <0.2 | 0.2 - 5.5 |
| Lactic acid (g/l) | <200 | 309 | 120 | 143 | 200 - 400 |
| Citric acid (mg/l) | 284.00 | 312 | 230 | 390 | 200 - 220 |
| **Secondary metabolites** | | | | | |
| Acetic acid (mg/l) | 305.00 | 177 | 571 | 302 | 100 - 942 |
| Acetoin (mg/l) | 17.00 | 43 | 10 | 10 | 10 - 15 |
| Ethyl acetate (mg/l) | 59.00 | <50 | <50 | <50 | 50 - 60 |
| Ethyl lactate (mg/l) | <150 | <150 | <150 | <150 | 150 - 160 |
| Fumaric acid (mg/l) | 5.00 | 8 | <5 | <5 | <5 |
| Gluconic acid (mg/l) | <400 | <400 | <400 | <400 | 400 - 424 |
| Putrescine (mg/l) | <50 | <50 | <50 | <50 | <50 |
| Cadaverine (mg/l) | <50 | <50 | <50 | <50 | <50 |
| HMF (mg/l) | <5 | <5 | <5 | <5 | <5 |
| Furfural (mg/l) | <2 | <2 | <2 | <2 | <2 |
| **Higher alcohols/Fermentation products** | | | | | |
| | | | | | |
| Methanol (mg/l) | 87 | 101 | 44 | 41 | 30 - 265 |
| 1,3-propanediol (mg/l) | <40 | <40 | <40 | <40 | <40 |
| 2,3-butanediol (mg/l) | 429 | 557 | 388 | 310 | 100 - 994 |
| 2-methylpropanol (mg/l) | 99 | <70 | <70 | <70 | 70 - 138 |
| 2-phenylethanol (mg/l) | 39 | 51 | 61 | 39 | 25 - 97 |
| 3-methylbutanol (mg/l) | 255 | 179 | 152 | 139 | 100 - 322 |
| Acetaldehyde (mg/l) | 82 | 94 | 31 | 22 | 10 - 15 |
| Pyruvic acid (mg/l) | <20 | <20 | <20 | <20 | 20 - 31 |
| Galacturonic acid (mg/l) | 606 | 689 | 306 | 307 | 160 - 250 |
| Succinic acid (mg/l) | 749 | 924 | 668 | 613 | 50 - 500 |
| **Amino acids** | | | | | |
| 4-aminobutanoic acid (mg/l) | <120 | <120 | <120 | <120 | 120 - 140 |
| Alanine (mg/l) | <35 | <35 | <35 | <35 | 35 - 50 |
| Arginine (mg/l) | <150 | <150 | <150 | <150 | 150 - 170 |
| Proline (mg/l) | 543 | 303 | 162 | 255 | 150 - 300 |
| **(Poly)phenols** | | | | | |
| Caftaric acid (mg/l) | 18 | 20 | 163 | 108 | 15 - 20 |
| Epikatechin (mg/l) | <30 | <30 | <30 | <30 | 30 - 40 |
| Gallic acid (mg/l) | <25 | 25 | <25 | <25 | 25 - 40 |
| Shikimic acid (mg/l) | 20 | 55 | 24 | 22 | 20 - 25 |
| Trigonelline (mg/l) | 12 | <10 | 12 | 10 | 10 - 11 |

In comparison with the analytical values of the white wines included in the database of Bruker BioSpin GmbH and made with the normal white wine making process, it can be stated that the examined skin-fermented white wines had a lower content of tartaric acid and a higher content of citric acid, galacturonic acid, succinic acid and caftaric acid. Tartaric acid, malic acid and citric acid come from the grapes, while galacturonic acid and succinic acid are formed during fermentation. The results show that by the end of the fermentation, a greater part of tartaric acid is removed in the form of tartar than in the case of a normal white wine, and malic acid can also break down due to the presence of the natural lactic acid bacterial flora. Shikimic acid, to which a beneficial physiological effect is attributed, turned out to be characteristic of the variety, because a significant concentration difference compared to the other wine samples could only be measured in the case of the Altesse amphora wine. Caftaric acid (caffeoyltartaric acid) is a derivative of hydroxycinnamic acid and the ester of caffeic acid and tartaric acid, and is one of the most important phenolic compounds in the flesh of the grape berry. As a result of prolonged soaking and fermentation on the skins, higher values can be detected in skin-fermented white wines compared to normal white wines, with five times higher values measured in ceramic eggs. If reduced glutathione (GSH) is present in the must, caftaric acid-ortho-quinone reacts with this first, forming 2-glutathionylcaftaric acid (grape reaction product, GRP). GRP is colorless, does not react with polyphenol oxidase and no browning occurs.

Comparing amphora and ceramic egg wines using NMR analysis and the MANOVA statistical method, the following findings can be presented:

- Measurement data from the individual wine samples, which apparently show no difference, have been omitted. The other parameters were evaluated by group, since one of the conditions of MANOVA is that the number of variables examined together cannot be higher than the number of observations (that is, more than 3, because this was the number of observations per vessel type).
- In addition, however, the variables met the other conditions of multivariate analysis of variance: the residues are normally distributed and their standard deviation is homogeneous with two exceptions where it is slightly affected: in the case of fumaric acid and methylbutanol. There are no extremes or outliers in one dimension (there is a suitable exchange in 4 cases) and, based on the Mahalanobis distance, in several dimensions, there is no multicollinearity between the final groups, however, due to multicollinearity, fumaric acid, galacturonic acid and 2-methylpropanol were not examined separately, because it would not have given a new, evaluable result compared to the other variables examined in the given group.
- No differences were found in the quantity of monovalent, non-higher alcohols (ethanol, methanol) depending on the storage vessel type ($F(2;3)=2.681$; $p=0.641$).
- In the case of organic acid content of grape origin (tartaric acid, malic acid, citric acid), when examined together, there is no significant difference between the wines by storage vessel type ($F(2;3)=6.856$; $p=0.130$). However, when looking at tartaric acid ($F(2;3)=23.115$; $p<0.05$) or malic acid ($F(2;3)=36.914$; $p<0.05$) alone, there is a difference: wines stored in ceramic eggs have a higher tartaric acid content and a lower malic acid content compared to amphora batches.
- In the case of organic acids formed during fermentation (lactic acid, acetic acid, succinic acid), when examined together, there is no significant difference between the wines by storage vessel type ($F(2;3)=2.064$; $p=0.343$). However, when looking at lactic acid ($F(2;3)=11.755$; $p<0.05$) or succinic acid ($F(2;3)=10.814$; $p<0.05$) alone, there is a difference: wines stored in ceramic eggs have a lower content of lactic acid and succinic acid compared to amphora batches. When examined outside of the model, the amount of fumaric acid does not differ ($t(4)=4.303$; $p=0.238$), while the amount of galacturonic acid differs ($t(4)=4.303$; $p<0.05$) by storage vessel type, it being lower in the case of ceramic eggs.
- Regarding fermentation byproducts (acetoin, acetaldehyde), a significant difference was found when examining the factors together ($F(2;3)=36.718$; $p<0.05$). The acetaldehyde content was found to be lower in the ceramic egg ($F(2;3)=36.718$; $p<0.05$). The same can be said for the amount of acetoin, which was close to the significance limit ($F(2;3)=6.852$; $p=0.059$).
- When higher alcohols (2,3-butanediol, 2-phenylethanol, 3-methylbutanol) were examined together, there was no difference ($F(2;3)=6.826$; $p=0.130$), while when butanediol was examined independently, the result was close to the significance limit ($F(2;3)=7.383$; $p=0.053$), it being lower in ceramic eggs.
- When polyphenols (shikimic acid, trigonelline, caftaric acid) were examined together, no significant differences were detected ($F(2;3)=13.,606$; $p=0.069$), but the amount of caftaric acid was significantly higher in ceramic eggs, if the values were assessed individually ($F(2;3)=36.977$; $p<0.05$).
- A statistically verifiable difference was found in the amount of proline based on an independence test, it being lower in ceramic eggs ($t(4)=2.770$; $p<0.05$). It is characteristic of free amino acids that proline is present in wines in almost 50%, the proportion of arginine is 10%, this ratio remains the same in amphora wines, but in ceramic eggs the proportion typical of Tokaj wines (30-25%) can be observed **[23]**.

## 6. Conclusions

Natural winemaking technology is the representation in wine of an approach that demonstrates, on the one hand, the close-to-nature dedication of its maker, and on the other hand, the imprint of the characteristics of the vineyard soil. Hygiene plays a very important role, without which the use of a chemical-free technology becomes impossible. The insistence on naturalness and sustainability can justify trying out the possibilities offered by different storage vessels and endows the wines produced in this way with added value. Each storage vessel adds to and shapes the chemical composition of the wine. They can also be important factors in market positioning, not only because they are special and unique, but also because the ideological values associated with them (the grape harvest, separated from mother earth, can complete its life journey of becoming wine in a similar environment) can endow these types of wine with a distinctive character.

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