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Investigation of the Use of Enzymes with Early Flavour Releasing Effects on Furmint Grapes in the Tokaj Wine Region

Keywords: aroma-releasing enzymes, Furmint, climate change, pectin degradation, Tokaj, wild yeast

1. Abstract

Furmint is the most important grape variety of the Tokaj wine region. Its advantages include less sensitivity to the drought stress caused by climate change and retaining its acidity, however it is quickly depleted of aromatics because it does not produce various terpenic compounds. The use of early aroma-releasing enzymes and a combination of non-Saccharomyces and Saccharomyces yeasts can provide a technological solution for the winemaking process, allowing the release of aroma precursors during fermentation and the development of richer and more complex flavours with the help of yeast strains. The present study describes the effect of the aroma-developing enzyme preparations (Trenolin®FastFlow, Trenolin®BouquetPLUS) and special yeast strains (Oenoferm®Klosterneuburg, Oenoferm®Wild&Pure) of Erbslöh GmbH on the aroma composition of Furmint grape varieties in the Tokaj wine region.

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2. Introduction

Climate change is not sparing the wine sector either, with average temperatures rising steadily, with an increase of 0.5-3°C expected by 2050, 12-15 fewer frost days, and approximately 14 more days reaching the heat-warning level. Changes in the form, amount, and frequency of precipitation will occur, with less rainfall overall but more intense, leading to soil erosion and changes in topography (MKIK-GVI, 2015). The effects of climate change are also creating increasing stress for vine plants, and this suggests that the world vine and wine landscape will change, with the boundaries of certain areas suitable for viticulture shifting northwards, grape varieties being selected and new varieties being planted. Winemaking practices will have to be changed to cope with higher sugar and lower acidity, osmotic conditions due to higher sugar concentration will reduce the fermentation capacity of Saccharomyces cerevisiae strains, Saccharomyces bayanus will be used and different wild yeast selections will be introduced (Szendei, 2021). Despite the high adaptability of the vine, extreme weather conditions shorten the periods between phenological phases, so that the ripening and harvesting of the grapes takes place earlier and at higher temperatures. The ripening grape is susceptible to the combination of extremely high temperatures and solar radiation, as it can only cool it surface to a minimal extent by evaporation. The sugar content, and thus the alcohol content of the wine, increases, while the acidity decreases and the pH rises.

Despite the high alcohol content, there is still a risk of harmful microbiological activity above pH 3.8. The presence of more and more pests is to be expected, and the increasing UV-B radiation, together with nutrient supply problems, accompanied by drought stress, will lead to an increase in potassium, calcium, and polyphenol content, and the presence of fewer and fewer aroma precursors (Bene, 2024).

The Tokaj wine region must also prepare to counter the negative effects of climate change, and how to do this in a way that preserves traditions is a major responsibility for the professionals of the present day. The work carried out contributes to this process by investigating the technological possibilities for improving the organoleptic parameters of Furmint grapes, and by seeking answers to the question of whether the use of enzymes or yeast plays a greater role in the development of aroma characteristics and whether the use of enzymes for early aroma development has a positive effect on aroma components.

3. Characteristic features of the Furmint grape variety

A very special Tokaj grape variety with mineral-rich and pronounced flavours and high acidity, it is essential for the production of late-harvest and Tokaj specialities. Its role in terms of botrytization is outstanding. The white grape varieties which are well-botrytized in the world are Sauvignon Blanc, Riesling, Semillon, Chenin Blanc, Pinot Blanc, Muscat Blanc, Chardonnay, Picolit, Olaszrizling, Müller-Thurgau, Ruländer, Silvaner, Furmint, Hárslevelű, Yellow Muscat, Kövérszőlő, Kabar; in Arad-Hegyalja, the red grape Kadarka is used.

Several basic conditions must be fulfilled for the grapes to be enriched:

1. the wet weather inducing the fungal infection must have ripened the grapes to full maturity;

2. at the same time, the berries must be intact and free from damage;

3. a period of a few days of rain and humidity is followed by a long dry period;

4. the enzymatic responses of the grape variety to the presence of Botrytis allow specific biochemical processes to take place (Bene, 2004).

For some grape varieties, those with tough and low waxy skins, thin cuticles and looser quaternary structures are favoured, because nodule growth must be limited and fungal metabolism can proceed in a controlled manner (Gabler et al., 2003).

In terms of aromatic compounds, it is a neutral grape variety, with fewer terpenic alcohols in its must, and its volatile compounds are predominantly composed of six-carbon aldehydes and alcohols, caproic acid, benzyl alcohol and α-butyrolactone (Kállay, 1998). When fermented on the skins, it often shows herbal notes, with nettle, mint and chamomile in its aroma and flavour (Bene, 2020).

4. The most important flavour compounds in grapes and must

The grape skins and pulp contain compounds that are the so-called primary flavouring agents and are essential for the aroma and flavour of the wine made from them, as well as precursors that can be used to form new flavourings by enzymatic or acid hydrolysis and then added to the must after pressing.

Aromatic substances can be grouped according to their origin and occurrence and their chemical structure.

They can be according to their origin:

1. Primary aromas (aroma compounds specific to each grape variety)

2. Prefermentative aromas (formed during grape processing)

3. Fermentative or fermentation aromas (products of the fermentation process)

4. Maturation aromas (aromas formed during the storage of wines as a result of contact with oxygen or contact with different containers).

In terms of chemical composition they can be:

1. Aldehydes and ketones, acetals

2. Esters

3. lactones and other oxygen-containing heterocyclic compounds

4. terpenes, terpenic alcohols and simple alcohols

5. Nitrogen-containing compounds

6. sulphur-containing compounds

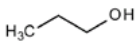
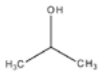
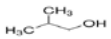
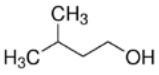
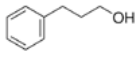
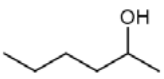
7. Polyphenols (Kállay, 1998).

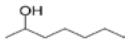
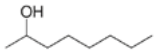
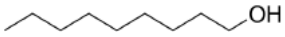
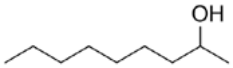
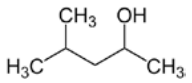
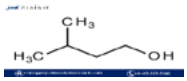
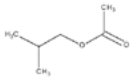
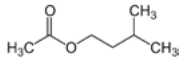
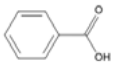
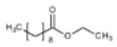
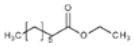
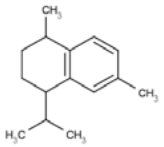
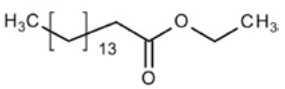
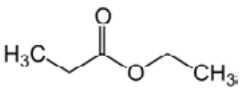
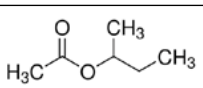
The organoleptic characteristics of fragrant grape varieties are determined by monohydroxy-terpenic alcohols (linalool, α-terpineol, nerol, geraniol, citronellol, hotrienol) and terpene derivatives (1-dienediol, 2-dienediol, triol, endiol, cis-linalool oxide) (Ferreira-Lopez, 2019).

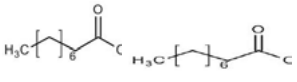
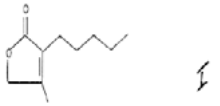
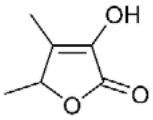
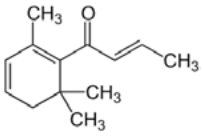
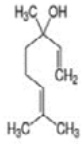
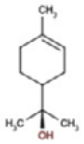
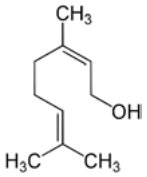
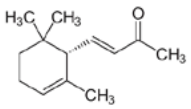
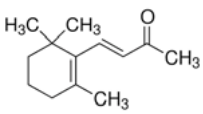
In the case of the non-illuminated neutral species, damascenone, -ionone, ethyl hexanoate, ethyl octanoate, as well as hexanol, decanoic acid and (E,Z)-2,6-nonadienal) compounds were isolated in the highest amounts by different gas chromatography-mass spectrometry studies (Fan et al., 2010).

The most commonly occurring volatile aroma compounds in wines and their organoleptic characteristics are shown in Table 1.

Table 1: The most important volatile aroma compounds in grapes, must and wine (Source: Ribéreau-Gayon et al., 2006 and Ferreira-Lopez, 2019, own ed.)

Compound name	Constituent formula	Organoleptic profile
Alcohols		
propan-1-ol		rubber
propan-2-ol (Isopropyl-alcohol)		fruit
butan-1-ol		camphor
2-methylpropan-2-ol (Isobutyl-alcohol)		potate and soya
3-methylpropan-1-ol (Isoamyl-alcohol)		pear and banana
2-phenylethanol		rose and floral
hexan-1-ol		fresh-cut grass
hexan-2-ol		citrus
heptan-1-ol		fragrant

heptan-2-ol		fruit
octan-1-ol		rose and citrus
octan-2-ol		mushroom and butter
nonan-1-ol		citrus
nonan-2-ol		cucumber and citrus
decan-1-ol		floral
4-methylpentan-2-ol		oil
3-methylbutan-1-ol (Isopentyl alcohol)		fruit and pear
Esters		
2-methylpropyl-acetate (Isobutyl-acetate)		fruit and floral
3-methylpropyl-acetate (Isoamyl-acetate)		banana and apple
Benzoic acid, 2-hydroxy-methyl ester		sweet mint
Ethyl-decanoate		pear
Ethyl-octanoate		fruit and floral
1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene (Trans-calamenene)		herb and garlic
Ethyl-hexadecanoate		wax
Ethyl-propanoate		pineapple
Pentyl-propanoate		apple

Etyl-9-hydroxy-nonanoate		botrytis aroma
Lactons		
2-vinil-2-metil-5-tetrahydroxyfuranone		raisin
4,5-dimethyl-tetrahydro-2,3-furane-dion (sotolon)		botrytis and sherry aromas
Terpenoids		
1-(2,6,6-trimethyl-cyclohexa-1,3-dien)-2-buten-1-one (Damascenone)		rose
3,7-dimethyl-1,6-octadien-3-ol (Linalool)		floral and citrus
2-(4-methylcyclohexohex-3-en-1-yl) propan-2-ol (Terpineol)		lilac and acacia
2,6-dimethyl-2,6-octadiene-8-ol (Nerol-oxide)		floral and orangeflower
(3E)-4-(2,6,6-trimethylcyclohexen-1-yl)-3-buten-2-one (α-ionone)		floral
(3E)-4-(2,6,6-trimethylcyclohexen-1-yl)-3-buten-2-one (β-ionone)		violet

5. Flavour-releasing enzymes

Many enzymes can be used as auxiliaries in winemaking: pectinases, cellulases, glucosidases, glucanases, lysozymes, macerases, polygalacturonases, lyases, depending on whether the application is in white or red wine production and the purpose of the enzyme. The aromatic compounds and or their precursors in grapes are present in free or bound form (Carro et al., 1996), so the use of different enzymes can enrich the aroma composition, for example, the amount of volatile phenols or terpene derivatives can be considerably increased and thus be more organoleptically perceived (Hampel et al., 2014).

For white grapes, the breakdown of the pectin chain (Figure 1) is one of the most important applications, it can lead to easier pressing and better filterability. Enzymes with cellulase, hemicellulase activity can enable aroma and flavour precursor release and, complemented by β-glucosidase activity, can remove glucose and

disaccharide-bound moieties. The degradation of so-called spiky moieties is difficult because of the wide range of groups of compounds that can bind to the backbone, so it is necessary to be careful in enzyme selection and to use one with the broadest possible spectrum of activity.

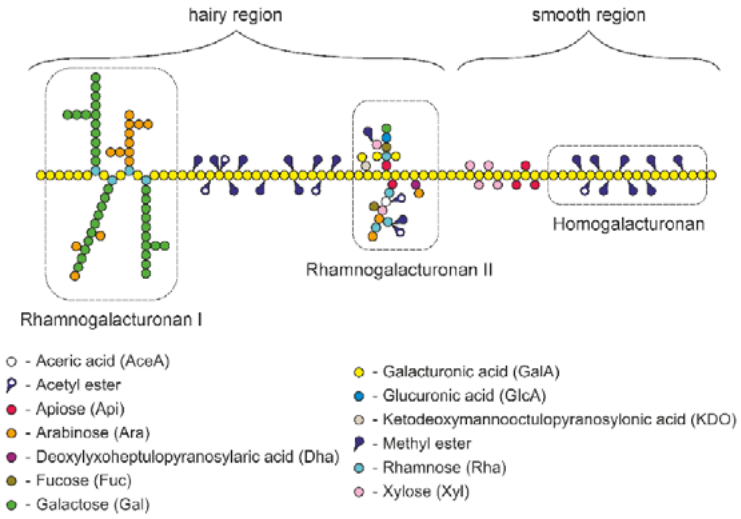


Figure 1. Structure of pectins (Homogalacturonan part: linear, unbranched, smoothened part; Rhamnogalacturonans I and II: spiky, branched part) (Source: Pancerz et al., 2022)

Another important criterion for the choice of the enzyme is that it should preferably be free of deoxidase (cinnamoyl esterase), because white wine musts contain large amounts of hydroxycinnamic acid derivatives, which form esters with tartaric acid and may produce bitter, phenolic, medicinal flavours due to the harmful cinnamic esterase enzyme activity, thanks to vinylphenols (4-vinylphenol, 4-vinylguaiacol) (Kovács-Kovács, 2007).

In red grapes, macerase enzymes are preferred because they can achieve colour and tannin extraction by their cellulase/hemicellulase activity to open the cell wall and vacuolar membrane.

6. Special purpose-oriented wine yeasts

The use of special-purpose yeasts is becoming increasingly important in oenological practice, in addition to the recommendation for different grape varieties, the excellent fermentation-conducting properties are of paramount importance, along with the need for complex wild yeast aromatics, low alcohol content in fruit wines, acid-producing activity, suitability for co-inoculation (yeast and lactic acid bacteria culture) for malolactic fermentation. To achieve all these objectives, yeast strains are commercially available from various manufacturers, but in the absence of adequate nutrient supply, these strains cannot perform the functions expected from their use. Different yeast strains respond in the same way to different sub-optimal environmental factors: metabolic activity is reduced, reserve nutrients and stress-protective molecules accumulate, and their cell wall is strengthened (Bauer and Pretorius, 2000). For the fermenting yeast, the low temperature, potential nitrogen deficiency, high osmotic pressure of high initial sugar content, and the toxicity of alcohol, which increases with sugar fermentation, can simultaneously trigger the development of an increased stress state that can be prevented or corrected by appropriate nutrient management (Bene, 2023). One of the undesirable effects of climate change is the impoverishment of aromatics and acids, which can be reduced by the use of aroma-detecting and early aroma-detecting enzymes (Rodriguez-Nogales et al, 2024), and a richer and more complex flavour can be achieved with non-Saccharomyces wine yeast cultures (Bene-Kiss, 2023). The use of wild yeast strains Torulaspora delbrueckii, Metschnikowia pulcherrima, Pichia kluyveri, Lachancea thermotolerans in the oenological production of white wines, red wines and sparkling base wines is also gaining ground (Szendei, 2021).

6. Material and method

The Furmint grape harvest date: 28 September 2023, Bodrogkisfalud, Kisvár- vineyard of estate producer Zsuzsanna Bene. 20q of grapes were harvested, sulphurized with 10 g/hl Sterisol 600, destemmed, crushed in a maceration tank and treated with 5 ml/hl Trenolin®FastFlow special liquid enzyme, after 2 hours of action, pressed except 50 litres of mash, then filled into glass bottles and 1 amphora, followed by two yeast preparations: Oenoferm®Klosterneuburg and Oenoferm®Wild&Pure inoculation, followed by treatment with Trenolin®Bouquet enzyme preparation in different doses.

For the 50 litres set aside, an additional 15 ml/hl of Trenolin®BouquetPlus enzyme was added, and after waiting 2 hours, they were squeezed separately and separated to inoculate with the 2 different yeast strains.

After rehydration, the yeast strains were fed with 20 g/hl Vitaferm®Ultra F3 and were then transferred to the glass flasks and 1 amphora (**Table 2., Figures 2. and 3.**).

Each sample received 30 ml/hl of Vitaferm®Liquid nutrient each day.

Regarding temperature ranges, 18-20oC was the optimal fermentation range for both yeasts, controlled by thermometer and tempering by room cooling, with water jacket cooling applied for overheated glass flasks (**Figure 2.).**

Table 2. Sample adjustments with different treatments

Sample name	Treatment/ specified yeast	Treatment/ Trenolin®Bouquet Plus enzyme	Treatment/others
OK1	O.Klosterneuburg	0 ml/hl	-
OK2	O.Klosterneuburg	5 ml/hl	-
OK3	O.Klosterneuburg	10 ml/hl	-
OK4	O.Klosterneuburg	15 ml/hl	-
OK5	O.Klosterneuburg	15 ml/hl	addition of 1 kg of ripe, destemmed grape berries
OK6	O.Klosterneuburg	15 ml/hl	treated with 15 ml/hl Trenolin®Bouquet Plus enzyme preparation for 2 h prior to pressing
WP1	O.Wild&Pure	0 ml/hl	-
WP2	O.Wild&Pure	5 ml/hl	-
WP3	O.Wild&Pure	10 ml/hl	-
WP4	O.Wild&Pure	15 ml/hl	-
WP5	O.Wild&Pure	15 ml/hl	addition of 1 kg of ripe, destemmed grape berries
WP6	O.Wild&Pure	15 ml/hl	treated with 15 ml/hl Trenolin®Bouquet Plus enzyme preparation for 2 h prior to pressing
K13 (kontroll)	spontaneous fermentation	10 ml/hl	-
K14	spontaneous fermentation	0 ml/hl	amphora fermented, addition of 1 kg of ripe, destemmed grape berries

Figure 2. a, the set of 14 samples b, the implementation of temperature measurement

Figure 3. a, fermentation with grapes in an amphora b, fermentation with grapes in a glass balloon

7.1. Presentation of the treatment products

Sterisol 600 (Ever s.r.l.): a concentrated sulphurising agent containing ammonium bisulphite, which is effective in preventing oxidation in grape processing, inhibiting the harmful activity of acetic acid bacteria and also acting as an assimilable nitrogen source as a fermentation nutrient.

Trenolin®FastFlow enzyme (Erbslöh GmbH): It has an intensive pectin degradation effect, improving the pressability of white and red wines. Contains an arabinogalactan II hydrolysis enzyme component, which enables the removal of pectin fractions that are difficult to break down.

Trenolin®Bouquet^{Plus} enzyme (Erbslöh GmbH): an enzyme with specific β-glucosidase activity, capable of early aroma release in white grape varieties, capable of releasing terpenic alcohols with intense fragrance and aroma.

Vitamon®Liquid liquide nutrients (Erbslöh GmbH): Continuous feed with DAP and thiamine.

Vitaferm®Ultra F3 nutrients (Erbslöh GmbH): It consists of complex nutrients, DAP, thiamine, inactive yeast and yeast cell wall components.

Oenoferm®Klosterneuburg specified yeast (Erbslöh GmbH): A yeast strain with good alcohol tolerance, Saccharomyces cerevisae strain capable of producing white pepper, nutty, nutty flavour notes.

Oenoferm®Wild&Pure specified yeast (Erbslöh GmbH): Torulaspora delbrueckii+Saccharomyces spp, can produce higher levels of monoterpenes and fruit esters (Erbslöh OenoGuide, 2022).

7.2. Fermentation monitoring

The chemical composition was analysed and the fermentation was monitored by NMR (Nucleic Magnetic Resonance) spectroscopy at Diagnosticum Zrt. Laboratory in Szerencs.

1H NMR technique (Godelmann et al., 2013) : 1H NMR spectra were recorded at 26.85°C using 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. Sample preparation and assay parameters for the targeted assay were as follows: pH adjustment to pH 3.1 with an automatic BTPH system, addition of tetramethylsilane, NMR spectra calibrated with a reference point, tetramethylsilane (TMS) signal set to 0, relaxation delay 4 s, sampling time 3.98 s, spectral width 8223.68 Hz.

7.3. Aromatic compounds examination

The analysis of the aromatic compounds was carried out by GC-MS HS-SPME, gas chromatography-mass spectrometry, and vapour space analysis with solid-phase microextraction in the wine laboratory of Cellarius Ltd. in Pécs.

The sampling procedure used capture volatile and semi-volatile components in the air. For the extraction of volatile compounds, 65 µm PDMS/DVB fibre was used, which was conditioned according to the manufacturer's instructions before the measurements (65 µm PDMS/DVB: 250 °C for 0.5 h). This sampling fibre is the one that allows the most volatile compounds to be bound (Stoppacher et al., 2010). Samples were stored at 23 °C. The SPME sampling time was 15 min, with desorption in the gas chromatograph injector at 240 °C for 5 min.

Measurements were performed using a Shimadzu GCMS-QP2010 Ultra AOC-5000 Plus gas chromatograph coupled to a sample feeder. Helium 6.0 (99.9999% purity) was used as a carrier gas. The helium flow rate was 1.51 ml/min. Separations were performed using ZB-5MS and ZB-WAXplus capillary columns (30.0 m x 250 µm x 0.25 µm and 30.0 m x 250 µm x 0.25 µm, Phenomenex), the former with 5% phenyl and 95% methylpolysiloxane and the latter with polyethylene glycol. The temperature of the so-called transfer line connecting the gas chromatograph and the mass spectrometer was set at 240 °C (the same as the final column temperature). The m/z (mass per unit charge) range tested was between 50 and 400 m/z. Shimadzu GCMSsolution software was used to control the parameters of the gas chromatography-mass spectrometry system, to search for components, analyse the mass spectra, further evaluate the data and perform a full comparison of the chromatograms. The identification of the chromatographic peaks, i.e. the components obtained, was performed using the NIST Mass Spectral Search (NIST/EPA/NIH/Mass Spectral Library) Version 2.0 and Wiley FFNSC 2 Mass Spectral Library.

Statistical analysis was performed using Orange Data Mining Toolbox version 3.37.0 (Demšar et al., 2013).

7.4 Profile analysis - Wine aroma profile sensory analysis

The sensory analysis was carried out with the help of 4 winemaker-tasters, evaluating separately the olfactory characteristics on a scale of 1 to 5 on 6 criteria: intensity of aroma, complexity, herbal, fruity, green leaf/ cooked apple, fresh cut grass notes; flavour characteristics separately on a scale of 6 criteria from 1 to 5:

alcohol, acidity, herbal, fruity, buttery, minty notes. The tasting took place in the tasting room of Tokajbor-Bene Winery.

The results were evaluated using PanelCheck 1.4.2 software.

8. Results

8.1. Chemical composition

The fermentation is not monitored by the traditional analytical method, but by instrumental measurement, both because the latter involves significantly less sample use and because it allows more parameters to be analysed in a shorter time. The limitation of the measurement is the carbon dioxide formed during fermentation, therefore the chemical composition of the samples was compared in the fermented new wine state (Table 3).

Regarding the basic chemical parameters (alcohol, sugar), all samples fermented to dryness in 10 days, none of the batches had residual sugar content and the same alcohol yield was obtained with spontaneous fermentation and inoculation with yeast. The application of Oenoferm®Wild&Pure resulted in lower tartaric acid values. The citric acid value was found to be a function of the use of the specific yeast, with lower levels detected in OK and WP samples compared to spontaneous fermentation.

The production of acetic acid was characteristic of the spontaneously fermented batches (K13, K14), with the highest values obtained in the amphora fermentation of all samples.

Table 3: Analytical parameters of the samples after fermentation

Wine sample	K13	K14	OK1	OK2	OK3	OK4	OK5	OK6	WP1	WP2	WP3	WP4	WP5	WP6
Measured values														
Standard parameters														
Alcohol (v/v%)	11,1	11,1	11,2	11,1	11,2	11,1	11,2	11,2	10,9	10,9	10,9	10,9	10,9	10,9
Sugar (g/l)	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
Malic acid (g/l)	1,9	2,0	1,4	1,5	1,5	1,6	1,4	1,4	1,6	1,6	1,5	1,6	1,6	1,3
Tartaric acid (g/l)	4,6	4,5	4,8	4,9	4,7	4,4	4,3	4,4	3,5	3,3	3,3	3,9	3,4	3,6
Citric acid (g/l)	246	245	232	217	220	217	212	203	218	218	237	231	226	204
Lactic acid (mg/l)	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
Degradation products														
Acetic acid (mg/l)	147	170	122	121	115	135	124	125	120	125	123	121	124	115
Gluconic acid (mg/l)	<400	<400	<400	<400	<400	<400	<400	<400	<400	<400	<400	<400	<400	<400
Fermentation products														
2,3-butanediol (mg/l)	254	286	253	254	252	254	259	250	327	318	320	399	433	448
2-methyl-propanol (mg/l)	<70	<70	84	76	71	83	71	81	80	85	85	83	82	82
2-phenylethanol (mg/l)	48	49	79	71	76	71	75	69	62	61	64	62	64	61
3-methyl-butanol (mg/l)	206	209	308	275	269	282	265	255	274	248	219	217	214	215
Acetaldehyde(mg/l)	21	33	14	15	12	11	<10	14	13	16	16	16	15	16
Pyruvic acid(mg/l)	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Galacturonic acid (mg/l)	566	556	475	490	518	551	547	527	472	499	519	518	553	548
Succinic acid (mg/l)	869	868	941	984	937	1000	964	965	969	1000	909	949	953	968
Phenolic acids														
Caftaric acid (mg/l)	39	36	35	35	36	39	39	50	37	35	35	37	37	50
Gallic acid (mg/l)	<25	<25	27	28	28	28	30	30	<25	<25	<25	<25	26	<25
Shikimic acid (mg/l)	40	41	36	36	35	35	35	36	36	32	30	35	37	35

For higher alcohols, 2,3-butanediol was higher in the amphoteric sample compared to the control and in all WP samples. For 2-methylpropanol and 3-methylbutanol, an increase was observed in both samples with specific yeast (OK, WP), the amount of 2-phenyl ethanol also showed an increase in the amphora sample (K14) compared to the control, but a higher rate can be achieved with the use of specific yeast, leading to the aromatic enrichment. The aldehyde production showed an increase in amphora, while it remained more balanced with the use of wood yeast. Specific yeast use led to a decrease in galacturonic acid, while an increase in succinic acid content was observed.

As regards phenolic acids, it can be concluded that yeast strains did not influence their quantity, whereas shikimic acid is a specific varietal characteristic, which can be detected in higher quantities during spontaneous fermentation. For caftaric acid, for both yeast strains, batches (OK6, WP6) that were separately pressed and treated with higher enzyme formulations showed significantly higher production compared to spontaneously fermented samples and batches with lower enzyme usage. Oenoferm®Klosterneuburg yeast showed higher activity in gallic acid production.

Overall, the role of yeast strains is stronger than the effect of enzyme use in the differences in these parameters.

8.2. Flavour component examination

The GC assay detected 97 aroma compounds (**Table 4**).

The composition per compound group is shown in **Figure 4**.

Table 4. Flavour components detectable in different samples (unit of measurement is the normalised area, the area relative to an internal standard characterising the ratios)

	k14	k13	ok1	ok2	ok3	ok4	ok5	ok6	wp1	wp2	wp3	wp4	wp5	wp6
Aldehydes	**0,30**	**0,37**	**0,34**	**0,47**	**0,40**	**0,37**	**0,28**	**0,70**	**0,35**	**0,61**	**0,51**	**0,42**	**0,53**	**0,40**
Benzaldehyde	0,16	0,14	0,20	0,22	0,22	0,20	0,16	0,20	0,20	0,22	0,18	0,16	0,19	0,13
Butanal, 3-methyl-	0,04	0,10	0,05	0,10	0,04	0,04	0,02	0,35	0,03	0,14	0,17	0,08	0,13	0,12
Hexanal <n->	0,02	0,03	0,02	0,04	0,03	0,03	0,01	0,04	0,03	0,04	0,03	0,03	0,04	0,04
Nonanal	0,07	0,10	0,07	0,10	0,11	0,10	0,08	0,11	0,08	0,20	0,11	0,15	0,17	0,10
Vanillin	0,00	0,00	0,01	0,01	0,00	0,00	0,00	0,00	0,01	0,01	0,01	0,01	0,01	0,00
Alcohols	**389,69**	**394,74**	**509,20**	**485,52**	**470,06**	**448,58**	**470,92**	**450,92**	**505,45**	**515,30**	**480,67**	**481,87**	**430,70**	**419,35**
Benzyl alcohol	1,19	1,05	0,80	1,48	1,01	0,88	0,94	1,18	1,78	0,72	0,58	0,75	0,71	0,75
BHT	0,05	0,09	0,09	0,12	0,07	0,07	0,14	0,17	0,17	0,14	0,21	0,14	0,15	0,12
Butyl alcohol	0,20	0,23	0,25	0,22	0,21	0,20	0,21	0,12	0,23	0,24	0,24	0,24	0,22	0,17
Heptanol <n->	0,30	0,37	0,15	0,20	0,23	0,19	0,31	0,41	0,23	0,23	0,15	0,18	0,22	0,21
Hepten-3-ol	0,29	0,39	0,27	0,31	0,34	0,39	0,37	0,36	0,29	0,32	0,33	0,35	0,32	0,27
Hex-(3Z)-enol	0,64	0,73	0,74	0,87	0,87	0,86	0,84	0,72	0,76	0,91	0,90	0,91	0,81	0,70
Hexanol	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00
Hexanol <2-ethyl->	0,11	0,14	0,13	0,16	0,15	0,14	0,12	0,13	0,15	0,17	0,13	0,14	0,13	0,13
Hexanol <ethyl->	0,05	0,06	0,05	0,06	0,06	0,06	0,06	0,07	0,07	0,09	0,07	0,08	0,07	0,07
Hexanol <n->	19,36	22,65	22,21	22,92	23,11	22,22	21,21	16,37	20,08	22,52	23,40	23,39	19,58	14,43
Hydroxycitronellol	0,09	0,08	0,06	0,11	0,11	0,07	0,11	0,12	0,09	0,09	0,07	0,12	0,08	0,12
Isopentyl alcohol	254,12	260,79	336,22	319,00	303,65	288,36	305,17	298,42	335,14	344,19	313,89	316,96	275,42	273,28
Octanol <n->	0,69	0,71	0,89	1,07	0,86	0,88	0,93	0,92	1,21	1,06	0,81	0,81	0,80	0,80
Pentanol <4-methyl->	0,30	0,34	0,44	0,38	0,40	0,36	0,41	0,31	0,42	0,36	0,34	0,26	0,27	0,23
Phenethyl alcohol	112,29	107,10	146,89	138,64	138,96	133,87	140,10	131,63	144,83	144,26	139,54	137,55	131,95	128,06
Others	**0,18**	**0,45**	**0,15**	**0,71**	**0,10**	**0,10**	**0,02**	**1,63**	**0,24**	**1,69**	**1,62**	**0,47**	**0,90**	**0,98**
1,3-Dioxolane, 2,4,5-trimethyl-	0,18	0,45	0,15	0,71	0,10	0,10	0,02	1,63	0,24	1,69	1,62	0,47	0,90	0,98
Esters	**256,00**	**366,20**	**318,72**	**263,76**	**318,99**	**279,47**	**289,91**	**224,61**	**334,61**	**335,50**	**289,10**	**253,77**	**239,31**	**193,25**
Acetate <isobutyl->	0,29	0,32	0,63	0,65	0,58	0,57	0,59	0,66	0,58	0,62	0,63	0,67	0,62	0,69
Acetate <pentyl->	24,14	19,74	10,46	15,26	26,88	20,30	24,94	26,34	14,89	31,29	38,60	41,70	39,93	28,85
Benzoic acid <2-[[[4-(4-hydrox\	0,02	0,02	0,02	0,03	0,02	0,02	0,02	0,02	0,13	0,03	0,03	0,02	0,02	0,02
Benzoic acid, 2-hydroxy-, meth	0,12	0,23	0,09	0,14	0,18	0,22	0,28	0,35	0,08	0,14	0,20	0,26	0,22	0,37
Benzyl carbyl butyrate	0,05	0,07	0,07	0,07	0,06	0,06	0,08	0,04	0,07	0,06	0,06	0,05	0,06	0,03
Butyrate <2-methyl-, phenyleth	0,00	0,00	0,00	0,03	0,02	0,03	0,02	0,02	0,67	0,61	0,51	0,59	0,33	0,60
Butyrate <3-hydroxy-, ethyl->	0,02	0,04	0,04	0,03	0,04	0,03	0,03	0,03	0,03	0,03	0,02	0,02	0,02	0,02
Butyrate <ethyl->	1,62	2,04	1,38	1,33	1,34	1,29	1,38	1,04	1,67	1,77	1,56	1,50	1,35	1,06
Butyrate <isopentyl->	0,07	0,07	0,08	0,05	0,07	0,06	0,06	0,08	0,09	0,09	0,07	0,07	0,06	0,07
Calamenene <trans->	0,37	0,67	0,56	0,62	0,69	0,70	0,53	0,55	0,41	0,46	0,62	0,56	0,49	0,40
Crotonate <(E)-, ethyl->	0,05	0,09	0,06	0,04	0,04	0,04	0,04	0,02	0,04	0,04	0,03	0,02	0,02	0,02
Decanoate <ethyl->	52,65	97,79	76,08	58,15	75,20	62,04	62,64	37,27	62,82	62,46	41,76	27,60	35,99	19,06
Decanoate <methyl->	0,22	0,39	0,27	0,21	0,27	0,22	0,24	0,15	0,21	0,21	0,12	0,10	0,14	0,08
Dodecanoate <ethyl->	2,64	6,91	4,76	2,98	3,27	2,36	1,83	1,44	3,03	4,00	2,06	1,42	1,68	1,18
Formate <hexyl->	0,52	0,54	0,55	0,48	0,53	0,47	0,63	0,42	0,54	0,58	0,36	0,41	0,49	0,44
Furoate <2-ethyl->	0,12	0,15	0,17	0,18	0,17	0,17	0,15	0,14	0,16	0,19	0,19	0,18	0,15	0,13
Heptanoate <ethyl->	0,44	0,64	0,51	0,42	0,56	0,52	0,50	0,41	0,76	0,78	0,67	0,65	0,53	0,46
Hex-(2E)-enoate <ethyl->	0,54	0,69	0,53	0,40	0,59	0,49	0,45	0,36	0,44	0,50	0,49	0,43	0,39	0,42
Hex-(3Z)-enoate <ethyl->	0,01	0,01	0,01	0,02	0,01	0,01	0,01	0,01	0,02	0,02	0,01	0,02	0,02	0,05
Hex-(3Z)-enyl acetate	0,21	0,27	0,27	0,31	0,29	0,32	0,26	0,20	0,29	0,30	0,29	0,31	0,22	0,21
Hexanoate <2-hydroxy-, ethyl-:	0,22	0,23	0,41	0,37	0,34	0,37	0,69	0,92	1,21	1,06	0,81	0,81	0,80	0,80
Hexanoate <ethyl->	43,50	56,09	42,80	38,94	41,80	40,39	38,29	33,12	57,69	55,74	49,09	43,06	35,58	37,14
Hexanoate <isopentyl->	0,49	0,80	0,75	0,48	0,66	0,60	0,62	0,42	0,94	0,80	0,64	0,55	0,51	0,45
Hexanoate <methyl->	0,10	0,10	0,07	0,07	0,07	0,07	0,07	0,07	0,10	0,10	0,09	0,07	0,07	0,09
Hexyl acetate	0,94	1,28	1,71	1,70	1,37	1,49	1,22	0,51	1,87	1,71	1,44	1,28	0,99	0,44
Isoamyl acetate	14,49	17,31	36,78	32,62	29,22	28,32	29,64	23,13	33,89	28,26	23,94	24,37	21,56	26,21
Isobutyrate <ethyl->	0,18	0,20	0,36	0,43	0,33	0,40	0,35	0,43	0,44	0,48	0,51	0,52	0,38	0,48
Isovalerate <ethyl->	0,09	0,11	0,28	0,33	0,22	0,29	0,27	0,28	0,26	0,22	0,19	0,18	0,15	0,19
Isovalerate <phenethyl->	0,00	0,00	0,03	0,07	0,02	0,04	0,03	0,04	0,64	0,57	0,46	0,55	0,32	0,52
n-Caprylic acid isobutyl ester	0,18	0,31	0,31	0,26	0,30	0,27	0,25	0,19	0,32	0,25	0,20	0,20	0,13	0,18
Neryl propionate	0,14	0,19	0,15	0,15	0,18	0,17	0,18	0,12	0,13	0,13	0,16	0,14	0,14	0,10
Octanoate <ethyl->	107,24	153,42	131,33	101,37	127,32	111,45	116,99	89,39	144,42	134,66	116,10	98,21	90,05	65,16
Octanoate <isopentyl->	0,69	1,28	1,38	0,95	1,16	1,01	0,99	0,75	1,46	1,25	0,90	0,76	0,73	0,70
Octanoate <methyl->	0,46	0,62	0,43	0,33	0,44	0,38	0,41	0,37	0,27	0,29	0,29	0,26	0,30	0,26
Palmitate <ethyl->	0,00	0,00	0,05	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,02	0,02
Phenethyl hexanoate	0,18	0,28	0,18	0,16	0,14	0,16	0,18	0,08	0,30	0,22	0,18	0,16	0,18	0,10
Phenylacetate <ethyl->	0,18	0,22	0,33	0,32	0,31	0,33	0,32	0,34	0,31	0,29	0,30	0,30	0,28	0,30
Phthalic acid, 3-methylphenyl 2	0,98	0,93	2,86	1,49	1,55	1,44	1,97	1,49	0,73	1,52	1,94	1,91	1,35	1,45
Propanoate <ethyl->	1,54	1,74	1,32	1,73	2,13	1,79	2,18	2,92	2,11	3,18	3,02	3,31	2,47	4,11
Propanoate <pentyl->	0,03	0,05	0,05	0,03	0,05	0,05	0,05	0,05	0,06	0,04	0,06	0,06	0,05	0,04
Succinate <diethyl->	0,19	0,27	0,46	0,43	0,45	0,43	0,42	0,34	0,37	0,35	0,40	0,37	0,41	0,29
Tetradecanoate <ethyl->	0,06	0,06	0,10	0,07	0,06	0,06	0,05	0,05	0,09	0,11	0,07	0,06	0,05	0,07
Valerate <ethyl->	0,02	0,02	0,02	0,02	0,02	0,02	0,03	0,02	0,03	0,03	0,03	0,03	0,03	0,02
ketons	**0,28**	**0,37**	**0,39**	**0,48**	**0,47**	**0,51**	**0,48**	**0,63**	**0,37**	**0,42**	**0,39**	**0,37**	**0,32**	**0,39**
3-Heptanone, 5-methyl-	0,05	0,06	0,05	0,05	0,06	0,06	0,05	0,08	0,05	0,06	0,06	0,06	0,05	0,07
Acetophenone	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Acetylvaleryl	0,07	0,07	0,14	0,15	0,13	0,14	0,14	0,16	0,10	0,11	0,11	0,11	0,10	0,12
Heptan-2-one	0,02	0,03	0,03	0,04	0,04	0,03	0,04	0,05	0,02	0,03	0,04	0,03	0,03	0,04
Heptyl methyl ketone	0,10	0,17	0,13	0,20	0,20	0,25	0,20	0,29	0,14	0,18	0,14	0,13	0,10	0,12
acids	**56,10**	**74,50**	**50,11**	**48,91**	**48,46**	**42,37**	**43,77**	**34,65**	**31,53**	**29,75**	**25,06**	**24,01**	**23,60**	**26,86**
Butyric acid	1,09	0,92	0,78	0,75	0,68	0,67	0,69	0,69	0,58	0,77	0,87	0,89	0,78	0,60
Decanoic acid <n->	20,69	29,24	18,29	16,61	17,81	14,93	14,77	11,17	6,59	6,44	4,77	4,93	4,37	5,21
Dodecanoic acid	0,18	0,49	0,14	0,07	0,11	0,08	0,06	0,06	0,08	0,08	0,04	0,03	0,03	0,04
Hexanoic acid	3,66	4,70	3,77	4,02	3,63	3,49	3,76	2,72	3,61	3,41	3,26	3,03	2,87	3,02
Octanoic acid <n->	29,90	38,54	26,64	26,91	25,64	22,63	23,86	19,58	20,31	18,68	15,65	14,64	15,01	17,61
Propanoic acid <2-methyl-, 1,3	0,59	0,60	0,49	0,55	0,59	0,56	0,63	0,42	0,36	0,37	0,47	0,49	0,53	0,37
hydrocarbons	**0,87**	**1,11**	**0,97**	**0,88**	**1,12**	**1,05**	**1,31**	**1,10**	**1,18**	**1,21**	**1,13**	**1,02**	**1,33**	**1,00**
Tetradec-1-ene	0,04	0,08	0,09	0,05	0,08	0,08	0,07	0,07	0,09	0,11	0,09	0,09	0,09	0,08
Benzene, 1-ethyl-2,3-dimethyl-	0,05	0,06	0,06	0,07	0,06	0,05	0,06	0,06	0,07	0,06	0,06	0,05	0,05	0,06
Naphthalene, 1,7-dimethyl-	0,11	0,16	0,11	0,12	0,14	0,14	0,13	0,13	0,08	0,08	0,10	0,07	0,08	0,09
Pentadecane <n->	0,12	0,19	0,17	0,11	0,17	0,15	0,15	0,13	0,16	0,17	0,16	0,15	0,12	0,13
Pseudocumene	0,21	0,23	0,22	0,25	0,25	0,22	0,26	0,25	0,30	0,26	0,25	0,22	0,20	0,23
Styrene	0,16	0,08	0,06	0,06	0,09	0,07	0,34	0,08	0,08	0,10	0,11	0,10	0,51	0,09
Undecan-2-one	0,05	0,12	0,07	0,08	0,10	0,12	0,09	0,15	0,10	0,13	0,08	0,08	0,06	0,08
Undecane <n->	0,13	0,19	0,20	0,12	0,23	0,22	0,21	0,25	0,31	0,30	0,27	0,27	0,22	0,26
terpenoids	**6,57**	**8,29**	**7,82**	**8,28**	**8,37**	**8,04**	**8,09**	**7,18**	**7,03**	**7,33**	**8,07**	**8,11**	**7,09**	**6,61**
Calacorene <alpha->	0,18	0,27	0,19	0,19	0,26	0,25	0,22	0,24	0,24	0,29	0,25	0,22	0,22	0,21
Damascenone <(E)-, beta->	3,22	4,04	3,88	4,21	4,24	4,11	3,91	4,01	3,22	3,27	3,70	3,69	3,22	3,39
Farnesene <(E)-, beta->	0,15	0,30	0,27	0,28	0,26	0,23	0,23	0,20	0,30	0,35	0,37	0,40	0,27	0,23
Farnesene <(E,E)-, alpha->	0,07	0,13	0,12	0,12	0,12	0,10	0,10	0,08	0,12	0,14	0,15	0,18	0,11	0,10
Linalool	1,27	1,27	1,51	1,54	1,44	1,36	1,55	1,05	1,61	1,64	1,74	1,76	1,67	1,26
Linalool oxide <cis->	0,06	0,07	0,04	0,05	0,05	0,06	0,07	0,05	0,02	0,03	0,04	0,04	0,05	0,04
Nerol oxide	0,17	0,20	0,13	0,13	0,16	0,16	0,18	0,13	0,09	0,09	0,12	0,12	0,12	0,09
Nerolidol <(Z)->	0,29	0,64	0,65	0,63	0,55	0,47	0,41	0,38	0,67	0,74	0,72	0,67	0,42	0,42
Ocimene <(E)-, beta->	0,08	0,10	0,07	0,08	0,08	0,09	0,10	0,07	0,06	0,07	0,08	0,08	0,08	0,07
Pinene <alpha->	0,05	0,06	0,05	0,05	0,05	0,05	0,06	0,04	0,04	0,05	0,05	0,06	0,05	0,04
Terpinene <alpha->	0,13	0,14	0,17	0,18	0,19	0,19	0,20	0,17	0,14	0,13	0,16	0,16	0,15	0,13
Terpineol <alpha->	0,65	0,71	0,51	0,57	0,63	0,63	0,73	0,51	0,34	0,34	0,46	0,49	0,51	0,41
Terpinolene	0,20	0,24	0,16	0,18	0,22	0,22	0,24	0,18	0,11	0,12	0,16	0,16	0,16	0,13
Vetivenene <beta->	0,06	0,12	0,08	0,07	0,11	0,10	0,08	0,09	0,07	0,08	0,08	0,08	0,07	0,08
Total sum	**710,00**	**846,02**	**887,70**	**809,01**	**847,96**	**780,48**	**814,78**	**721,42**	**880,75**	**891,81**	**806,55**	**770,04**	**703,79**	**648,83**

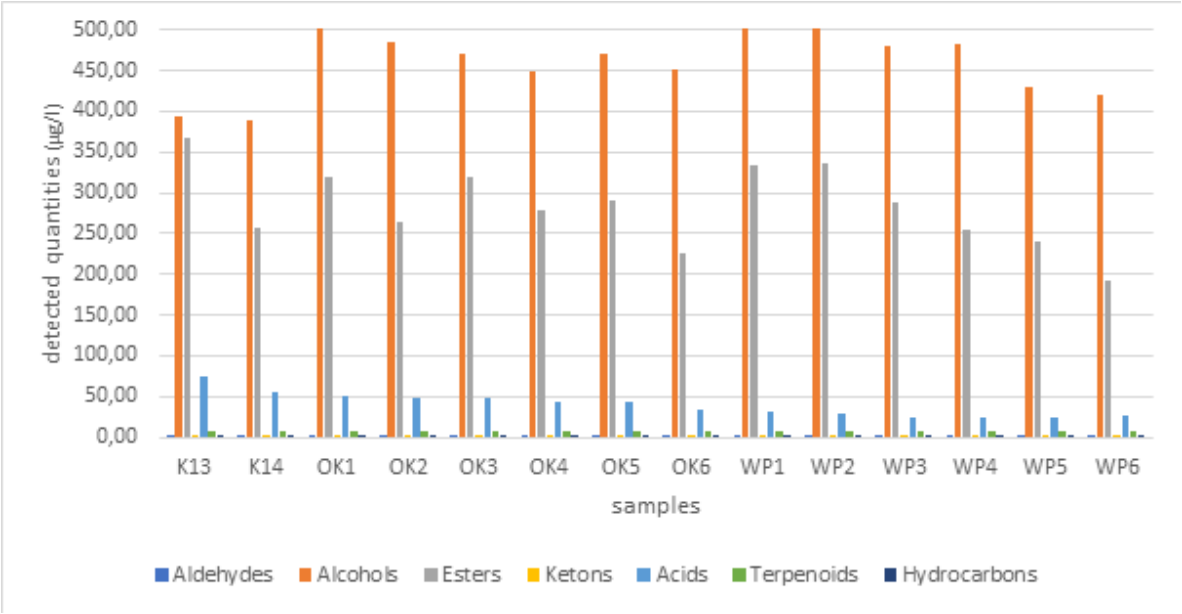


Figure 4. Detectable aromatic compound groups in each sample

In terms of the total amount per group of compounds, conclusions can be drawn on yeast use, because with different flavour-detecting enzymes, it is not the amount but the composition that varies.

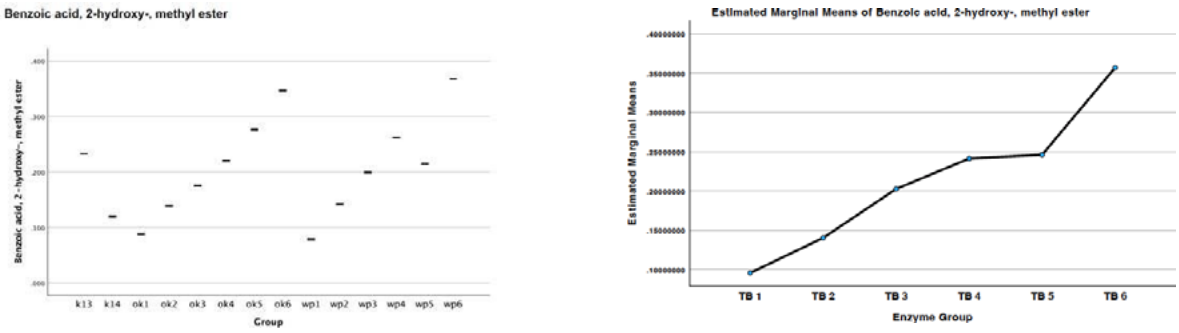
The samples are the richest in different alcohols and esters, with yeast use increasing the number of detectable alcohols and decreasing the number of esters compared to the control. A decrease in acids was also observed for each sample.

Alcohols (isopentyl alcohol, 2-phenyl ethanol, hexanol) and esters (isopentyl acetate, isoamyl acetate, ethyl decanoate, ethyl hexanoate) were the most abundant. Among the acids, decanoic acid and octanoic acid (caprylic acid), and among the terpenes, damascenone and linalool were detected in higher amounts, as reported in the literature for neutral grape varieties (Fan et al., 2010).

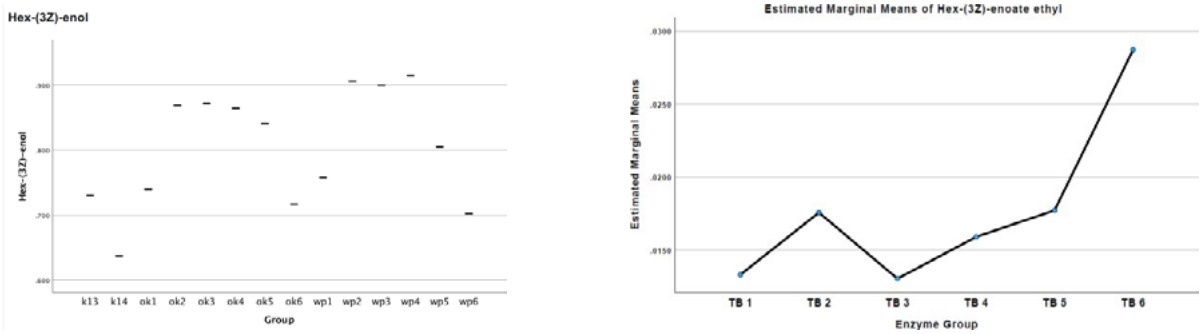
Statistical analysis was used to monitor changes in aroma composition and the following conclusions can be drawn:

- benzoic acid, ethyl-(3Z)-hexanol, pentyl acetate, phenyl acetate (mint, fruit, pear, apple, honey fruit notes) (Figure 5.) are compounds that are specifically enzyme use dependent, the higher the dose of the flavour detecting enzyme used, the higher the values detected (higher for pentyl acetate, but falling back at double the dose, which draws attention to the fact that it is reasonable to increase the enzyme dosage, that an application above a certain amount does not necessarily mean further improvement, and that with phenylacetate it is also observed that the amount increases with enzyme use but does not increase further with increasing dosage);

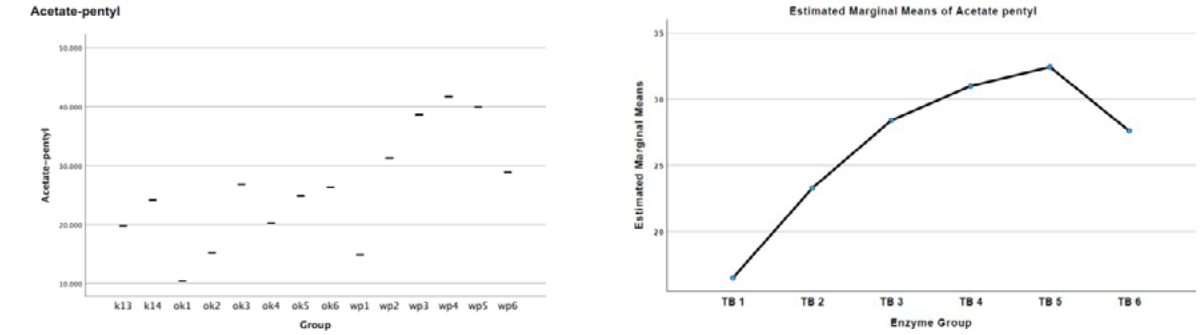
Benzoic acid (k-ok p<0.001,k-wp p<0.001, ok-k p<0.001)



Hex-(3Z)-enol (k-ok p<0.001, k-wp p=0.023, ok-k p<0.001, ok-wp p=0.006)



Acetate-pentyl (k-ok p<0.001, k-wp p<0.001)



Phenylacetate (k-ok p<0.001, k-wp p<0.001, ok-wp p=0.010)

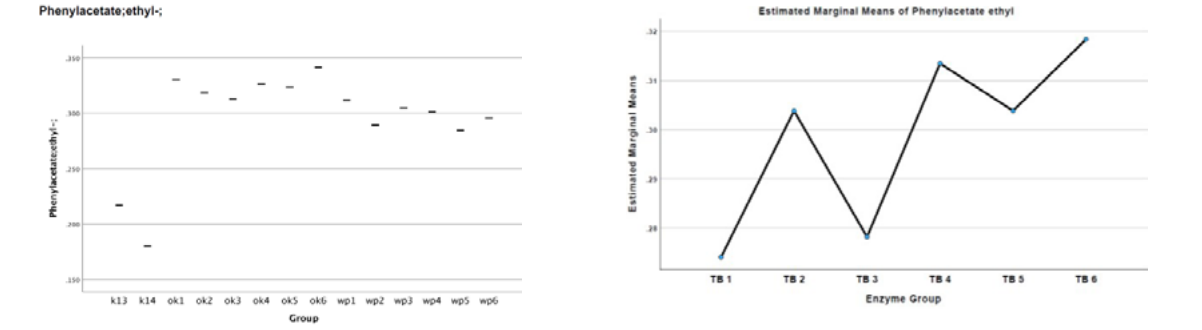


Figure 5. Values measured for benzoic acid, hex (3Z)-enol, acetate pentyl,and phenylacetate for each sample (Enzyme groups are indicated: TB1 0 ml/hl, TB2 5 ml/hl, TB3 10 ml/hl, TB4 15 ml/hl, TB5 15 ml/hl + berries, TB6 separately pressed and 2x15ml/hl; treatments (Group) k13,k14, ok1,ok2,ok3,ok4,ok5,ok6,wp1,wp2,wp3,wp4,wp5,wp6)

- isovaleric acid, (fruit aroma and flavour) are compounds whose quantity does not depend on the enzyme used, the yeast strain used increases their value, and they are not or only very slightly detectable in spontaneous fermentation (Figure 6.);

Phenethyl isovalerate (k-ok p<0.001, k-wp p=0.015, ok-wp p=0.005)

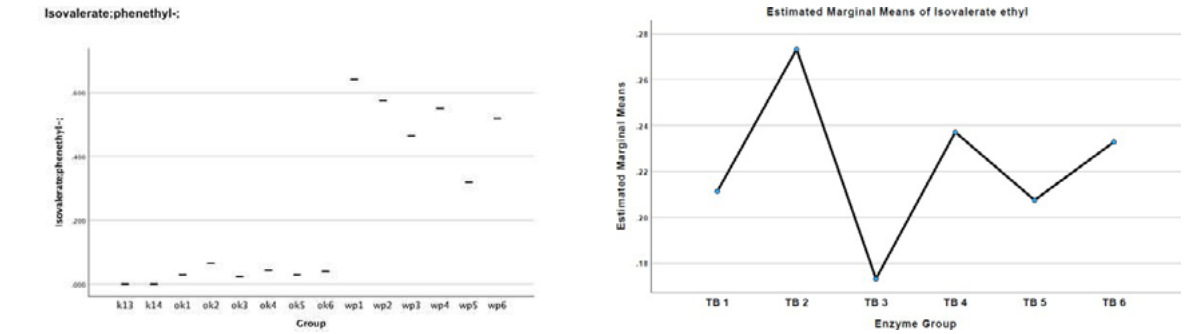


Figure 6. The amount of isovaleric acid in each sample (Enzyme groups are indicated: TB1 0 ml/hl, TB2 5 ml/hl, TB3 10 ml/hl, TB4 15 ml/hl, TB5 15 ml/hl + berries, TB6 separately pressed and 2x15ml/hl; treatments (Group) are k13,k14, ok1, ok2,ok3,ok4,ok5,ok6,wp1,wp2,wp3,wp4,wp5,wp6)

- the amount of ethyl propanoate (pineapple fragrance) increased with enzyme use, but the value is highly dependent on the yeast strain used, with higher values being achieved with Oenoferm®Wild&Pure yeast (Figure 7.).

Ethyl propanoate (k-wp p=0.039, ok-wp p=0.033)

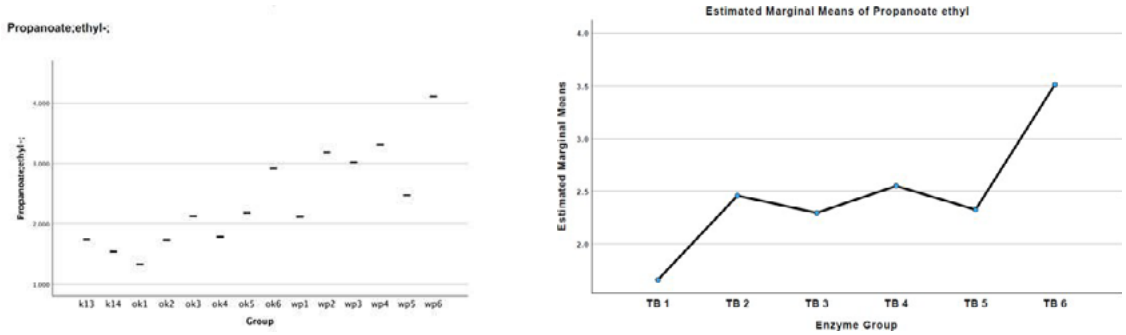


Figure 7. Quantities detectable for ethyl propanoate in each sample (Enzyme groups are indicated: TB1 0 mL/hl, TB2 5 mL/hl, TB3 10 mL/hl, TB4 15 mL/hl, TB5 15 mL/hl + berries, TB6 separately pressed and 2x15 mL/hl; treatments (Group) are k13,k14, ok1,ok2,ok3,ok4,ok5,ok6,wp1,wp2,wp3,wp4,wp5,wp6)

8.3. Profile analysis

The result of the assessment for the smell test is shown in **Figure 8.**

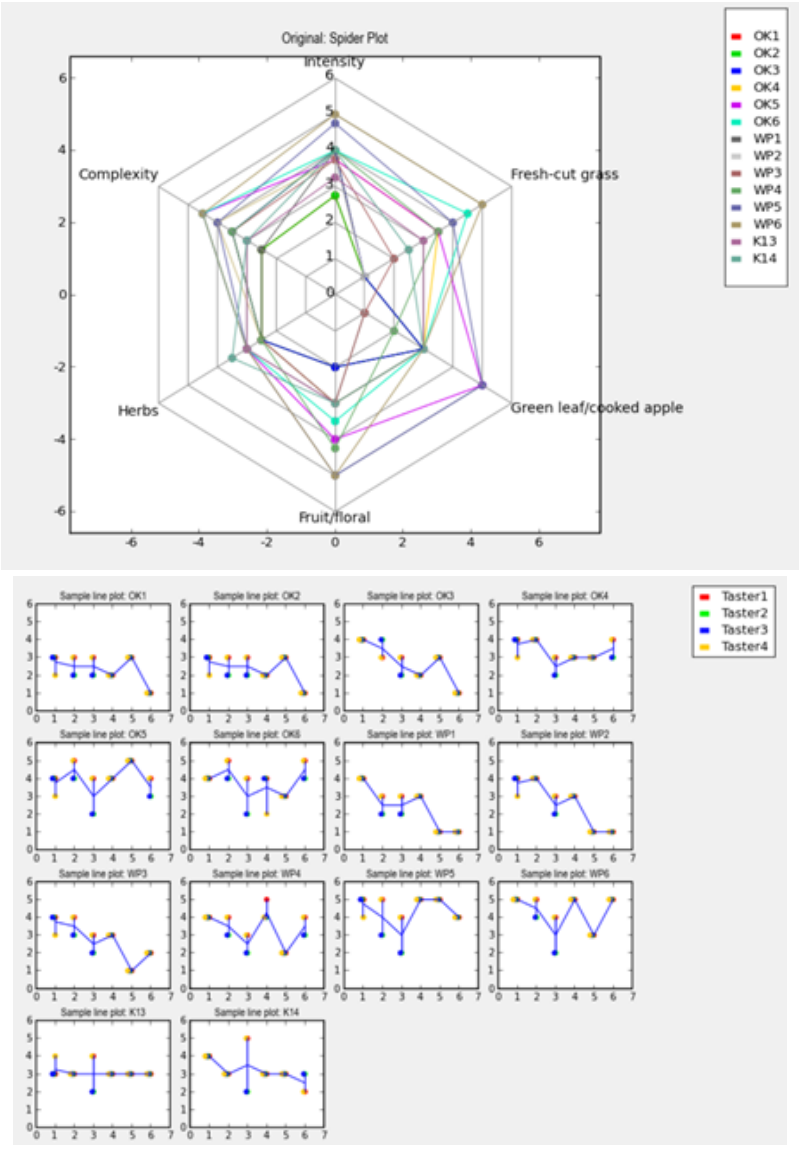


Figure 8. Results of Spider and Line-Plot analysis (Attributes (horizontal axis): intensity (1), complexity (2), herbs(3), fruit/flower (4), green leaf/cooked apple (5), fresh cut grass (6) ; values (vertical axis): 1-5)

Based on the sensory analysis of the smell, the samples are very different from each other except for OK1 and OK2 and none of them resembles the control K13.

The green leaf/cooked apple (OK5, WP5) samples are the highest, with a reduced herbal character in these batches. Herbal aromas are strongest in amphora (K14) and least in batches fermented with O.Klosterneuburg yeast.

The fresh-cut grass scent was not a function of yeast use but rather triggered by increased enzyme use in OK6, and WP6.

Both aroma intensity and complexity increased with enzyme use.

In OK4, OK5, OK6, WP4, WP5, and WP6 samples, both fruity and floral notes were perceived more by the tasters.

An illustration of the taste test results is shown in Figure 9.

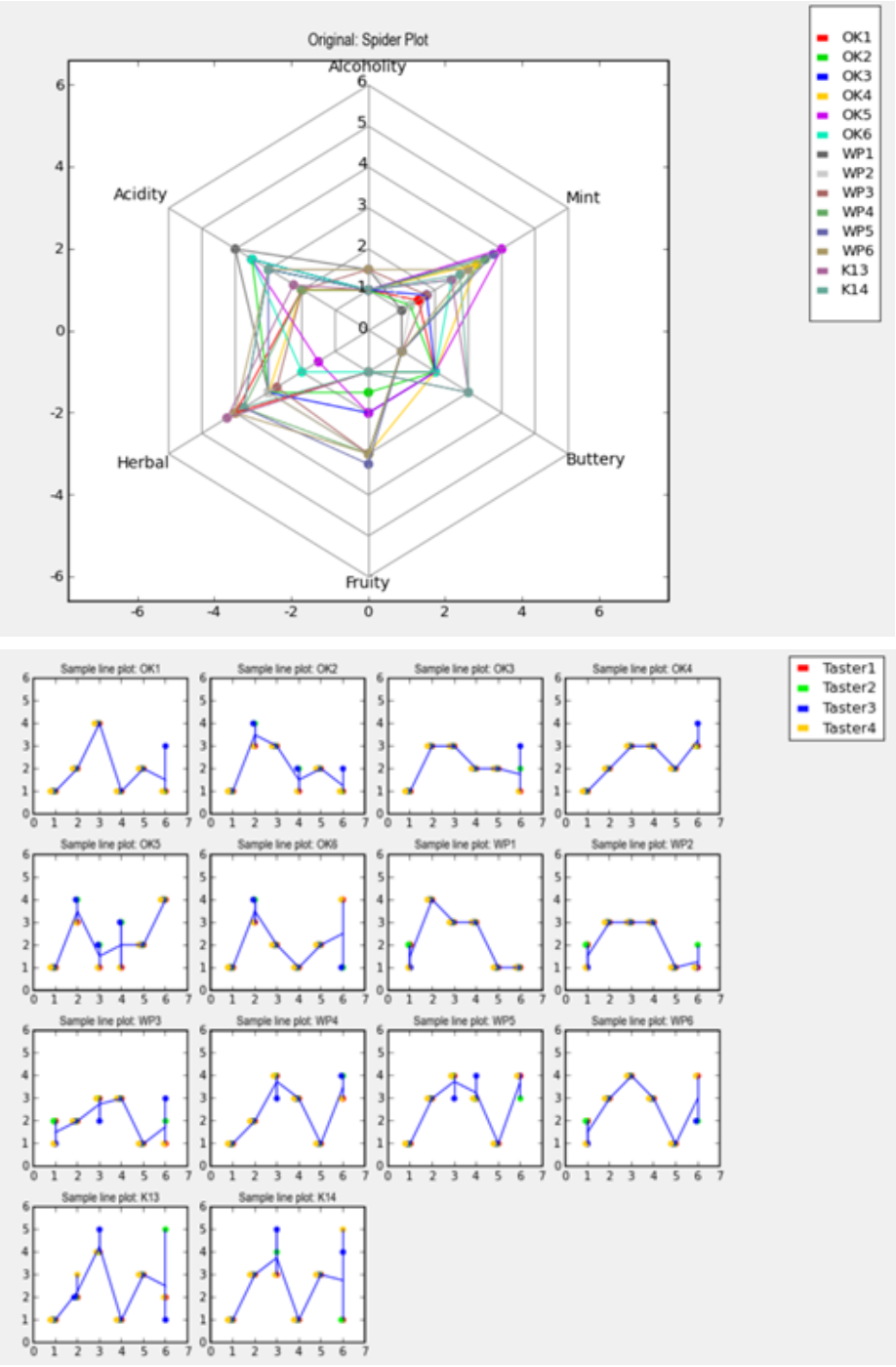


Figure 9. Results of the Line-Plot analysis (Attributes (horizontal axis): alcoholicity (1), acidity (2), herbal (3), fruity (4), buttery (5), minty (6) ; values (vertical axis): 1-5)

In the sensory evaluation, the samples also differ in terms of the scores given to the flavour notes, with similarities being found in the scores given to the WP5 and WP6 items.

The tasters gave the widest range of scores for the mint flavour, with only the OK5 sample giving the same high score.

Mint and herbaceousness contrasted, with the samples in which the judges tasted mint more strongly not dominated by herbaceousness in the taste (OK4, OK5).

In the control K13 and amphora K14 samples, the judges did not perceive the fruitiness at all, neither in batches OK1, OK2, OK5, OK6, but they did perceive it in OK6.

The buttery, sweet taste was scored higher by the tasters for control K13 and amphora K14.

9. Conclusion

The negative effects of climate change are also affecting the Furmint grape variety, even though it can maintain its acidity, aroma depletion is evident and a positive effect can be achieved by using different yeasts. The question arises as to how far the complexity of this aromatic world can be increased by using an early aroma-detecting enzyme. The study carried out is a pilot study, anticipates the importance of enzyme use and confirms the justification for the use of a specific yeast as opposed to spontaneous fermentation. It is no longer certain whether the vine can withstand the different stress situations and whether the aroma precursors that were present before are now formed in the same quantities and remain in a bound state, impoverishing the organoleptic characteristics. The possibilities offered by large-scale instrumental measurements not only support these findings, but can also greatly assist in the choice of appropriate oenological adjuvants. In modern winemaking technology, there is also a place for the use of early aroma-detecting enzymes in the neutral Furmint grape variety, where fruity, pear and apple notes can be enhanced and the herb mint can be added. However, further studies are needed on vintage, yeast and enzyme applications, and to adapt the results to practical applications.

10. References

Bauer, F.F., Pretorius I.S. (2000): Yeast Stress Response and Fermentation Efficiency: How to Survive the Making of Wine - A Review. South African Journal of Enology and Viticulture, 21(Special Issue):27-51. DOI: https://doi.org/10.21548/21-1-3557

Bene Zs. (2004): Aszúbogyók élesztő- és penészbiotájának tanulmányozása Tokaj-hegyalján. PhD-értekezés, BCE, Budapest. https://docplayer.hu/5558375-Aszubogyok-eleszto-es-peneszbiotajanak-tanulmanyozasa-tokaj-hegyaljan-doktori-ertekezes-bene-zsuzsanna.html

Bene Zs. (2020): Héjon erjesztett Furmint borok polifenol összetétele. Szőlő-levél 10(2):63-76. https://ww.szolo-level.hu/Szolo-level_tavaszi_kiadvány_2020

Bene Zs. (2023): Borászati fajélesztők fenolsav termelésének vizsgálata botrítizált alapanyag esetében. Borászati Füzetek 33(4): 29-35.

Bene Zs. (2024): Kutatások a klímaváltozás tükrében. Borászati Füzetek 34(1):26-28.

Bene, Zs. – Kiss, I. (2023): Investigation of using different specified yeasts and early protein stabilization for Tokaji dry wines. Bio Web of Conferences. 68 p.02010 (2023). https://www.bioconferences.org/articles/bioconf/full_html/2023/13/bioconf_oiv2023_02010/bioconf_oiv2023_02010.html

Carro, N.; López, E.; Günata, Z.Y.; Baumes, R.L.; Bayonove, C.L. (1996): Free and glycosidically bound aroma compounds in grape must of four non-floral Vitis vinifera varieties. Analusis (24): 254–258. DOI:https://doi.org/10.3390/biom9120818

Demsar, J., Curk, T., Erjavec, A., Gorup, C., Hocevar, T., Milutinovic, M., Mozina, M., Polajnar,

M., Toplak, M., Staric, A., Stajdohar, M., Umek, L., Zagar, L., Zbontar, J., Zitnik, M., Zupan, B. (2013): Orange: Data Mining Toolbox in Python, Journal of Machine Learning Research 14 (2013): 2349–2353.

Fan,W.L.; Xu, Y.; Jiang,W.G.; Li, J.M. (2010): Identification and Quantification of Impact Aroma Compounds in 4 Nonfloral Vids vinifera Varieties Grapes. J. Food Sci. (75)S81–S88. DOI:https://doi.org/ 10.1111/j.1750-3841.2009.01436.x

Ferreira, V., Lopez, R. (2019):The Actual and Potencial Aroma of Winemaking Grapes. Biomolecules 9(12),818. https://doi.org/10.3390/biom9120818

Gabler, F.M., Smilanick, J. L., Mansour, M., Ramming, D. W., Mackey, B. E. Correlations of morphological, anatomical and chemical features of grape berries with resistance to Botrytis cinerea. Phytopathology, 93: 1263-1273. (2003).

Godelmann, R., Fang, F., Humpfer, E., Schutz, B., Bansbach, M., Schafer, H., Spraul, M. (2013): Targeted and Nontargeted Wine Analysis by H^{-1} NMR Spectroscopy Combined with Multivariate Statistical Analysis. Differentiation of Important Parameters: Grape Variety, Geographical Origin, Year of Vintage. Journal of Agricultural and Food Chemistry 61 (23) 5610-5619.DOI: https://doi.org/10.1021/jf400800d

Hampel, D.; Robinson, A.L.; Johnson, A.J.; Ebeler, S.E. (2014): Direct hydrolysis and analysis of glycosidically bound aroma compounds in grapes and wines: Comparison of hydrolysis conditions and sample preparation methods. Aust. J. Grape Wine Res. (20):361–377. DOI: https://doi.org/10.1111/ajgw.12087

Kállay M. (1998): Borászati kémia. – Eperjesi, I., Kállay, M., & Magyar, I. (1998): Borászat (Winemaking) Mezőgazda Kiadó, Budapest, pp.309-312.

Kovács, T., Kovácsné, B.O. (2007): Borászati pektinbontó és macerációs enzimek (Rendezőelvek és információk az eligazodáshoz a kaotikussá vált piacon). Borászati Füzetek 17(3):24-27. https://www.kokoferm.hu/resources/docs/Pektin. MKIK-GVI 2015. A klímaváltozás várható gazdasági hatásai Magyarországon 2020-2040 / Expected economic effects of the climatic change in Hungary, 2020-2040. Kézirat. https://www.gvi.hu

Pancerz, M., Kruk, J., Ptaszek, A. (2022): The Effect of Pectin Branching on the Textural and Swelling Properties of Gel Beads Obtained during Continuous External Gelation Process Appl. Sci. *12*(14), 7171. https://doi.org/10.3390/app12147171

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2006): Handbook of Enology. Volume 2. The Chemistry of Wine. Stabilization and Treatments. 2nd Edition John Wiley and Sons Ltd., New Jersey. ISBN: 978-0-470-01038-9

Rodríguez-Nogales, J.M., Fernández-Fernández, E., Ruipérez, V., Vila-Crespo, J. (2024): Selective Wine Aroma Enhancement through Enzyme Hydrolysis of Glycosidic Precursors. Molecules *29*(1), 16. https://doi.org/10.3390/molecules29010016

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R., Schuhmacher, R. (2010): Identification and profiling of volatile metabolites of the biocontrol fungus Trichoderma atroviride by HS-SPME-GC-MS. Journal of Microbiological Methods 81(2):187-93. DOI: https://doi.org/10.1016/j.mimet.2010.03.011

Szendei, G. (2021): Speciális fajélesztőtörzs a klímaváltozás okozta borászati problémák enyhítésére. Szőlő-levél 11(3):77-80. https://ww.szolo-level.hu/Szolo-level_tavaszi_kiadvány_2021