Article

Possibilities for Depleting the Content of Undesirable Volatile Phenolic Compounds in White Wine with the Use of Low-Intervention and Economically Efficient Grape Processing Technology

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Featured Application: Support for ripening processes and prevention of premature aging of wine with the use of low-intervention grape processing technology.

Abstract: The influence of the technological processes of grape processing on the content of hydroxycinnamic acids and volatile phenolic substances in wine was studied. The method of targeted oxygenation was applied in grape processing technology of the Welschriesling and Chardonnay grape varieties. The content of volatile phenolic substances was determined by gas chromatography, the content of hydroxycinnamic acids by liquid chromatography, and the basic analytical parameters of the wine by FTIR spectrometry. The method of targeted must oxygenation had a statistically significant effect on the content of hydroxycinnamic acids and volatile phenolics in the wine. In all three monitored years (2015–2017), the content of 4-vinylphenol and 4-vinylguajacol in the wine significantly decreased. A significant dependence between the content of hydroxycinnamic acids and volatile phenolics was found. The experiment showed that a 1% increase in the content of hydroxycinnamic acids in the Chardonnay variety could result in an average increase in the content of monitored volatile phenolics by 3.6% (3 years' data). Naturally reducing the content of hydroxycinnamic acids, with the application of technological processes, eliminated the oxidative processes during wine maturation. Sensory undesirable volatile phenolic substances were consequently formed in lower quantities, and there was no negative impact on the favourable sensory properties of wine. It was not necessary to use the polyvinylpolypyrrolidone adsorbents.

Keywords: targeted must oxygenation; volatile phenolic compounds; bretty; wine maturation; costs; profit

1. Introduction

Wine presents a complex of various chemical components that play a key role in the sensory perception of the final product. In the winemaking process, the quantity and composition of many of these components can be consciously modified by the choice of production method [1]. The components contributing to the final organoleptic properties of wine are well known, and their direct impact on sensory properties has been described

in many studies. Grape and wine contain a number of flavour precursors belonging to groups of glycosides, amino acid conjugates, odourless volatiles, hydroxycinnamic acids, and many others. They mainly originate in the grape berry but also from oak or other materials involved in winemaking [2]. Polyphenols are very common in the chemical composition of grapes; hydroxycinnamic acids especially, which are the most widely distributed phenolic acids in plants, present the third most abundant class of soluble phenolics in grape berries, particularly in grape pulps [3,4]. Hydroxycinnamates are a class of aromatic acids derived from a cinnamic acid that differs in the number and location of hydroxyl groups on the aromatic ring, resulting in different individual compounds [5]. These compounds have been described as potent antioxidants with a chain-breaking action through radical scavenging activity [6]. In grape musts/wines, there were not only nonesterified hydroxycinnamic acids found—such as caffeic, p-coumaric, and ferulic acids— but, predominantly, the corresponding tartaric acid esters, such as caftaric, p-coutaric, and fertaric acids, respectively [7–9]. The esters are water-soluble and have a bitter taste; however, they do not significantly affect the overall taste of wine, only partially its acidity. Caftaric and coutaric acids correlate with the enzymatic browning of grape must [10,11]. In the winemaking process, the corresponding hydroxycinnamic acids are decarboxylated by hydroxycinnamate decarboxylase by yeasts (especially by *Brettanomyces bruxellensis*, *B. anomalus*, and *Dekkera* spp.) to vinylphenols and subsequently reduced by vinylphenol reductase to the corresponding volatile ethylphenols (4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol) [12,13]. Vinylphenols (4-vinylphenol and 4-vinylguaiacol) are formed in selective, enzymatic decarboxylation by yeast during fermentation, from p-coumaric and ferulic acids [12,14,15]. In the process of fermentation, the role of the yeast strains applied in wine fermentation is also important factor. The use of *Saccharomyces* yeast strains is connected with formation of little or no ethylphenols [15,16]. In white wines, the vinylphenols are dominant, while ethylphenols are found mostly in red wines [17]. The volatile phenols (especially 4-ethylphenol and 4-ethylguaiacol) have characteristic aromas which, above a certain concentration threshold, have a negative effect on the overall aroma of a wine, but at a low concentration have been cited as contributing positively to aroma complexity [10,18]. At present, the effect of ethyl and vinylphenols, as well as other metabolites in wine that are linked with the off-flavours of wine and thus detracting from wine quality, are the subject of live debates worldwide [19]. These volatile phenols are associated with the so-called "Brett" aroma, mainly affecting high-quality red wines. The wine spoilage is due to the production of a "horse sweat" taint in bulk or bottled wines. The phenolic off-odours have been described as "medicinal", "phenolic", "rancid", "sweaty", "smoke", "Band-aid®", "barnyard", "leather", or "horse sweat" [19,20]. Hydroxycinnamic acids and flavanols were also examined in several studies due to their effect on the sensory properties of white wine, focusing on oxidative aromas or the browning rate [21,22].

Various technological approaches, including preventive and remedial treatments, have been studied to decrease the chance of wine contamination with volatile phenols. Treatment of wines with commercial fining agents, including mineral, synthetic, animal, and vegetable proteins, has been realized. The application of activated carbon in the removal of 4-ethyphenol and 4-ethylguaiacol from red wines has been described by Filipe-Ribeiro et al. [23], achieving a maximum efficiency of 74%. On the other hand, activated carbon changed some aspects of the wine's chemical composition, colour, and perception of some attributes of the headspace aroma components. The ability of esterified cellulose fibres to reduce 4-ethylphenol and 4-ethylguaiacol was studied by Larcher et al. [24]. A 31–32% reduction in both phenols was achieved on average in defective red wines. The most efficient was an application of regenerable polymer cellulose acetate propionate. Treatment of wine did not affect the colour and polyphenol content and it improved the wine quality. Adsorbents based on polyvinylpolypyrrolidone (PVPP), activated charcoal, and zeolite were also used as fining agents in contaminated red wines by Lisanti et al. [25]. Treatment for preventing volatile phenol formation in wines during winemaking have included preventive actions such as proper sanitization in all stages of the winemaking procedure to

minimize the spread of *Dekkera/Brettanomyces* [26], control of enzymatic activity (avoidance of the use of cinnamoyl esterase activity contaminated enzymes [27]), sulfiting followed by a complete fermentation process [13], and treatment of must with cinnamyl esterases before the fermentation with *S. cerevisiae* strains to eliminate the free hydroxycinnamic acids with subsequent impacts on volatile phenols formation [28]. Other physical and chemical methods have been studied in winemaking operations to decrease the development of volatile phenols due to inhibition of microbial activity, such as manipulation with pH, use of dimethylcarbonate, fungal chitosan, sorbic acid, benzoic acid, oenological tannins, and many others chemical alternatives to SO_2 [26]. Nowadays, the wine industry does not have any easy and affordable technique to rescue wines contaminated by ethylphenols, exhibiting a phenolic off-odour [25].

As the polyphenols are the relatively unstable, reactive components in the wine, the technology of winemaking requires to know the content and properties of such compounds due to their ability to influence colour and the archival potential of the wine in negative way. For that reason, improvements in the present knowledge and practice on the prevention of this problem in the wine industry has a high importance. One of the most essential aspects in the natural prevention of oxidation and browning of wine is the gentle processing of grapes and elimination of phenolics in the production of rosé and white wines before the start of alcoholic fermentation [29].

In our study, targeted must oxidation technology, as a low interventional method for elimination of hydroxycinnamic acids, resulting in depleting the content of the undesirable volatile phenolic substances 4-vinylguajacol, 4-vinylphenol, and potentially also 4-ethylphenol and 4-ethylguajacol in the white grape varieties Chardonnay and Welschriesling, was studied as a novel approach to the prevention of the formation of these undesirable off-flavours.

2. Materials and Methods

2.1. Plant Material

The grapes of the Chardonnay came from the Modra wine district, Modra municipality, from the Little Carpathian wine-growing region. The grapes of the Welschriesling variety were cultivated in the Želiezovce wine district, Farná municipality, in the Nitra wine-growing region. The grapes were collected in the years 2015, 2016, and 2017. The impact of climate variations on the physical and chemical parameters was assessed due to this long-term experiment.

2.1.1. Welschriesling

Welschriesling is a grape variety commonly cultivated throughout central and eastern Europe. This grape variety, notorious for its neutrality, is convenient for production of different wine quality categories. In most cases, the wines produced are light and fairly neutral, characterized by a gentler aroma profile. In our latitudes, the Welschriesling grape variety is known for its higher acidity that it retains also in warmer climate cultivation conditions. The age of the vineyard was eleven years.

2.1.2. Chardonnay

Chardonnay, currently a very popular white wine grape variety in the world, is used for the production of high-quality wines, characterized by a unique bouquet. Chardonnay is considered as an easy variety to cultivate, which is the reason for its mass popularity worldwide. In our latitudes, the variety is known for its typically more pronounced aroma, which is characterized by floral tones. The wines produced are elegant and delicate, with balanced aroma and taste properties [30]. The age of the vineyard was eight years.

2.1.3. Characterisation of Experimental Sites

The Želiezovský wine district belongs to the Nitra wine region and includes 20 wine-growing municipalities. The Welschriesling grape variety was grown in the wine-growing

municipality Farná, where there is 2100 to 2200 h sunshine per year. The total atmospheric precipitation during the growing season ranges from 650 to 680 mm. The sum of the active temperatures is 3000–3100 °C.

The Modranský wine district belongs to the Small Carpathians wine region and includes 5 wine-growing municipalities. The Chardonnay grape variety was grown in the wine municipality Modra, where there is 2000 to 2200 h sunshine per year. The total atmospheric precipitation during the growing season ranges from 400 to 450 mm. The sum of the active temperatures is 3060–3100 °C.

2.2. Technological Processes

The sugar content in the individual grape varieties at the grape harvesting time ranged from 180 to 220 g L^{-1}. The grapes were collected manually for the experiment every year in September, during the second and third week. The must was produced from the same grape for each variety, and each variable in a triple batch of 1000 L for both the control and the experimental variants. Each variant (WRO, WRC, CHO, and CHC) was realized in triple replicates in each experimental year. Each experimental variant replication was analysed to provide data for statistical evaluation. The article presents the mean and standard deviation values from the measurements. In both variants and each year of the experiment, the sugar content of the Welschriesling variety was adjusted to 210 g L^{-1} before fermentation. No adjustment was required for the Chardonnay variety. The grapes were grinded, and then dry ice in amount of 50 mL hL^{-1} was applied. The control variant of the must was mixed with 15 mL hL^{-1} of pectolytic enzymes and with 35 mg L^{-1} of sulphur dioxide, according to manufacturer's recommendation. The must was prepared by a pneumatic press, applying the maximum pressure applied of 0.15 MPa. Elimination of indigenous undesirable microflora and a reduction in proteins were performed by application of bentonite for 24 h and static decanting. Sodium–calcium bentonite was applied in an amount of 60 g hL^{-1}.

In the variant with the targeted oxygenation technology, the same amount of dry ice and pectolytic enzymes was added as mentioned above in the control variant. The must was prepared by pressing on a pneumatic press. No sulphur dioxide nor any other antioxidants were added during the fermentation phase. The sludge was removed by gravity using sodium–calcium bentonite for elimination of indigenous undesirable microflora and a reduction in proteins at a dose of 60 g hL^{-1}; this process was finished after 16 h. Oxygen was not introduced into the wine under atmospheric air pressure. The acidification took place naturally. No sulphur dioxide is added to the must until the desludging phase; thus, the phenolic substances are able to react with air oxygen spontaneously. Duration of the targeted oxygenation was 24 h. By removing the sludge, some oxidized polyphenolics were eliminated from the must. The next phase of the technological process of wine production was the same in the both variants. The dry wine yeast commercial preparation (*Saccharomyces cerevisiae*) was applied in an amount of 15 g hL^{-1}. The process of fermentation took 12 days at a temperature of 14−15 °C (±1.0 °C). When the fermentation was completed the wine remained on the yeast lees during two weeks without oxygen access. When bottling both variants, we added 40 mg L^{-1} of SO_2. The wines were further stored at a temperature of ±20 °C. In variants where we observed the risk of starting malolactic fermentation, we added another dose of SO_2 (40 mg L^{-1}) after 21 days. The sulphur dioxide content in the wine reflected its added amount. The design of the experimental variants is shown in Figure 1.
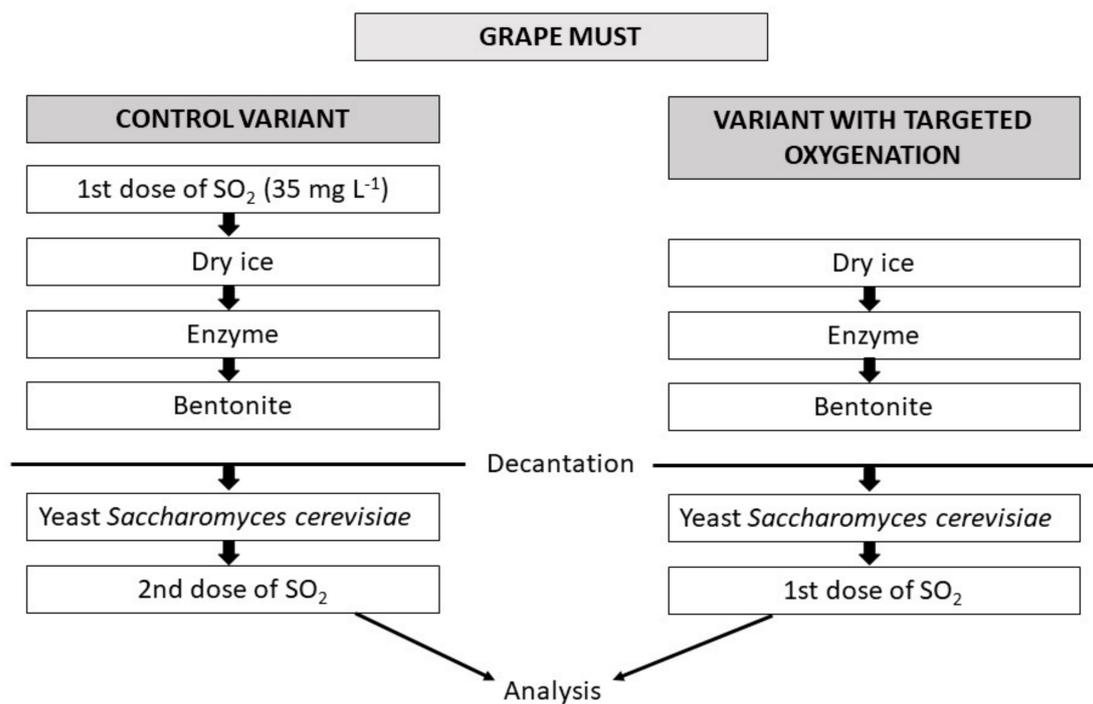
Figure 1. Experimental design of the control variant and variant with targeted oxygenation.

2.3. Evaluation of the Organoleptic Quality

The organoleptic quality was evaluated according to the international union of oenology and STN EN ISO 8586 [31] using the 100-point system. Six experienced evaluators with the relevant certificates assessed the products of the experimental variants. The results are based on the arithmetic mean, with exclusion of the extreme values. Sensory evaluation of the wine was based on the following sensory parameters: appearance (purity and colour), aroma (positive intensity of aroma, purity, and aroma harmony), and taste (positive intensity, purity, harmony, and persistence). The last parameter was the overall impression. We used sensorial evaluation combined with the specific descriptors of the aroma and the taste profiles of the wine. The aroma profile descriptors to describe the wine sample were as follows: ripe grape, blossoming meadow, healthy hay, linden blossom, green apple, pepper, almond, walnut, vanilla, minerality/quartz, copper, tropical fruit, chlorophyll, vegetation, oxidation, yeast decomposition, and others/error.

2.4. Statistical Evaluation

The statistical evaluation was realized in the program Statgraphics Centurion XVII (StatPoint Inc., Warrenton, VA, USA, 2016, version 17.01.03) and Excel software. The one-way ANOVA method was used for the analysis of the differences between the oxygenated and control variants. The conditions for the method used were preserved and the significance level was set at 0.05. The statistical evaluation was chosen for evaluation of the differences between the chemical parameters of the variants within one grape variety and one vintage, and also to analyse the differences between the individual hydroxycinnamic acids, the sums of hydroxycinnamic acids, and the volatile phenols, as the presence of these compounds needs to be considered as a complex due to their synergic effect on wine sensory parameters. Statistical evaluation of the sums of the hydroxycinnamic acids is required due to the impossibility of evaluation of the individual hydroxycinnamic acid contribution to the formation of individual volatile phenols. We also evaluated individual differences among the individual hydroxycinnamic acids and individual volatile phenols.

The method applied for evaluation of the influence of the variant and the year on the content of the monitored substances in the wine was by a two-factor analysis of variance. The statistical significance of the results was determined by the least significant difference

test (LSD test $p \leq 0.05$). The test is performed to identify differences in the average values of the observed trait between the factor(s) levels. Other statistical methods used were regression and correlation analyses, specifically, nonlinear models. Based on the value of the determination index and the size of the residues, the most suitable type of model was chosen.

2.5. Wine Analysis

2.5.1. Samples and Sample Preparation

Samples of wine were kept at 4 °C prior to the analysis. Before FT-IR and HPLC analysis, the residues of carbon dioxide were removed from the wine samples by vacuum filtration and centrifugation ($3000 \times g$; 6 min). The HPLC analysis of hydroxycinnamates required sample dilution in a ratio 1:1 by 100 mM $HClO_4$, and a diluted sample was directly injected into the HPLC system. For GC-MS analysis of the volatile compounds, wine samples were extracted with methyl t-butyl ether according to the method described by Baron et al. [32].

2.5.2. Chemicals

The hydroxycinnamates (caffeic acid, caftaric acid, p-coumaric acid, ferulic acid, and fertaric acid) were purchased as single-component standards from Sigma Aldrich (Sigma Aldrich Chemie GmbH, Steinheim, Germany); acetonitrile (HPLC gradient grade), methanol (HPLC grade), and perchloric acid (ACS reagent grade) were purchased from Sigma Aldrich (SigmaAldrich Chemie GmbH, Steinheim, Germany); standard wine for FT-IR analysis (Bruker Optic GmbH, Ettlingen, Germany) was purchased from OK Servis BioPro, Ldt; and deionized water was used for the sample and mobile phase preparation.

2.5.3. FT-IR Analysis

Determination of the basic chemical parameters in wine samples was performed with the use of the ALPHA Bruker Optik GMBH analyzer. Fourier Transform Infrared Spectroscopy (FT-IR) with the ATR (Attenuated total reflect) measurement procedure was applied. The method allows simultaneous analysis of different parameters with one measurement. The analyser was calibrated to a wine and must sample by setting the parameters for the measurements of wines. The measurement of the wine calibration spectra and the calibration settings according to reference values were performed by the accredited (DAkkS) Institute Heidger (Osann Monzel, Germany). The calibration data consisted of over 2000 red and white wines. The Root Mean Square Error of Prediction (RMSEP) was determined. Wine samples were analysed after filtration and centrifugation without further modification. Approximately 10 mL of the wine sample was injected into the flow-through cell. The sample was heated to 40 °C, and then the spectrum was recorded in the wavelength range 0.78–1000 nm. The instrument evaluated the sample within 70–100 s, and the results were recorded. The precision of the method was checked by analysing the standard wine for analysis (Bruker Optic GmbH, Germany) with reference values of individual parameters.

2.5.4. RP-HPLC-DAD Analysis

Concentrations of the individual phenolic compounds were determined by a method published by Kumšta et al. [33]. The chromatographic system LC-10A (Shimadzu, Kyoto, Japan) was equipped with a binary pump LC-10ADvp (Shimadzu, Kyoto, Japan), a column thermostat with a manual injection valve with a 20 μL injector loop, a diode array detector (DAD, SPD-M10Avp; Shimadzu, Kyoto, Japan), and a personal computer with the chromatographic software LC Solution (Shimadzu, Kyoto, Japan). The chromatographic separations were performed on a reverse-phased column Alltima C18-3 μm, 3×150 mm (Alltech, Deerfield, IL, USA) equipped with a guard column (3×7.5 mm i.d.) filled with the same sorbent. The column thermostat was kept at 60 °C. The separation conditions were as follows: a mobile phase A was 15 mM $HClO_4$, and B consisted of 15 mM $HClO_4$ in

a 10% MeOH and 50% ACN mixture. The flow rate was 0.6 mL/min. The gradient program started with 96% A and 4% B, and the gradient increased to 100% B in 40 min, followed by the column equilibration 4 min post time. Data were recorded in the wavelength range of 200–520 nm. The retention time and spectrum profile of the standards were used to identify the compounds in the samples.

2.5.5. GC-MS Analysis

The volatile phenolic compounds were analysed by gas chromatography coupled to mass spectrometry (GC-MS). A Shimadzu gas chromatograph GC-17A (Shimadzu, Kyoto, Japan) coupled with a mass spectrometer detector QP-5050A (Shimadzu, Kyoto, Japan) was used. The apparatus is equipped with the autosampler (AOC-5000) and the operation program LabSolutions, GC MS solution, Version 1.20. Separation was performed by a DB-WAX column (30 m × 0.25 mm × 0.25 μm; stationary phase was ethylene glycol). The detector voltage was 1.5 kV and the temperature program and MS conditions were set as described in Baron et al. [32]. Identification of the compounds was performed on the base of the MS spectrum and by matching the measured peaks with the retention times using the NIST 107 library.

3. Results

The results of the basic physical–chemical analyses of the wines are shown in Table 1. The technology applied in the experimental variants would not have a significant effect on the wines' physical and chemical parameters. The differences in some parameters are only in the level of the determination error. The result was expected, and we can consider it as a positive. The physical–chemical characterization of the wines provides information on the basic wine parameters compared to the control variant. Statistical evaluation was performed to show the potential differences in those parameters that could be affected by the fermentation process. In some cases, statistically significant differences were found as shown in Table 1. Total sugar content showed higher values in most of the oxygenated variants in both wine varieties. Oxygen treatment during white wine fermentation influences mostly the kinetics of fermentation rather than style of wine. Impact on ethanol content did not show any trend in the comparison of experimental variants. Wine pH ranged from 3.07 ± 0.04 to 3.39 ± 0.00 and significant differences were found in three cases during the experimental period. Acetic acid as a parameter showed no changes during the whole experiment.

3.1. Influence of Technology and Vintage on Content of Hydroxycinnamic Acids

Evaluation of the hydroxycinnamic acids was based on the content of the total caffeic acid (including caffeic acid and caffeic acid ethylester), total coumaric acid (including coumaric acid, coutaric acid, and cumaric acid ethylester, respectively), total ferulic acid (containing ferulic acid and ferulic acid ethylester), and caftaric and fertaric acids (Table 2). Caffeic and caftaric acids are the most abundant representatives of hydroxycinnamic acids in grape must. They present the primary substrate for the enzymes polyphenoloxidase in the redox processes of wine production and a substrate for cinnamate reductase that has a key role in the production of undesirable volatile phenolics in wine [12,13].

Table 1. Basic physical and chemical parameters of the wine variants in experimental years 2015, 2016, and 2017.

Variant		SO$_2$ Free (mg L^{-1})	SO$_2$ Total (mg L^{-1})	Ethanol (% vol)	Total Acidity (g L^{-1})	Total Sugar (g L^{-1})	pH	Malic Acid (g L^{-1})	Acetic Acid (g L^{-1})	Tartaric Acid (g L^{-1})	Glucose (g L^{-1})	Fructose (g L^{-1})
2015	WRC	17.00 ± 1.63	70.67 ± 4.50	12.32 ± 0.27	6.16 ± 0.18	0.81 ± 0.05 [b]	3.12 ± 0.00	0.97 ± 0.05	0.31 ± 0.02	2.38 ± 0.02	0.12 ± 0.01	0.94 ± 0.04
	WRO	6.33 ± 0.47	47.33 ± 0.47	11.86 ± 0.31	6.04 ± 0.05	1.13 ± 0.12 [a]	3.13 ± 0.00	0.91 ± 0.05	0.35 ± 0.01	2.56 ± 0.09	0.06 ± 0.01	0.83 ± 0.02
	CHC	27.67 ± 0.94	69.33 ± 2.87	12.07 ± 0.09	7.34 ± 0.13	0.77 ± 0.05 [b]	3.15 ± 0.02	2.23 ± 0.04	0.38 ± 0.03	2.47 ± 0.05	0.15 ± 0.03	1.03 ± 0.03
	CHO	58.33 ± 4.50	136.00 ± 0.00	12.18 ± 0.09	7.55 ± 0.04	1.17 ± 0.05 [a]	3.07 ± 0.04	1.91 ± 0.00	0.37 ± 0.02	2.51 ± 0.04	0.27 ± 0.00	0.67 ± 0.06
2016	WRC	25.67 ± 0.94	82.33 ± 1.25	12.49 ± 0.01 [a]	5.87 ± 0.03 [a]	2.52 ± 0.00 [b]	3.27 ± 0.00 [b]	1.90 ± 0.04	0.31 ± 0.01	2.39 ± 0.01	0.29 ± 0.00	1.27 ± 0.02
	WRO	14.33 ± 0.47	68.33 ± 2.05	12.29 ± 0.08 [b]	5.27 ± 0.03 [b]	1.99 ± 0.04 [a]	3.35 ± 0.04 [a]	1.93 ± 0.02	0.26 ± 0.04	2.01 ± 0.00	0.16 ± 0.02	1.06 ± 0.03
	CHC	28.67 ± 2.87	62.33 ± 1.25	12.54 ± 0.03	6.35 ± 0.03	1.67 ± 0.05 [b]	3.21 ± 0.03	2.33 ± 0.03	0.34 ± 0.03	2.74 ± 0.02	0.32 ± 0.01	1.32 ± 0.04
	CHO	36.33 ± 2.05	49.33 ± 2.05	12.65 ± 0.07	6.27 ± 0.05	1.87 ± 0.05 [a]	3.25 ± 0.01	2.14 ± 0.04	0.36 ± 0.02	2.72 ± 0.01	0.37 ± 0.02	1.34 ± 0.01
2017	WRC	19.33 ± 1.25	76.33 ± 2.05	12.24 ± 0.02 [b]	5.87 ± 0.04	2.14 ± 0.05 [b]	3.39 ± 0.00 [a]	1.57 ± 0.02	0.41 ± 0.00	2.12 ± 0.05	0.57 ± 0.02	1.14 ± 0.03
	WRO	14.33 ± 2.05	57.33 ± 2.05	12.34 ± 0.02 [a]	5.81 ± 0.09	2.24 ± 0.03 [a]	3.34 ± 0.01 [b]	1.74 ± 0.01	0.43 ± 0.01	2.04 ± 0.02	0.44 ± 0.02	1.21 ± 0.02
	CHC	29.33 ± 2.05	58.67 ± 1.25	12.51 ± 0.01	6.24 ± 0.04 [b]	1.87 ± 0.06 [a]	3.19 ± 0.00 [a]	1.89 ± 0.03	0.38 ± 0.06	2.54 ± 0.04	0.47 ± 0.03	0.87 ± 0.02
	CHO	44.33 ± 0.47	47.67 ± 2.87	12.55 ± 0.06	6.34 ± 0.00 [a]	1.72 ± 0.04 [a]	3.15 ± 0.01 [b]	1.94 ± 0.04	0.36 ± 0.01	2.41 ± 0.08	0.45 ± 0.05	0.94 ± 0.08

Note: Values express the mean ± SD; WRC—Welschriesling control variant; WRO—Welschriesling variant with targeted must oxygenation; CHC—Chardonnay control variant; CHO—Chardonnay variant with targeted must oxygenation; [a,b]—mean values of individual parameters in columns with different superscript have a statistically significant difference ($p < 0.05$) between the variants within one variety and one vintage.

Table 2. Hydroxycinnamic acids content in the experimental variants in vintages 2015–2017.

	Variant	Total Caffeic Acid (mg L^{-1})	Total Coumaric Acid (mg L^{-1})	Total Ferulic Acid (mg L^{-1})	Caftaric Acid (mg L^{-1})	Fertaric Acid (mg L^{-1})	Sum of Hydroxycinnamic Acids (mg L^{-1})
	WRC	38.80 ± 1.22 [a]	9.93 ± 1.22 [a]	3.45 ± 0.12	21.54 ± 0.27 [a]	2.44 ± 0.05 [a]	76.16 ± 2.45 [a]
2015	WRO	32.65 ± 1.22 [b]	6.95 ± 1.22 [b]	3.14 ± 0.12	17.74 ± 0.48 [b]	2.25 ± 0.00 [b]	62.73 ± 2.94 [b]
	CHC	47.23 ± 1.42 [a]	13.61 ± 0.62 [a]	2.83 ± 0.12 [a]	9.28 ± 0.17 [a]	1.49 ± 0.06	74.44 ± 2.38 [a]
	CHO	37.01 ± 0.14 [b]	11.48 ± 0.09 [b]	2.22 ± 0.02 [b]	7.59 ± 0.07 [b]	1.18 ± 0.00	59.48 ± 0.32 [b]
	WRC	42.01 ± 0.42 [a]	9.41 ± 0.22 [a]	3.32 ± 0.16 [a]	17.47 ± 0.37 [a]	2.8 ± 0.07 [a]	74.29 ± 0.69 [a]
2016	WRO	35.66 ± 0.56 [b]	7.67 ± 0.30 [b]	2.88 ± 0.08 [b]	16.47 ± 0.39 [b]	1.99 ± 0.08 [b]	64.67 ± 0.54 [b]
	CHC	37.94 ± 0.06 [a]	6.87 ± 0.10 [a]	5.82 ± 0.14 [a]	24.87 ± 0.23 [a]	4.98 ± 0.12	80.48 ± 0.26 [a]
	CHO	32.92 ± 0.43 [b]	5.24 ± 0.29 [b]	5.45 ± 0.12 [b]	20.18 ± 0.27 [b]	4.72 ± 0.13	68.51 ± 0.83 [b]
	WRC	35.22 ± 0.45 [a]	8.40 ± 0.17	4.66 ± 0.13 [a]	19.2 ± 0.41	3.68 ± 0.10	70.98 ± 0.88 [a]
2017	WRO	31.97 ± 0.13 [b]	6.68 ± 0.30	3.74 ± 0.23 [b]	16.66 ± 0.38	3.47 ± 0.16	62.52 ± 0.86 [b]
	CHC	37.47 ± 0.18 [a]	5.82 ± 0.15 [a]	5.53 ± 0.21	22.47 ± 0.29 [a]	4.72 ± 0.07 [a]	76.02 ± 0.32 [a]
	CHO	33.43 ± 0.43 [b]	4.88 ± 0.16 [b]	5.18 ± 0.16	20.25 ± 0.45 [b]	4.38 ± 0.11 [b]	68.11 ± 0.70 [b]

Note: Values express the mean ± SD; WRC—Welschriesling control variant; WRO—Welschriesling variant with targeted must oxygenation; CHC—Chardonnay control variant; CHO—Chardonnay variant with targeted must oxygenation; [a,b]—mean values of individual parameters in columns with different superscript have a statistically significant difference ($p < 0.05$) between the variants within one variety and one vintage.

We found a statistically significant decrease in the content of caftaric, caffeic, coumaric, and ferulic acids in the samples produced by targeted oxygenation of the must in all experimental years in the Chardonnay variety. The Welschriesling variety showed a significant decrease of total caffeic acid and total coumaric acid in all experimental years, and a decrease in caftaric and fertaric acid was observed in vintages 2015 and 2016. In the Welschriesling control variant (WRC), produced by the reductive method in vintage 2015, the sum of the hydroxycinnamic acids was 76.16 mg L^{-1}, and in the variant with targeted oxygenation (WRO) it was 62.73 mg L^{-1}. This represents a decrease of 17.63%, which was the highest decrease observed during the experiment in the Welschriesling variety.

The chardonnay variety showed the same decreasing trend for individual the hydroxycinnamic acids; however, in the case of fertaric acid, we did not observe a statistically significant difference in the experimental years 2015 and 2016. In the sum of the hydroxycinnamic acids, the highest difference (20.10%) was observed in 2015. The main contribution in this decrease was caused by the reduced content of total caffeic and total coumaric acids. Total caffeic acid, which represents the most important hydroxycinnamic acids in wine, was found in the Chardonnay variety from 2017 at a concentration level of 37.47 mg L^{-1} in the control variant CHC, and 33.43 mg L^{-1} in the targeted oxygenated variant CHO (Table 2).

Stefenon et al. [34] studied the effect of wine aging in the barrel on its lees from 60 to 360 days on the content of hydroxycinnamic acids. The acids were found in the range from 23.40 to 30.33 mg L^{-1}, while the length of the wine aging in the barrel on its lees did not affect these phenolic compounds' content.

The caftaric acid was identified as the most abundant hydroxycinnamic acid, representing up to 50% of these acids [35]. Our results, with the caftaric acid content ranging from 7.58 to 21.54 mg L^{-1}, are in agreement with the abovementioned findings.

Singleton and Trousdale [36] reported the following values of caftaric acid in the Chardonnay variety: 191 mg L^{-1} in the must immediately after pressing, 86 mg L^{-1} in the free run, and 73 mg L^{-1} in the must after oxygenation (12 h of maceration of mashed grapes). Gómez-Míguez et al. [37] determined 13.43 mg L^{-1} of caftaric acid in the free run of the must and 25.29 mg L^{-1} in the pressed fraction.

The year 2016 was significantly different in climatic conditions than the years 2015 and 2017: 2016 was a wet year, characterized by above-average precipitations; however, the years 2015 and 2017 were considered dry [38]. Above-average total precipitation, a lower number of sunny days, and a cold autumn caused lower ripeness of the grapes and lower concentrations of constituents in the musts, including hydroxycinnamic acids. The

assumption that a higher than average volume of water being taken up by the plant during the vegetation season leads to a lower concentration of many constituents than those under standard conditions was confirmed by Ailer et al. [39]. Significant differences in the sum of hydroxycinnamic acids were observed.

The highest differences were found especially in the case of caftaric acid. In the 2017 vintage, the caftaric acid was present in 17.7 mg L^{-1} in the control variant, but in 2016 its content was only 9.28 mg L^{-1}.

Statistical evaluation of the sums of hydroxycinnamic acids and volatile phenols was performed as the parameters (hydroxycinnamic acids and volatile phenolics as the sums) have a complex adverse effect on wine quality. Significant differences were found between the control and oxygenated variants within individual vintages (Table 2). In 2015, the *p* values were 0.0077 and 4.2×10^{-5} for WRC vs. WRO and CHC vs. CHO, respectively. In 2016, the *p* values were 0.0009 and 0.00063 for WRC vs. WRO and CHC vs. CHO, respectively, and in the last experimental year were 0.0001 for both the Welschriesling and Chardonnay variants.

3.2. Sensory Evaluation of Wine Quality from Individual Variants

In the sensory evaluation of the mature wine, without the influence of the variant, some results were in favour of the targeted oxygenation variant and some were neutral. This finding—that targeted oxygenation of the must does not have adverse effect on the wine sensory parameters—is considered a favourable result. Results from the sensory evaluation of the wines produced by reducing the technology or targeted must oxygenation are published in a previous study by Pokrývková et al. [29].

In summary, we observed the following differences: wines produced by the reductive method showed broad primary aromas of blossoming meadow, tropical fruit, linden blossom, and aromas of vanilla and green apple. The grassy, green, phenolic tones, which are a risk for the further maturation process of the wine, were disturbing. In the variant produced by the oxygenation method, the wines did not show grassy but spicy tones, which the commission assessed positively. Furthermore, the commission identified minerality (quartz), ripe apple, and varietal manifestation of ripe grapes as important descriptors.

The results of the sensory evaluation with use of the 100-point test are shown in Figure 2.

Unlike in red wines, the technology of white wine production has to take into account not only the positive antioxidant properties of the phenolic substances, but also their adverse effect on the oxidative and aging processes. It is necessary to naturally eliminate phenolic substances as the increased antioxidant content and high antioxidant activity do not affect the sensory profile of wine positively [40].

3.3. Influence of Targeted Must Oxygenation Technology on the Content of Volatile Phenolic Substances in Wine

In both varieties and variants with targeted oxygenation, a statistically significant reduction in the content of undesirable 4-vinylguaiacol and 4-vinylphenol was achieved. The results are shown in Table 3. These substances are found in young wine in very low concentrations, usually in µg L^{-1}. The olfactory thresholds of the volatiles are very low. Therefore, the results obtained are considered to be very positive, in favour of the sensory attributes of wines produced by the targeted oxygenation of must. Other undesirable volatile phenolic substances that may be present in the wine are 4-ethylphenol and 4-ethylguaiacol. Their content was in all variants at a non-detectable level. For this reason, we did not include them in the evaluation.
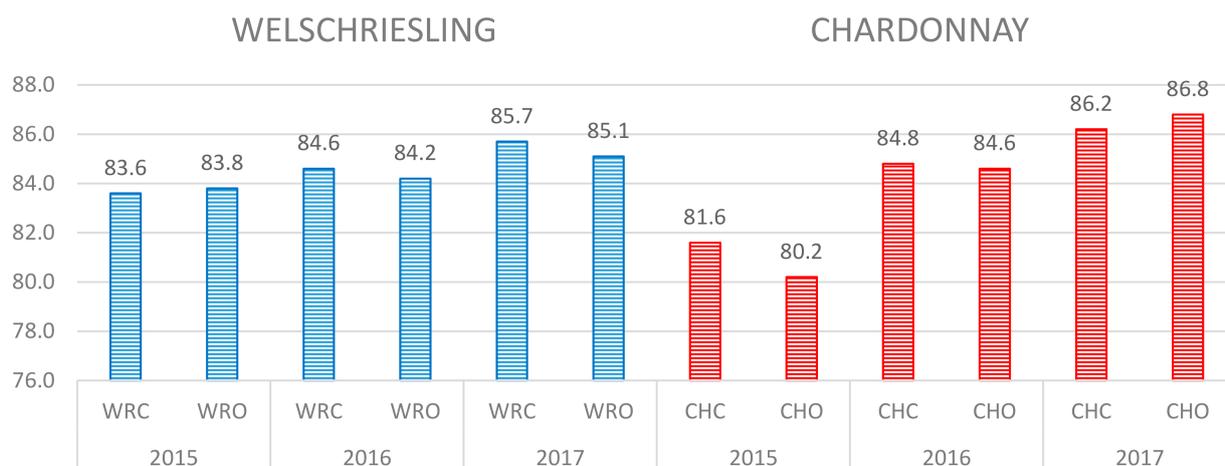
Figure 2. Results of sensory evaluation of individual variants of the Welschriesling and Chardonnay varieties based on the 100-point system according to STN EN ISO 8586, vintages 2015–2017. Note: WRC—Welschriesling control variant; WRO—Welschriesling variant with targeted must oxygenation; CHC—Chardonnay control variant; CHO—Chardonnay variant with targeted must oxygenation.

Table 3. The 4-vinylguaiacol, 4-vinylphenol, and sum of the volatile phenolics content in the experimental variants in vintages 2015–2017.

| Vintage	Variant	4-vinylguaiacol $\pm$ SD	4-vinylphenol $\pm$ SD	Sum of VPs $\pm$ SD
		$\mu g\ L^{-1}$	$\mu g\ L^{-1}$	$\mu g\ L^{-1}$
2015	WRC	67.00 $\pm$ 4.55 [a]	286.00 $\pm$ 9.42 [a]	353.00 $\pm$ 13.95 [a]
	WRO	28.67 $\pm$ 3.40 [b]	177.00 $\pm$ 6.98 [b]	205.67 $\pm$ 9.53 [b]
	CHC	334.67 $\pm$ 15.15 [a]	301.00 $\pm$ 10.42 [a]	635.67 $\pm$ 16.21 [a]
	CHO	210.00 $\pm$ 9.09 [b]	213.33 $\pm$ 8.81 [b]	423.33 $\pm$ 17.78 [b]
2016	WRC	26.00 $\pm$ 0.82 [a]	85.00 $\pm$ 6.16 [a]	111.00 $\pm$ 6.98 [a]
	WRO	18.00 $\pm$ 0.82 [b]	58.33 $\pm$ 2.05 [b]	76.33 $\pm$ 2.87 [b]
	CHC	181.33 $\pm$ 4.50 [a]	176.00 $\pm$ 5.10 [a]	357.33 $\pm$ 8.81 [a]
	CHO	164.67 $\pm$ 6.80 [b]	157.67 $\pm$ 5.19 [b]	322.33 $\pm$ 4.92 [b]
2017	WRC	59.00 $\pm$ 2.16 [a]	253.67 $\pm$ 9.10 [a]	312.67 $\pm$ 8.26 [a]
	WRO	40.67 $\pm$ 1.25 [b]	192.00 $\pm$ 6.48 [b]	232.67 $\pm$ 7.32 [b]
	CHC	268.67 $\pm$ 8.81 [a]	276.00 $\pm$ 7.48 [a]	544.67 $\pm$ 15.84 [a]
	CHO	241.67 $\pm$ 5.73 [b]	256.00 $\pm$ 6.53 [b]	497.67 $\pm$ 6.60 [b]

Note: Values express the mean $\pm$ SD; WRC—Welschriesling control variant; WRO—Welschriesling variant with targeted must oxygenation; CHC—Chardonnay control variant; CHO—Chardonnay variant with targeted must oxygenation; VPs—volatile phenolics; [a,b]—mean values of individual parameters in columns with different superscript have a statistically significant difference ($p < 0.05$) between the variants within one variety and one vintage.

We compared the sums of the volatile phenolics in individual varieties in individual vintages in order to find out if the oxygenation contributed to the decrease in volatiles. Significant differences were found in all variants and vintages. A decline in the sum of the volatile phenolics ranged from 9.8 to 41.7%. In vintage 2015, we observed the highest decline in content of both volatile phenolics. 4-vinylguaiacol decreased in Welschriesling by 57.2% and 4-vinylphenol by 38.1% between the experimental variants. In Chardonnay, the decrease in phenolics ranged from 9.2 to 37.3% for 4-vinylguaiacol and from 7.2 to 29.1% for 4-vinylphenol.

The findings are in agreement with those of Zhou et al. [41], in that the yeast *S. cerevisiae* produces vinylphenols in alcoholic fermentation, but not ethylphenols. Ethylphenols are formed from hydroxycinnamic acids by the action of the microflora of *Brettanomyces/Dekkera* spp., *Pichia* spp., *Torulaspora* spp., and *Zygosaccharomyces* spp., i.e., during long-term maturation and secondary contamination, especially in the case of red wines.

Microorganisms can metabolize the hydroxycinnamic acids in wine to sensory undesirable volatile phenolic substances. Our results have confirmed that by the natural reduction of the hydroxycinnamic acid content in winemaking, it is possible to eliminate oxidative processes during wine maturation and the formation of sensory undesirable volatile phenolic substances. In the variants with targeted oxygenation of the must, the statistically significant lower content of the sum of the hydroxycinnamic acids is of interest, and a significantly lower content of volatile phenolic substances than the control variants were additionally confirmed.

The correlation between the contents of the hydroxycinnamic acids and 4-vinylguaiacol ($\mu g \, L^{-1}$) with 4-vinylphenol ($\mu g \, L^{-1}$) in the targeted oxygenated group of the Chardonnay variety was determined through regression and correlation analyses. To describe the dependence between the content of the hydroxycinnamic acids and 4-vinylguaiacol ($\mu g \, L^{-1}$) with 4-vinylphenol ($\mu g \, L^{-1}$) in the targeted oxygenation variant of the Chardonnay variety, the power regression model ($R^2 = 0.748$) was chosen. The equation of the model shows that a 1% increase in hydroxycinnamic acids can result in an average increase in the content of volatile substances by 3.6%. In the control group, the power regression model ($R^2 = 0.9346$) to describe the dependence was chosen. The regression equations are shown in Figure 3. If we compare a targeted oxygenated group with a control group, it shows a significantly higher content of hydroxycinnamic acids in the control variants compared to the targeted oxygenated variants.
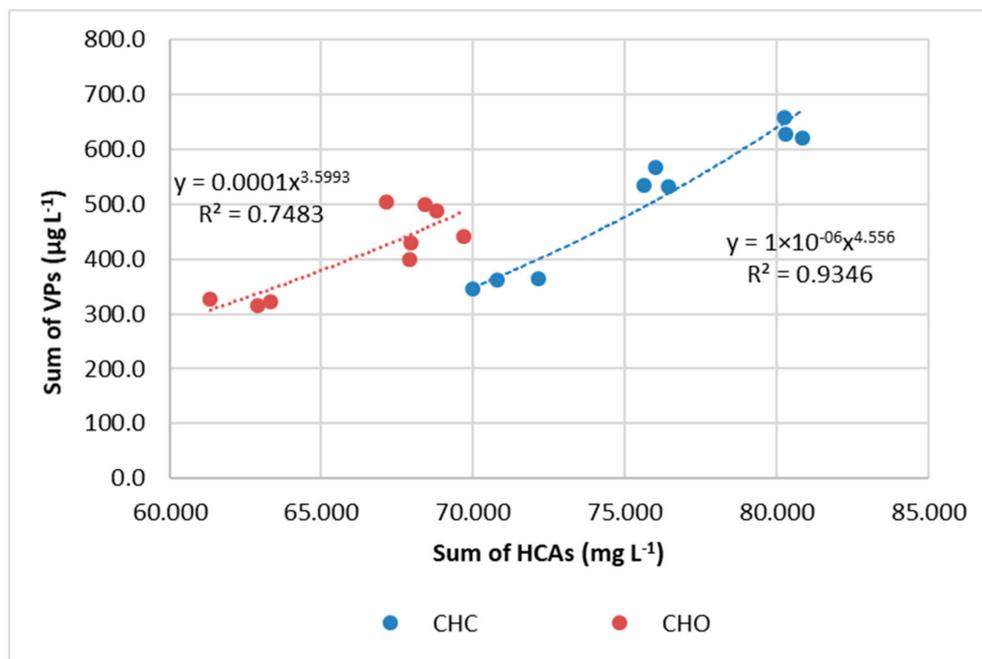
Figure 3. Correlation between the content of the volatile phenolics (VPs) in the Chardonnay experimental variants and the sum of the hydroxycinnamic acids (HCAs), vintages 2015–2017.

To describe the dependence between the content of hydroxycinnamic acids and 4-vinylguaiacol ($\mu g \, L^{-1}$) and 4-vinylphenol ($\mu g \, L^{-1}$) in the targeted oxygenated group of the Welschriesling variety, the power regression model was selected. The correlation is characterized by the coefficient of determination $R^2 = 0.5384$ (Figure 4). From the model, it can be expected that an increase in hydroxycinnamic acids by 1% can result in an increase of approximately 8.28% in the volatiles. The correlation in the control group is described by a third-degree polynomial function ($R^2 = 0.5718$), shown in Figure 4. If the variant of targeted oxygenation is compared to the control, a significantly higher level of hydroxycinnamic acids was confirmed in the control variant than in the targeted oxygenation variant.
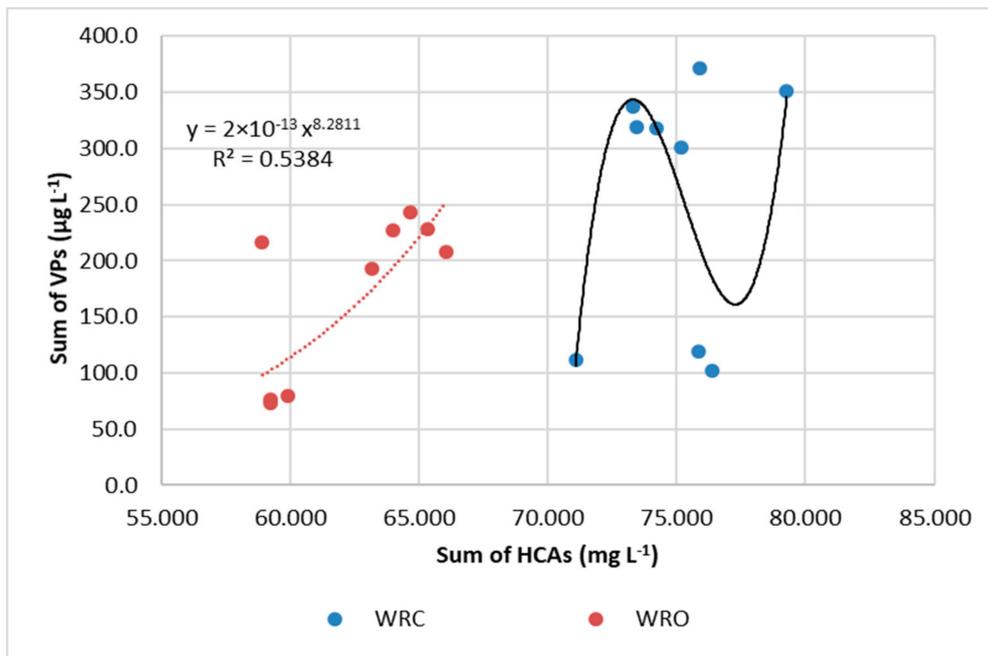
Figure 4. Correlation between the content of the volatile phenolics (VPs) in the Welschriesling experimental variants and the sum of the hydroxycinnamic acids (HCAs), vintages 2015–2017.

The assessment of the impact of the vintage, and the identification of the significance of the differences in hydroxycinnamic acids content between the two experimental varieties and their variants, were evaluated. To determine the influence of the vintage (2015, 2016, and 2017) and variants (WRC, WRO, CHC, and CHO) on the content of the hydroxycinnamic acids in wine, a two-factor analysis of variance (ANOVA) was applied. The analysis was performed by the Statgraphics statistical software. The significance of the differences was tested at the α = 0.05 significance level. The least significant difference (LSD) test for multiple comparisons was used to determine the significant differences between the factor levels. Comparing the content of the five monitored hydroxycinnamic acids in the Welschriesling in three experimental vintages showed no significant difference (p = 0.1676). However, significant differences were identified between the variants WRO and WRC (p = 2.1 × 10^{-8}. In Figure 5, the significant differences are shown.
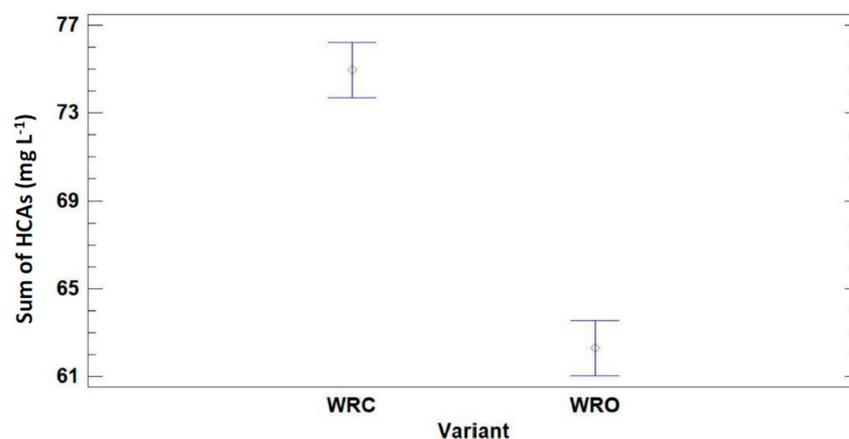
Figure 5. Significant differences between the Welschriesling variants and the sum of the hydroxycinnamic acids (HCAs).
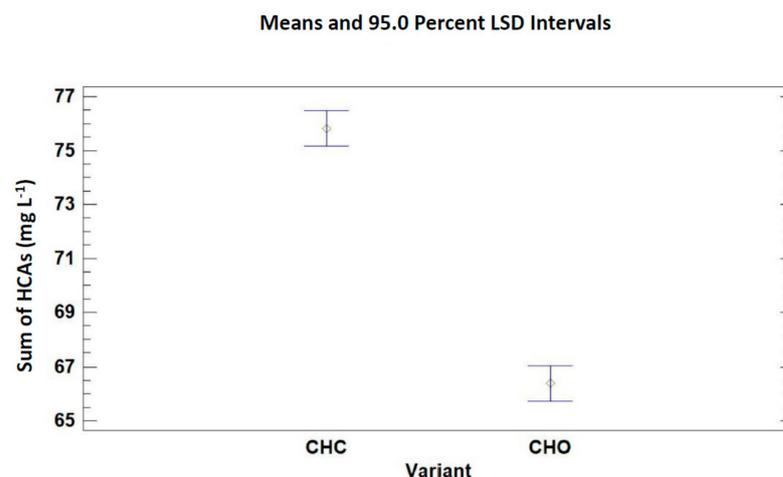
The statistically significant difference between the vintages in the content of the sum of the hydroxycinnamic acids in the Chardonnay variety was confirmed (p = 0.0000). In Figure 6 the results of the LSD test are present. The highest mean value of the sum of the hydroxycinnamic acids was measured in 2015, and the lowest in 2016.

Means and 95.0 Percent LSD Intervals
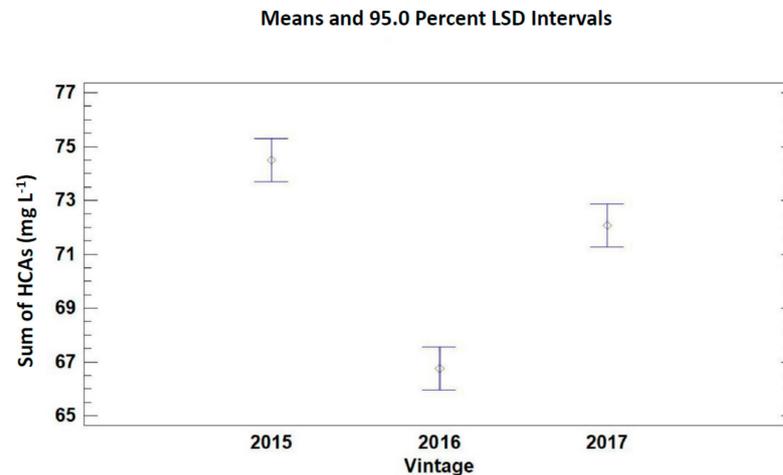
Figure 6. Significant differences in the Chardonnay variety in the vintages 2015–2017 based on the sum of the hydroxycinnamic acids (HCAs).

The year 2016 was characterized as wet, whereas the years 2015 and 2017 were dry [39]. Above-average total precipitation, a lower number of sunny days, and a cold autumn caused lower ripeness of the grapes and lower concentrations of constituents in the musts, including secondary metabolites such as hydroxycinnamic acids. The assumption that in the case of a higher water uptake by the plant during vegetation the concentration of many constituents is lower compared to the standard conditions was confirmed [39].
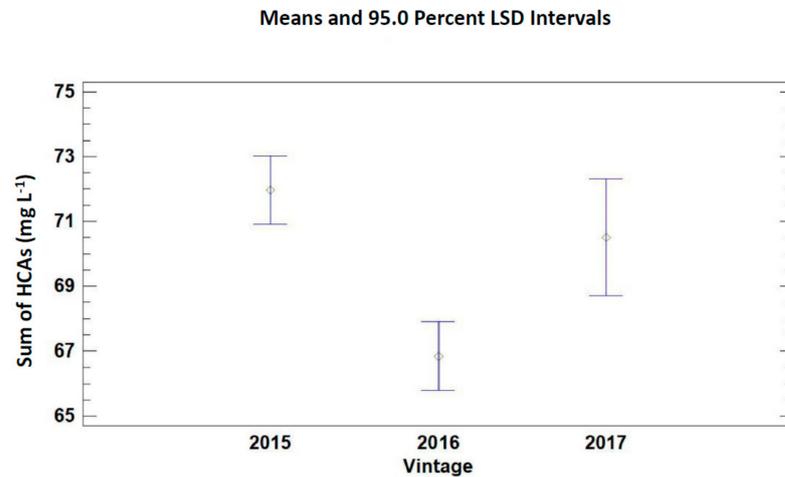
Significant differences in the sum of the hydroxycinnamic acids were identified between the Chardonnay variants (CHO and CHC, p = 4.74 × 10^{-5}) and visualized in Figure 7.

Means and 95.0 Percent LSD Intervals

Figure 7. Significant differences between the Chardonnay variants in the sum of the hydroxycinnamic acids (HCAs).

The statistically significant differences between the vintages and varieties, and between the variants within the variety, were studied. The statistically significant differences in the sum of the hydroxycinnamic acids in the wine of different vintages were confirmed (p = 0.0000). Based on this result, we specified the differences with the use of the LSD test.

Significant differences in the average values of the sum of hydroxycinnamic acids were found in 2016. The mean concentration of the sum of these hydroxycinnamic acids was the lowest, specifically 66.854 mg L^{-1} (Figure 8).

Figure 8. Significant differences between the sum of the hydroxycinnamic acids (HCAs) in wine and vintages in all variants and varieties.

A highly significant difference in the sum of the five hydroxycinnamic acids between the varieties and then between the variants within them was also confirmed ($p = 8.548 \times 10^{-11}$). Using the LSD test, significant differences were identified between the control and oxygenated variants (Figure 9). There are also significant differences between the varieties and within variants with targeted oxygenation WRO and CHO.
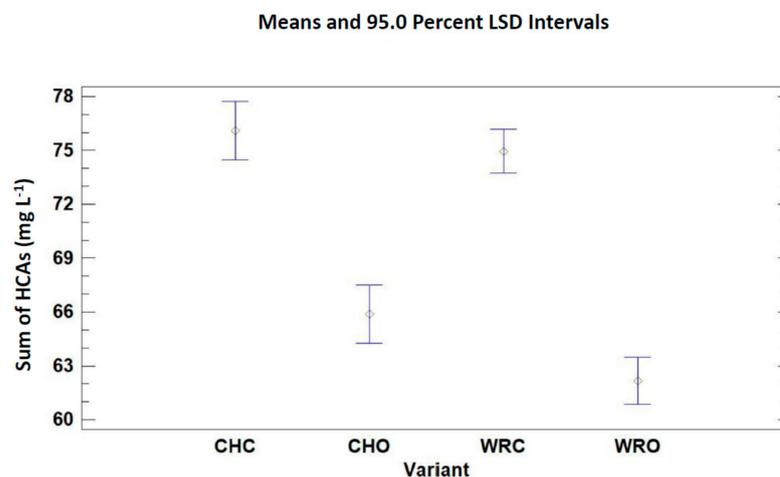
Figure 9. Significant differences between the sum of the hydroxycinnamic acids (HCAs) in wine in wine and the varieties and all vintages.

Viticulture and winemaking are a traditional part of the agri-food sector of the Slovak Republic. The marketing and promotion of Slovak wines are rarely based on the unique location of Slovakia. The conditions of the forty-seventh and forty-eighth latitudes are interesting. Slovakia is located on the northern border of economically efficient vineyard cultivation in Europe. Despite the climate risks, this region gives originality to the wines produced and can potentially be successful commercially. Slovak wines have a great chance of being unique, fresh, lively, aromatic, and expressive. Slovakia has not been self-sufficient in wine production for two decades, which is confirmed by the report of Meravá [42] summarized in Table 4. Therefore, the aim is not to produce large quantities

of wine. The winemaker's personality can play an unexpected role and can be the most valuable variable in the process of wine production. From an economic point of view, wine production presents opportunities and challenges in the contribution of wine routes to wine tourism [43]. Wine is considered an alternative investment [44] or an asset with value, for example to the bank as a loan guarantee [45]. Science also brings new knowledge to grape processing, resulting in a practice that is friendly to the product itself, the environment, the consumer, and the economy of the enterprise.

Table 4. Balance of resources and domestic wine consumption in the Slovak Republic (1000 hL).

Indicator	2014/15	2015/16	2016/17	2017/18
Stocks to 1.8.	394	457	390	454
Imports of wine and musts	1037	740	709	853
Production of wine from domestic raw materials	294	376	310	298
Total resources	1725	1573	1409	1605
Domestic wine consumption	1010	802	719	671
Domestic wine consumption cover of wine production from domestic raw materials in %	29	47	43	44

The economic comparison of the two variants included pressed must without removal of the must solids. For both variants, the costs of production inputs (electricity, water, must solids removal, and overhead costs) are taken into account in units of EUR 0.50 per 100 L.

In Variant A, the costs are counted using bentonite in the amount 100g per 100 L. The total costs for must-solids removal are € 2.20 per 100 L of must. In Variant B, the costs were extended (except for the use of an equivalent dose of bentonite) by potassium pyrosulphite at a dose of 7 g per 100 L of must. The total cost is € 2.21 per 100 L of must. However, the reduction did not eliminate the content of undesirable hydroxycinnamic acids by atmospheric oxygen.

Variant C, which present a hypothetic simulation of costs, counts with the extension of costs (excluding bentonite) by application of potassium pyrosulphite at a dose of 7 g per 100 L of must, and a reduction in the hydroxycinnamic acids with use of polyvinylpolypyrrolidone (PVPP) at a dose of 50 g per 100 L of must. The total cost is € 4.61 per 100 litres of must. By comparing Variants A and C, we found a cost-saving for removal of solids in the must in the amount of € 2.41 per 100 L of must. An overall comparison of the variants is visualized in Figure 10.
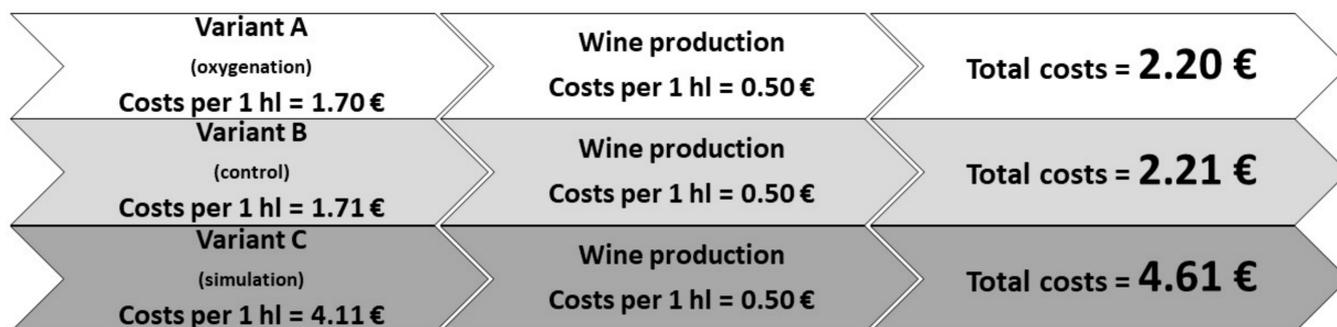
Figure 10. Wine production costs per 100 L for two variants (A and B) and a simulation (in the case of addition of PVPP agent—C variant). Note: Variant A—oxygenation during the removal of must solids with the use of atmospheric oxygen for the elimination of hydroxycinnamic acids content; Variant B—reductive removal of must solids with use of sulphur dioxide without elimination of hydroxycinnamic acids; Variant C—simulation based on removal of must solids with use of sulphur dioxide with the elimination of hydroxycinnamic acids with use of polyvinylpolypyrrolidone PVPP.

The oxidation method makes this process gentler, more natural, and ultimately more economical. Comparison of Variants A and B brought fewer savings, but when the solids

are removed, e.g., for 1 mil. litres of must, there is a difference in cost of € 10,000. The labour costs have not been included in the individual formulas, as these vary from country to country and across business entities. We do not mention here the brands of processing aids and additives as this research does not have commercial support.

In practice, it is necessary to find a procedure that allows systematic examination of the production costs, determining those that are functionally highly expensive, and finding cheaper alternatives that demonstrably meet customer requirements in terms of quality [46]. The example given by Pollak [46] showed that a 4% saving in costs can result in the profit increase of an enterprise by 33%.

4. Conclusions

The technology of the targeted oxidation of must to reduce the content of undesirable phenolic substances is an alternative that wine producers can use without risk of reducing the quality of their products. Natural oxygenation with atmospheric oxygen is a simple, gentle, and significantly more cost-effective solution than technological adsorbents. Application of the PVPP-based adsorbent for removing undesirable phenolic substances represents, at a dosage of 100 g hL^{-1}, an economic cost of up to € 30–50 per hL of wine sulfurization; the cost, and one additional manipulation, is not insignificant. The method of targeted oxygenation must be recommended for practice, especially in the case of neutral grape varieties in the production of white and rosé wines. If the targeted oxygenation is carried out simultaneously with the pre-fermentation maceration of mashed grapes, the wines will have a varietal character, be richer in aromatic substances, be more powerful, and the need for their sulfurization is reduced. In order to keep the SO$_2$ content low for a long time in the case of targeted oxidation, it is necessary to store the wine at temperatures up to 15 °C and in a protective gas atmosphere, which we recommend when using this technology in practice.

Alcoholic fermentation is healthier and smoother in an oxygenated must. Targeted oxygenation can also be a suitable technology to avoid the unwanted uniformity of the wine, which often arises from strictly reductive technology. By reducing the content of hydroxycinnamic acids, it is possible to reduce the formation of volatile phenolic substances, which negatively affect the sensory parameters of the wine, maturation processes, and archiving potential.

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