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Chemometric Approach Application in Modern Wine Studies

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Abstract: The wine market is affected by the origin of wines, but the current wine traceability system has some limitations. The idea of geographical authenticity and quality has increased as one of the most important parameters influencing consumers' preferences. Chemical parameters such as total polyphenolic content (TPC), alcohol and organic acid content (total acids, malic and tartaric acids), and antioxidant activity were observed in this work at 15 dry white wines of varieties Pinot blanc, Riesling, Sauvignon blanc, and Chardonnay, which originated from three different countries. FTIR and spectrophotometry methods (TPC and DPPH) were used and chemometric approaches such as ANOVA and PCA were selected as the most important for evaluation. In general, high levels of malic acid, TPC, and antioxidant activity were detected in Austrian wines. Pinot blanc, Chardonnay, and Sauvignon from the same producing region in Austria showed higher results. The higher overall acid concentration was a distinguishing feature of the Slovakian Sauvignon and Riesling varieties, particularly in the case of tartaric acid. Austrian samples showed significantly higher antioxidant activity compared to Hungarian Chardonnay (p = 0.002) and Sauvignon (p = 0.007), comparable to TPC. The application of statistical analysis was useful in demonstrating many discrepancies, particularly by application of the PCA method.

Keywords: wine; quality; chemical composition; origin; PCA

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1. Introduction

Wine is one of the products resulting from the complete or partial fermentation of fresh grapes or must. One of the quality parameters for which wine is appreciated is its geographical or varietal authenticity. Some attributes are protected by the legislation that defines the conditions that wine must meet, particularly regarding its quality and composition, as well as the treatments used [1]. Wines are considered to be a complex matrix since they contain ethanol, organic acids (such as malic, tartaric, citric, acetic, or lactic acid), saccharides [2], and others. Inorganic elements were measured in different wines, white and red, from four distinct wine-producing regions in Portugal in a study by Rodrigues et al. [2]. They state that there is difficulty in obtaining certified reference materials for the analysis of inorganic elements in wine samples.

The wine sector belongs to the one of the most economically important sectors. The wine market is affected by the origin of wines, but the current wine traceability system has some limitations [3], although geographic traceability is crucial. The idea of geographical authenticity has increased as one of the most important parameters influencing consumers' preferences [4]. Geographical origin has been recognized as an essential element in wine identification for a long time [5]. Consumers, producers, retailers, and government agencies urgently require the introduction of a comprehensive technology for origin tracking [4,6,7]. All agricultural products have specific geographic origins and consumers, and producers and retailers differentiate products based on it. Recent food policy initiatives within the European Union offer formal mechanisms for such differentiation via certification schemes. A study by Tregear et al. [6] evaluated geographic indication in foods from a consumer perspective. The findings highlight some important issues regarding the characteristics and perceived authenticity of regional foods.

Wine analysis attracts a lot of attention due to its importance in quality, authenticity, and adulteration [8]. Nowadays, wine studies have also been undertaken in order to perform wine categorization. To date, methods such as elemental and isotopic analysis, GC and liquid chromatography, mass spectrometry (MS), HPLC, sensory evaluation, and other methods with money and time-consuming operations have been used to distinguish wines based on their various geographical origins [4,9,10]. In contrast, molecular spectroscopy technologies have a promising future in batch detection applications for the wine industry [5,11,12] because of their environmental qualities and potential time and money savings associated with analysis. Particularly, Fourier transform infrared spectroscopy (FT-IR) has been demonstrated to be a successful method for describing the main elements in complex mixture systems and differentiating chemical components in very similar samples [13–15]. Notably, Raman spectroscopy coupled with chemometric analysis provides positive results in the recognition of mono-vine wines in terms of grape (validation test provided reliability of 93%), geographical provenance (reliability higher than 90%), and aging time (reliability higher than 80%). One of the biggest advantages is the direct analysis of wine, using just the glass container, without any pretreatment or purification process. The chemometric identification of variability between the different classes meant wines could be differentiated by the grape, geographical origin, and aging time. The more specific and user-friendly the analysis is, the more likely it is to be exploited by wine producers for certification. The application of Raman spectroscopy to distinguish a single producer will be the next challenge, with a higher impact in the commercial field [12].

In this study, white wines with Protected Designation of Origin (PDO) were produced from different wine varieties with an emphasis on their different origin from Slovakia (two different wine-growing regions) compared to the wines that originated from Austria and Hungary that were used, primarily focusing on chemical parameters affecting their antioxidant effect. We suppose that within the same variety, their quality should be comparable, but other aspects, such as the origin of their production, which is significant from both a consumer and a research perspective, may also have an impact.

2. Materials and Methods

For this study, samples of four different varieties of white wines, such as Riesling (Blanc), Pinot blanc, Chardonnay, and Sauvignon from Slovakia (SVK), Hungary (HUN), and Austria (AUT) from the 2020 vintage were observed. They were chosen as quality wines with PDO (protected designation of origin). Wines were collected and used for determinations at the same time. Samples were analyzed directly after opening the bottle to minimize the effect of air and storing them open.

Particularly, wines from Slovakia were collected from two different wine-growing regions, the Southern Slovak wine-growing region (SVK-S) and the Central Slovak wine-growing region (SVK-C).

Table 1 provides a summary of the wine samples used.

Table 1. Overview of the analyzed wines.

Sample of Wine	Origin	Variety
1	SVK-S—Southern Slovak wine-growing region	SB
2	SVK-S—Southern Slovak wine-growing region	RR
3	SVK-S—Southern Slovak wine-growing region	PB
4	SVK-S—Southern Slovak wine-growing region	CH
5	SVK-C—Central Slovak wine-growing region	RR
6	SVK-C—Central Slovak wine-growing region	PB
7	SVK-C—Central Slovak wine-growing region	CH
8	HUN	SB
9	HUN	RR
10	HUN	PB
11	HUN	CH
12	AUT	SB
13	AUT	RR
14	AUT	PB
15	AUT	CH

Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc, CH—Chardonnay. In relation to wine origin: SVK—Slovakia, HUN—Hungary, AUT—Austria.

2.1. Wine Origin

2.1.1. Slovakia

Slovakia is divided into six wine-growing areas. In this study, the wines from two officially recognized wine-growing regions were used, such as the Southern and Central Slovak wine-growing regions.

The southern Slovakian wine-growing region (SVK-S) has a higher level of homogeneity, which has an impact on the quality of the wine. The average altitude of this region is 140 m. The soils have a deeper profile and range from light (sandy) to medium-heavy.

The central Slovakian wine-growing region (SVK-C) is divided into 7 districts. Its climate is characterized by moderate temperatures and relatively low annual precipitation. The average altitude is 180 m and the soil base is represented by sediments, clayey, and sandstone bases, with medium–heavy soils.

2.1.2. Austria

The climate is deeply continental, with cold, snowy winters, warm summers, and sunny autumns. Niederösterreich (27.074 ha) (Lower Austria) is situated to the northeast along the river Danube, surrounding Vienna. Burgenland (11.772 ha) is to the east and Steiermark (5.086 ha), or Styria, is found in the south. Vienna (575 ha) is the world's lone capital with a significant wine industry.

Samples used in this work originated from the Steiermark region, just a variety Riesling from the Niederösterreich region [16]. The highest wine-growing region in Austria is in the southern Steiermark. Steiermark is an area of 5054 hectares, located in the southeast of the country. The southern Illyrian climate brings warmth during the daytime, whereas the cold nights result from the cooling effect of the steep hillsides. Around two-thirds of the area under the vine in Steiermark is located in the "Bergwein" (mountain wine) zone. The terrain here is hilly, almost mountainous, and many of the vineyards are planted on steep slopes to take advantage of sunlight and the prevailing winds. The Niederösterreich region is presented on 27,160 hectares, divided into three climatic regions: the Weinviertel, which is situated in the north, the Danube region on the west of Vienna, and Niederösterreich in the southeast [17].

2.1.3. Hungary

Approximately 73,000 hectares of vineyards in Hungary are spread over almost the entire country. There are 22 wine regions in Hungary. Hungary's hills are rich in volcanic soils and limestone–idyllic soil types for fine winemaking. Two varieties used in this work, Savignon blanc and Riesling, originated from the Tolna region. The wine-growing area is located in the central-western part of Hungary, southwest of Lake Balaton. There are dry summers with plenty of sunshine and mild winters. The vineyards cover about 2200 hectares of vines [18].

Variety Pinot blanc (Fehér burgundi) originated from the Sopron region. It is located in the west of the country; it has an area of 1630 hectares. Its vineyards are quite stony. Eroded brown forest soils dominate, there are also heavy clay and loess. It is the region with a moderate continental climate, lower median temperatures, and higher rainfall with cooler wetter summers [19].

2.2. Methods

2.2.1. Chemicals and Laboratory Equipment

A 0.5 L reference solution (Bruker Optics, O.K. Servis BioPro, Prague, Czech Republic) was used to calibrate the ALPHA analyzer (Bruker Optik GMBH, Ettlinger, Germany). Gallic acid (99%; Alfa Aesar Thermo Fisher GmbH, Kandel, Germany), Folin–Ciocalteu reagent (p.a., Centralchem, Bratislava, Slovakia), and Na_2CO_3 (p.a. 99%; Centralchem, Bratislava, Slovakia) were used for the determination of total phenolic content (TPC) in glass cuvettes (type S/G/10; Exacta + Optech GmbH, Munich, Germany). The UV/VIS spectrophotometer operated at 765 nm and 515.6 nm (type T80 UV/VIS Spectrometer, PG Instruments Ltd., OK Service, Prague, Czech Republic) was employed for both TPC and antioxidant activity determinations, respectively. For the antioxidant activity assay, DPPH radical (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and ethanol (96%, Centralchem, Bratislava, Slovakia) were used, and measurement was performed with the same type of glass cuvettes (type S/G/10; Exacta + Optech GmbH, Munich, Germany).

2.2.2. FTIR Analyses

By the FTIR determination selected parameters in wines included total acids, tartaric, and malic acids, and, as a supporting chemical parameter (for the PCA analysis), alcohol content was used. The ALPHA Bruker Optik analyzer was used for the measurements according to Jakabová et al. [20]. The calibration conducted was based on reference solution ranges for the wine as follows: alcohol 0.12–20.48%; total acids 2.9–13.5 g L^{-1}; malic acid 0.1–4.7 g L^{-1}; and tartaric acid 0.5–5.4 g L^{-1}.

2.2.3. Method of Total Phenolic Content (TPC) Determination

TPC determination by the modified Folin–Ciocalteu method was used, following Lachman et al. [21] (2003). Gallic acid was used as a standard and diluted with demineralized water to prepare a stock solution (1 g L^{-1}). The calibration curve in the range of 50–500 mg L^{-1} was prepared with the stock solution of gallic acid and used for TPC measurements with expression of the results as a gallic acid equivalent (GAE). The wines were injected into volumetric flasks in an amount of 50 µL. Folin–Ciocalteu reagent was diluted 2:1 with demineralized water and injected into volumetric flasks in the amount of 2.5 mL and after 3 min of reaction, 5 mL of Na_2CO_3 (diluted with demineralized water to obtain 20% solution) was added. Flasks were shaken and filled up to 50 mL volume. They were kept at room temperature for 2 h, and measurements were performed in glass cuvettes using a UV/VIS spectrophotometer at 765 nm.

2.2.4. Method of Antioxidant Activity (DPPH Method) Determination

The antiradical activity was determined using Brand-Williams et al. [22] with a few modifications. The DPPH stock solution was prepared (0.025 g DPPH radical) in ethanol in the flask. The stock solution was diluted with ethanol directly before the measurement in the ratio 1:9 to obtain an initial absorbance of 0.750. The initial absorbance was measured in a glass cuvette at 515.6 nm. The wine was injected into the glass cuvette and the mixture was stirred. The cuvettes were left for reaction for 10 min in the dark and the final absorbance was recorded. Triplicate measurements for each product were performed. The DPPH radical's % inhibition was used to express antioxidant activity based on the calculation published by Jakabová et al. [20].

2.2.5. Statistical Evaluation

Differences among individual parameters/samples of wines regarding their origin were evaluated by the ANOVA (Kruskal–Wallis with Dunn pairwise test) and principal component analysis (PCA). All calculations were performed with the use of the program XL STAT v. 2021.4.1 [23,24].

3. Results and Discussion

The geographical origin of wines is one of the most important requirements for their authenticity [25].

The total acid concentrations in samples ranged from 4.8 to 8.9 g L^{-1} (Sauvignon blanc). Differences (Figure 1) between HUN and SVK-S for Pinot blanc were found to be statistically significant (p = 0.002). In the case of Riesling, statistically significant differences were found between SVK-C and HUN (p = 0.002) and in Chardonnay samples between HUN and SVK-C (p = 0.002) while in the case of Sauvignon, SVK-S results were shown to be significantly higher than HUN (p = 0.006). Malic and tartaric acids represent more than 90% of the overall concentration of organic acids in wine.

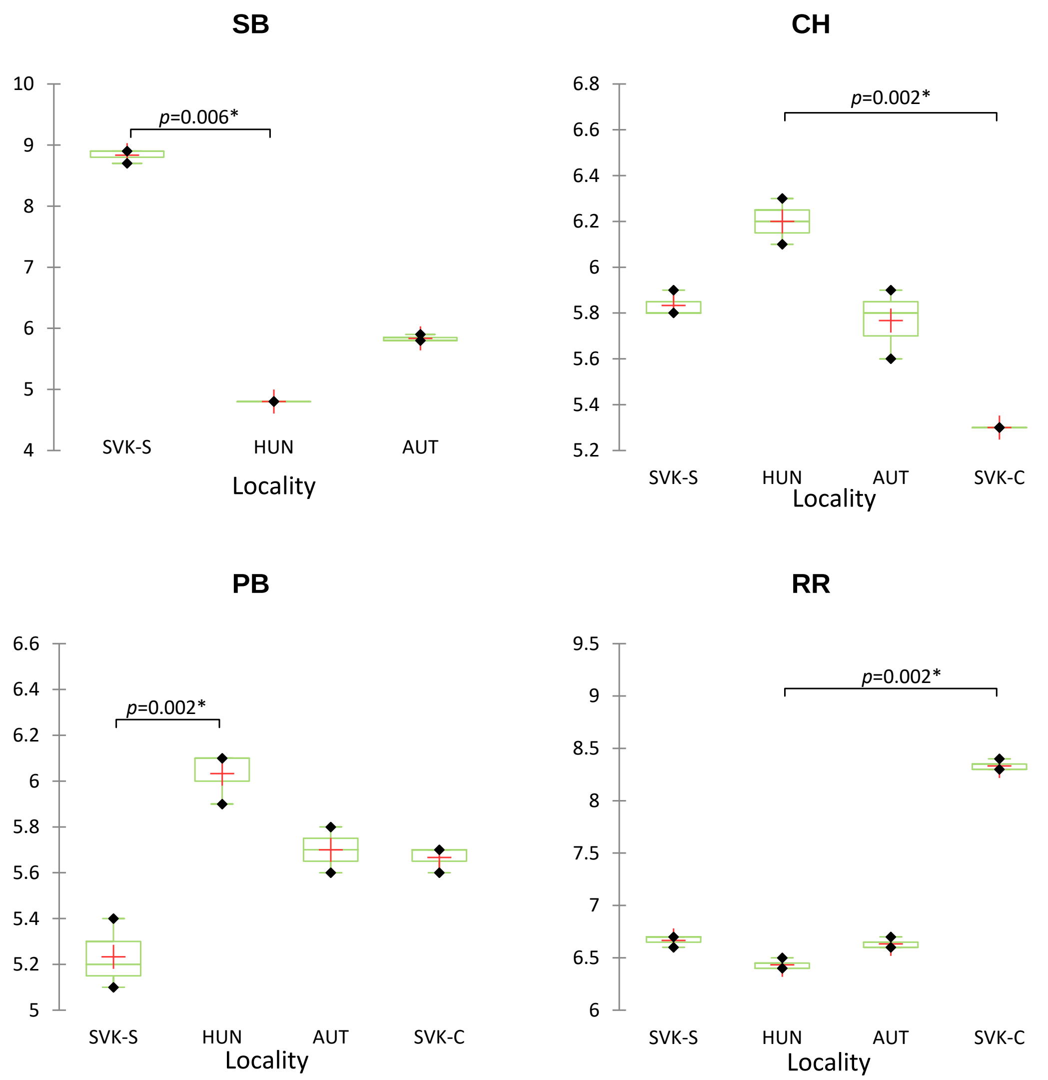

Figure 1. Statistical evaluation of total acid content (g L^{-1}) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the p value is statistically significant at the 0.05 level.

Total acid content includes organic acids in the grape or wine [26]. Tartaric and malic acids are two of the most significant wine descriptors; their presence in white wines was investigated.

The mean malic acid content ranged from 1.8 g L^{-1} (Riesling) to 3.9 g L^{-1} (Pinot blanc). Statistically significant differences (Figure 2) in malic acid content were observed in the case of the Sauvignon blanc variety between HUN and SVK-S (p = 0.008), similarly as in the case of total acids. In case of Chardonnay variety between SVK-S and HUN (p = 0.007) and between SVK-S and AUT (p = 0.014). At Pinot blanc between HUN and SVK-S (p = 0.002) the trend was similar to the total acids. In case of Riesling variety between AUT and SVK-C (p = 0.002) statistical significant differences were detected as well.

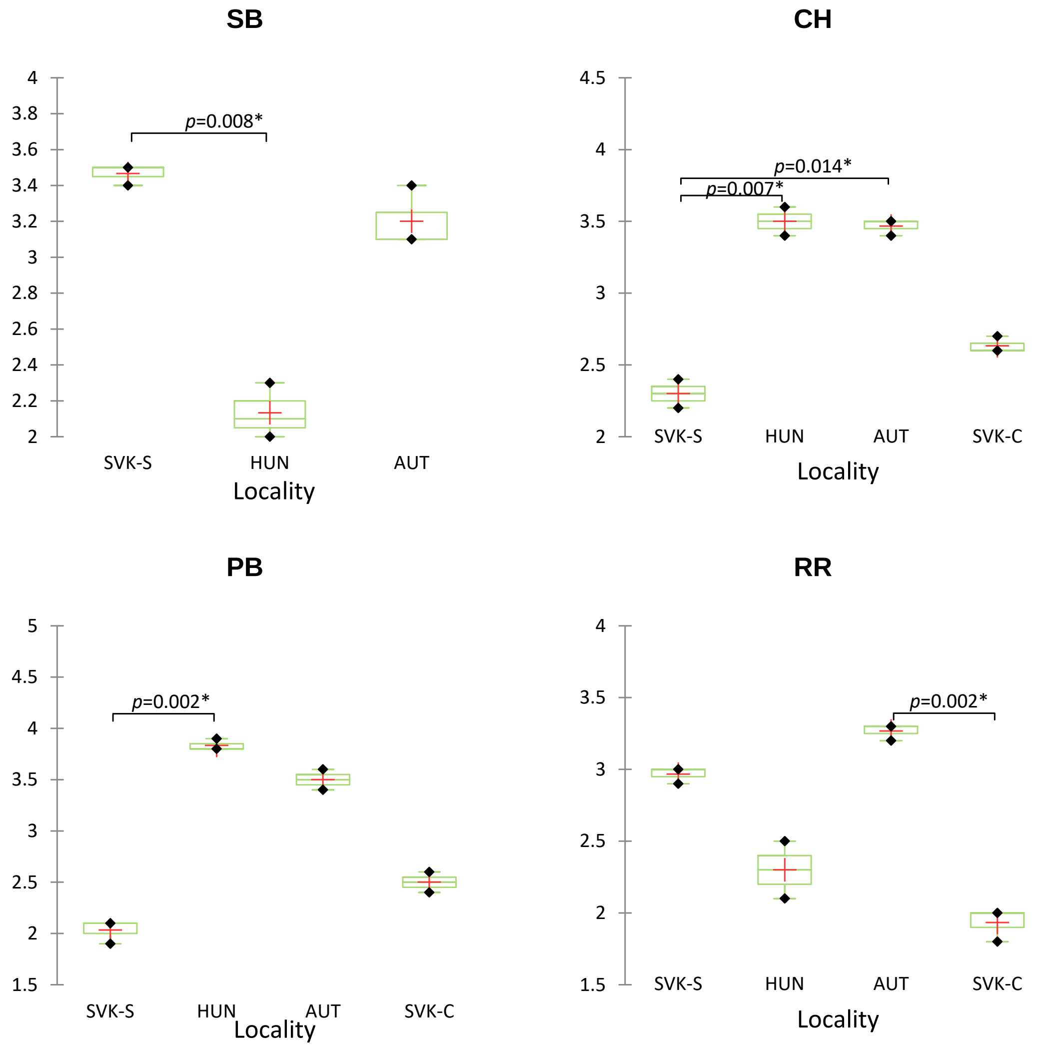

Figure 2. Statistical evaluation of malic acid concentration (g L^{-1}) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the p value is statistically significant at the 0.05 level.

About 50% of all the acids in wine are tartaric acids [27]. The mean content of tartaric acid in our wine samples ranged from 1.62 to 4.83 g L^{-1}. The lowest one was determined as Pinot blanc from Hungary, with the highest mean content in the sample of Riesling originating from SVK-C.

The statistical analysis atthe tartaric acid concentration of Sauvignon, showed significant differences (Figure 3) between SVK-S and HUN (p = 0.007), such similar trend as in the case of total acid content. In the case of Chardonnay between SVK-S and AUT (p = 0.002) and at

Pinot blanc variety between SVK-S and HUN (p = 0.024) and HUN and SVK-C (p = 0.005) statistically singificant changes were detected as well. Additionally, Riesling samples from AUT and SVK-C showed statistically significant differences (p = 0.002).

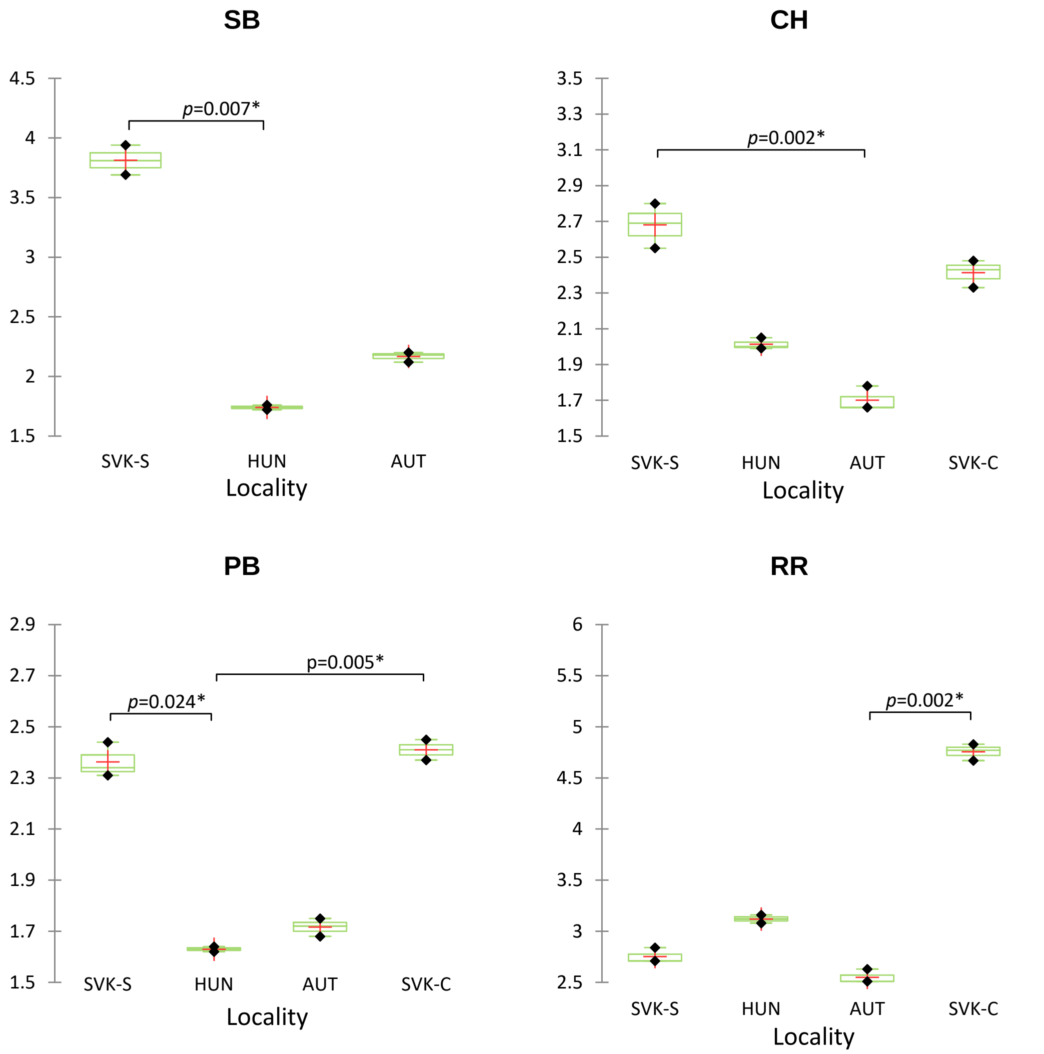

Figure 3. Statistical evaluation of tartaric acid concentration (g L^{-1}) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. t Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the p value is statistically significant at the 0.05 level.

The Kruskal–Wallis test combined with the Dunn pairwise test has revealed several differences among wine samples based on their geographical origin.

The mean alcohol content in our wine samples was determined to be the lowest in the sample of Sauvignon blanc from SVK-S (10.5%) and the highest (13.4%) in SVK-S wine of Pinot blanc. Statistical analysis (Figure 4) revealed significant differences in samples of Sauvignon blanc between SVK-S and HUN (p = 0.007), between HUN and SVK-S for Chardonnay (p = 0.002), between AUT and SVK-S for Pinot blanc (p = 0.005), and statistically significant differences between SVK-S and SVK-C for Riesling (p = 0.003).

Sauvignon from Austria showed the highest antioxidant activity (AA) among our wines (% inhibition DPPH), found to be 78.561%. The lowest results (30.8% DPPH inhibition) were detected in the sample of Pinot blanc from SVK-S. The southern Slovakian wine-growing region has a high level of homogeneity, which has an impact on the quality of the wine. The average altitude of this region is 140 m. The soils have a deeper profile and range from light (sandy) to medium–heavy.

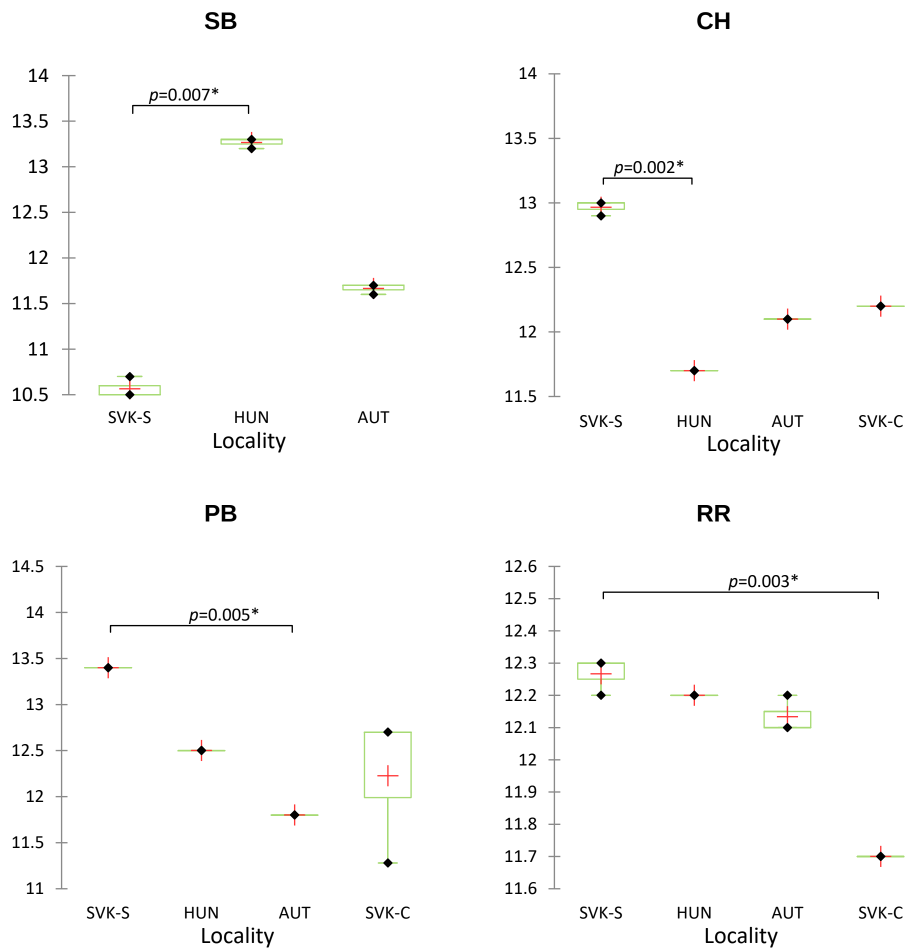

Figure 4. Statistical evaluation of alcohol content (%) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the *p* value is statistically significant at the 0.05 level.

Austrian samples of wines showed significantly higher (Figure 5) antioxidant activity compared to HUN at Chardonnay ($p = 0.002$) and Sauvignon varieties ($p = 0.007$, comparable with the case of TPC).

Statistical analysis showed significant differences in samples of Pinot blanc between two Slovakian regions, SVK-S and SVK-C ($p = 0.017$), HUN and SVK-C ($p = 0.007$), and between HUN and SVK-C for Riesling ($p = 0.002$). These results correspond with the results of Zanette et al. [28], who determined AA in the Cabernet Sauvignon variety (81.64%) and in dry white wine (37.47%) and also with the results of Li et al. [29] and Ma et al. [30]. TPC in wines ranged from 218.1 mg GAE L^{-1} to 455.3 mg GAE L^{-1}. The lowest TPC was recorded for the Pinot blanc variety from Slovakia-C and the highest in Riesling from Slovakia-S. Statistically significant differences (Figure 6) were observed at the Sauvignon variety between HUN and AUT ($p = 0.037$); in the case of Chardonnay, significant differences were found between AUT and SVK-C ($p = 0.002$), in the Pinot blanc variety, between SVK-C and AUT ($p = 0.002$), and significantly higher content of total polyphenols was found to be in SVK-S samples compared to SVK-C ($p = 0.002$). Ailer et al. [31] observed the effect of the ripening, location, and variety on analytical parameters and volatile phenolic compounds in white wines. Bajčan et al. [32] measured the TPC content in Riesling and Chardonnay wines using the Folin–Ciocalteu method, their values for TPC were on average 303.2 mg GAE L^{-1} for Riesling and 355.6 mg GAE L^{-1} for the Chardonnay variety.

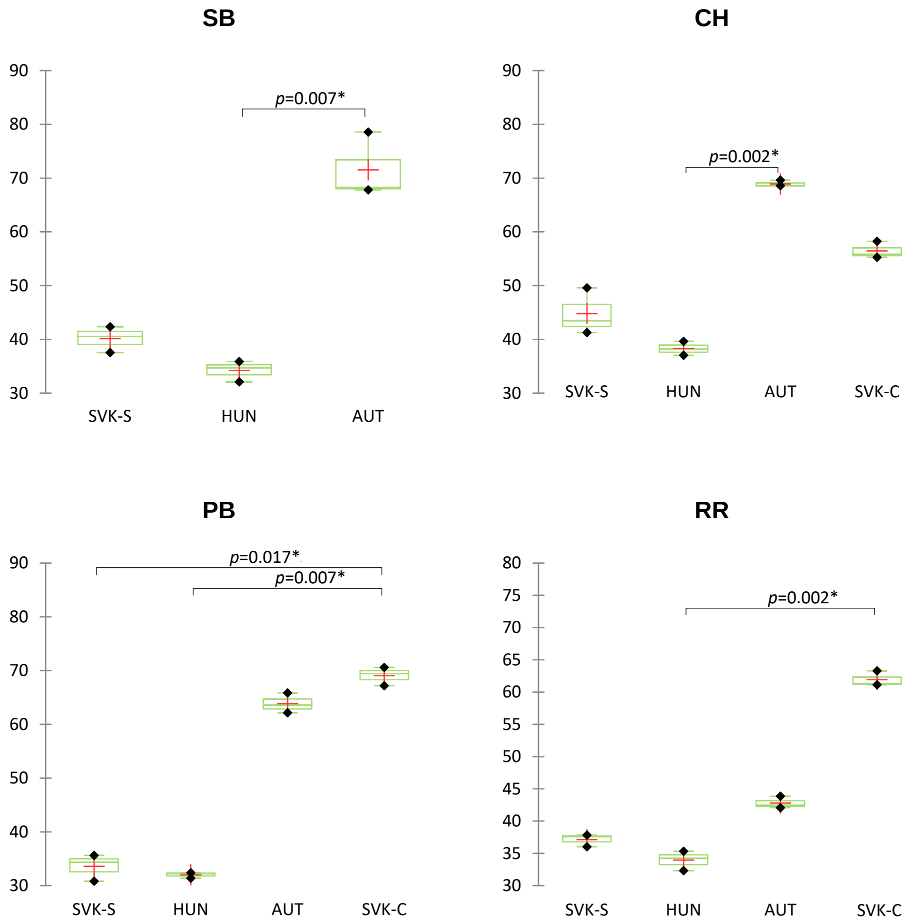

Figure 5. Statistical evaluation of antioxidant activity (% inhibition DPPH.) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the p value is statistically significant at the 0.05 level.

Jakabová et al. [20] examined TPC from 283 to 379 mg GAE L^{-1} at Pinot blanc, which corresponds with our results. These results are comparable to Brad et al. [33] and Ma et al. [30]; the content of TPC in wines Chardonnay ranged from 275 to 454 mg GAE L^{-1}.

There were wine varieties in this work, which originated from two officially different wine-growing regions of Slovakia, the South Slovak wine-growing region and the Central Slovak wine-growing region. It was Riesling, Pinot blanc, and Chardonnay that originated from these areas. A statistically significant difference in alcohol content (%) was found only in the case of the Pinot blanc sample ($p < 0.001$). Regarding the content of malic acid, TPC, AA, and the content of total acids, a statistically significant difference ($p < 0.001$) was recorded between all compared wines from the South Slovak wine-growing region and the Central Slovak wine-growing region. In the case of tartaric acid, a significant difference was found only between the Riesling samples ($p < 0.001$).

Feher et al. [34] efficaciously applied chemometric statistics for a description of white wines by their geographical origin, vintage, and variety. Principal component analysis in our case (Figures 7 and 8) was applied to describe the individual wines by their origin. The results of Bartlett's test indicate that the data were likely factorable (Chi-square (observed = 216.721), Chi-square (critical = 24.996), and $p \leq 0.001$. The Kaiser–Meyer–Olkin (KMO) test of sampling adequacy showed the middling suitability of the data for the complete model (KMO = 0.400).

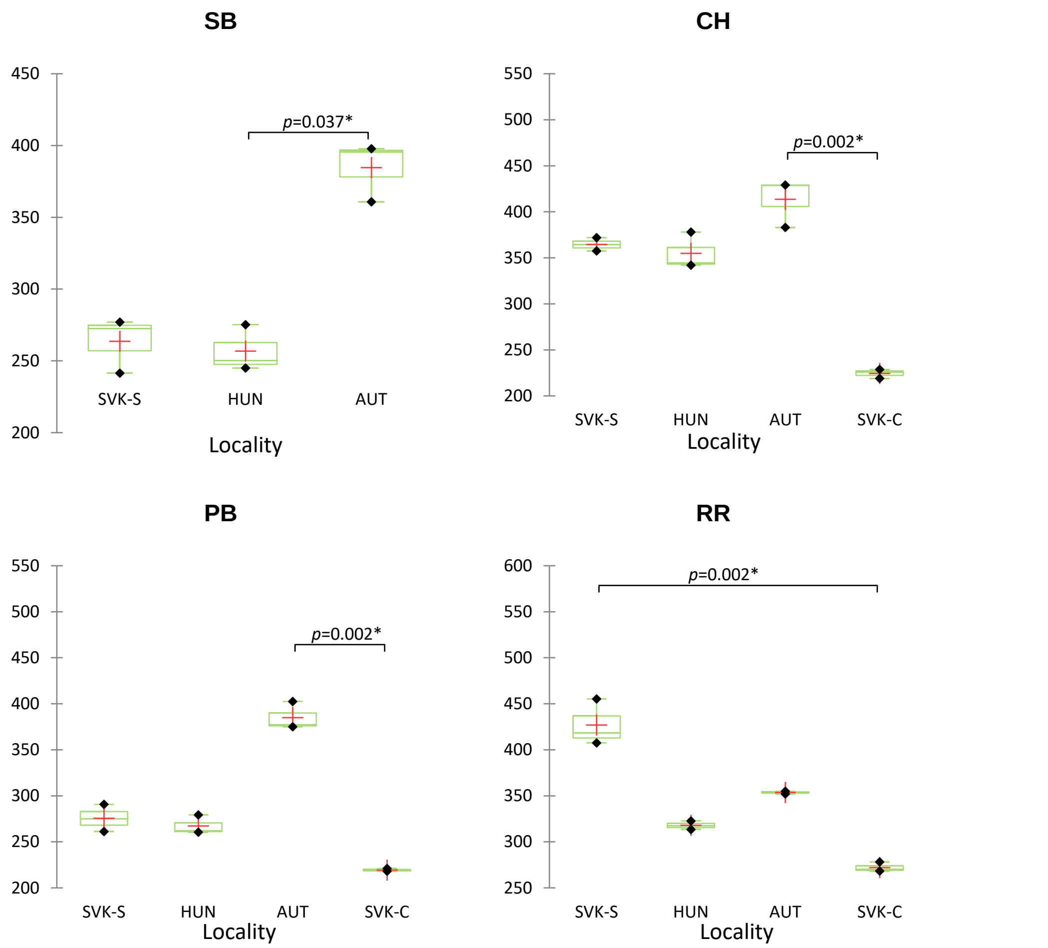

Figure 6. Statistical evaluation of TPC (mg GAE L^{-1}) in wines by their different origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc. Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria. * means that the *p* value is statistically significant at the 0.05 level.

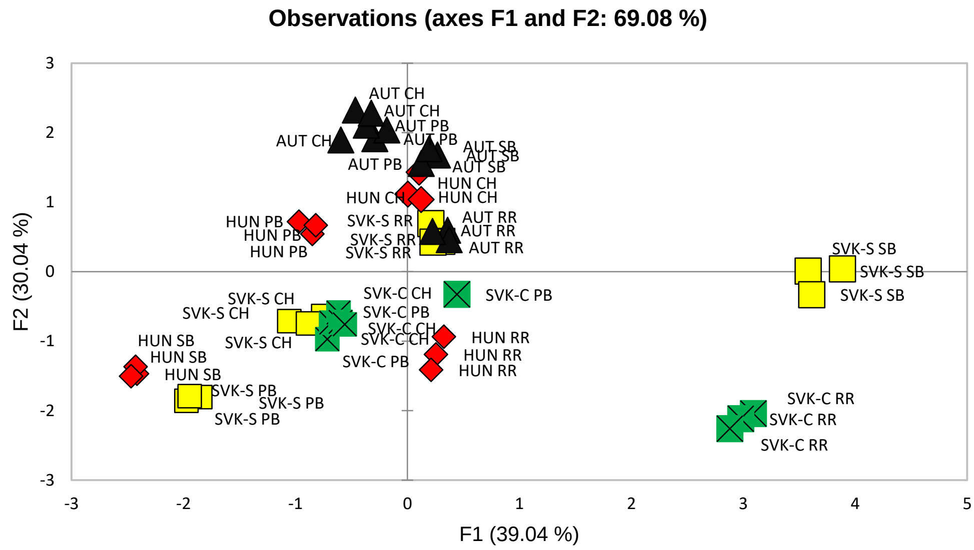

Figure 7. Application of PCA analysis to represent the different wine varietals according to their origin. Note: Variety: RR—Riesling (Blanc), PB—Pinot blanc, SB—Sauvignon blanc, CH—Chardonnay; Origin: SVK-S—Southern Slovak wine-growing region, SVK-C—Central Slovak wine-growing region, HUN—Hungary, AUT—Austria.

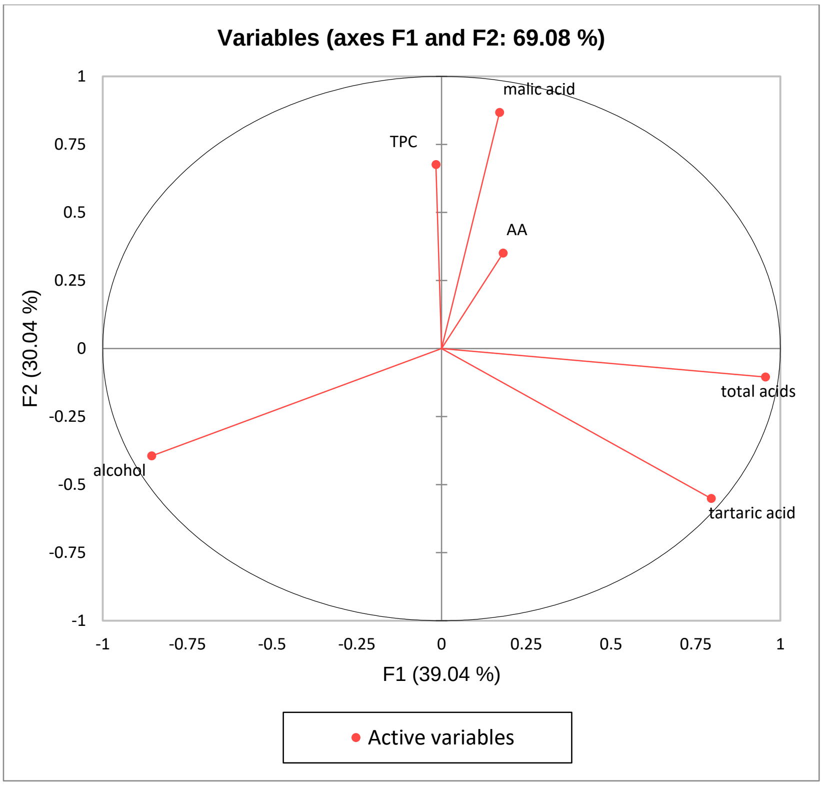

Figure 8. Representation of the main and supplementary parameters by the PCA evaluation.

The PCA indicated that 69.08% of the total variation can be condensed and explained by the first two principal components (PCs), with eigenvalues of 2.342 and 1.802. PC1, accounting for 39.04% of the inertia, contrasted tartaric acid and total acid content with malic acid, % inhibition, and TPC content whereas PC2, explaining 30.04% of the inertia, reflected the different content of TPC and alcohol.

High contents of malic acid, AA, and TPC can be observed in samples of Austrian wines. Higher values of these characteristics are typical, particularly for the Austrian varieties Chardonnay, Pinot Blanc, and Sauvignon.

The southern Slovakian wine-growing region (SVK-S) with Sauvignon blanc and Riesling varieties is distinguished by increased total acid content, particularly in the case of tartaric acid. The Riesling variety from different origins cannot be differentiated using the PCA analysis because the selected parameters are very similar.

Tethal et al. [35], in addition to reflecting the character of the site and subsoil, also emphasize the significant influence of the rootstock used on the analytical and sensory parameters of the wine. Hu et al. [36] also used principal component analysis (PCA) based on spectra and NIR to observe differences among wines and to perform the traceability of the geographical origin of Cabernet Sauvignon wines.

Regional products attract attention, which, as a result, contributes to growing producers' income and the development of the whole region as well. Locally produced wines could be characterized by a range of chemical parameters that are interesting from the health point of view, such as antioxidant activity, phenolic content, and organic acid content contributing to their antioxidant and antimicrobial activity. Particularly, some of these chemical parameters were selected in this study; as they are considered to be the most valuable at wine composition in terms of how they are affected by the country/region of production. Locally produced and offered wines with known health benefits and origin can be offered through innovation and in restaurant services, production, farm visits, and tastings related to the tours of the vineyards since the area of production raises a visible stamp on the originality and quality characteristics of products and is reflected in the final price [25].

4. Conclusions

High contents of malic acid, AA, and TPC were observed in samples of Austrian wines. Higher values of these characteristics are typical, particularly for the Austrian varieties of Chardonnay, Pinot Blanc, and Sauvignon. On the other side, the Slovakian wine-growing regions with Sauvignon blanc and Riesling varieties were distinguished by the higher acids.

Finding several distinctions among four varieties that originated from several separate localities/countries was made possible by using multivariate statistical approaches (PCA). It is important from both scientific and consumer points of view. On the other side, there were a few instances where no statistically significant difference was found, such as antioxidant activity and total acidity at Riesling wines. Possible consideration of testing additional statistical and/or chemometric methods besides PCA to identify or use more markers for region and/or wine quality is also recommended.

Locally produced wines could be characterized by a range of parameters that are interesting from the health point of view, such as antioxidant activity, phenolic content, and organic acid content, contributing to their antioxidant and antimicrobial activity. In this study, some of these chemical parameters were particularly tested; as they are considered to be the most valuable at wine composition and are affected by the country/region of origin. Local wines with known health benefits and origin can be valuable in innovation and in restaurant services, production, farm visits, and tastings related to wine tourism.

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