Investigation of geographic origin of wine from border regions: Results from investigation of two vintages

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Abstract. Investigation on discrimination of geographic origin in border areas is carried out by investigating and comparing authentic wine samples from the border areas of Austria, Czech Republic, Slovakia (and from Serbia) by applying different physico-chemical methods (e.g. IRMS, NMR, ICP-MS, ICP-OES, EPR, HPLC, UV-VIS, etc...). The comparison of the data sets from the participating countries and the different applied methods demonstrates, to which extent a differentiation can still be achieved in the border areas of the respective countries, which methods prove most efficient and sensitive and if a combination of methods leads to an increased sensitivity. Furthermore, we also investigate the potential causes for the differentiation, as the environment, different agricultural practices or enology. Analysis of two consecutive vintages shows significant variations between the two years, complicating direct comparison of regions beyond individual vintages. Still, several parameters are identified enabling correct and complete differentiation. However, some of them have been found to represent lab effects, whereas others are interpreted as resulting from different agricultural practices or environmentally driven. Only the latter two ones, if consistent, can be used for discrimination of geographic origin.

1. Introduction

Neighbouring geographic border areas are a difficult topic with respect of their characterization, as they very often are similar or almost identical on both sides of the border. Natural borders (e.g. mountain range, sea, desert,..) might facilitate the respective characterization, as such borders often result in significant differences with respect to environment, flora & fauna, etc.., due to their separating effect. On the other hand, however, political borders, drawn without taking into account natural barriers (or where the border is merely a river) usually have only minor natural (and other) differences between both sides of the border areas. Thus, correct classification of geographic origin in such border areas is a big challenge, especially, as identical (agricultural) products often gain different prices depending on which side of the border they were produced. Therefore, analytical tools are required to control the declared geographic origin of agro-products. The authenticity and declared geographic origin of wine in the EU is controlled by comparison of isotope patterns of commercial samples with the patterns of authentic samples from the EU wine database. The latter has been founded in 1991 by an EU-regulation. The authentic samples have to be collected, produced and analysed following standardized methods.

To investigate the possibilities for differentiation in border areas we chose the Austrian-Czech-Slovak border region. This region is characterized geologically by the Molasse basin containing sediments of Tertiary to Quaternary age, confined by the Bohemian Massive in the Northwest and by foothills forming the transition from the Alps to the Carpathians in the Southeast. Significant parts of the border are defined by the rivers Thaya and March (and Danube). Wine is produced on all sides of the borders, in the Austrian "Weinviertel" (wine district), in the Czech oblast Morava (Moravian region) and in the Slovak "Malokarpatska" (Little Carpathians) region. Among the main varieties are Grüner Veltliner/ Zelené Veltlínské, Müller-Thurgau, Welschriesling/Ryzlink vlašský, Sauvignon (Blanc), Rhein-riesling/Ryzlink rýnský, Pinot Gris/Rulandské šedé, Chardonnay; red varieties: Blauer Zweigelt, St. Laurent/Svatovavřinecké, and Blaufränkisch/ Frankovka modrá on all sides of the borders. The aim of the present study is the in-depth investigation of wine samples coming from the different sides of the borders in Austria, Czech Republic, Slovakia (and also Serbia) complemented by statistical analysis to identify methods and method combinations enabling a differentiation and correct classification of wine from the described border areas. Furthermore, if this can be achieved, it is followed by an evaluation of the causes for the differentiation.

Results of the first vintage investigated have already been published in Horacek et al., 2019 [1], here we present a comparison of the results 2016 and 2017.

2. Materials and methods

2.1. Sampling procedure

Samples of white and red wines of similar grape varieties from the Austrian – Czech – Slovak border region and Serbia were subjected to analysis. In 2016 10 samples from Austria (AT), 12 from Czech Republic (CZ), 5 from Slovakia (SLK) and 1 from Serbia were obtained. In 2017 9 samples from Austria, 12 from Czech Republic and 1 from Serbia were available. For the Austrian, Czech and Slovak samples the procedures for grape collection, vinification, processing and analysis were carried out according to the regulations for the authentic samples for the EU wine database (EU regulation EC No. 555/2008 and the Compendium of the OIV [2]). Several of the samples were exchanged among the participating institutes for laboratory intercomparison tests.

2.2. Physico-chemical analysis

A detailed methods description is found in Horacek et al., 2019 [1]. The primary chemical parameters of the musts were analysed using the FOSS Grape Scan 2000 (Rhine Ruhr, Denmark), Glucose, Fructose, volatile acidity, tartaric acid as well as the pH of the juice were measured by OIV official methods. Concentration of SO_2, alcohol content, and total acidity were evaluated according to the AOAC [2] and OIV methods, respectively. Major and trace element concentrations were measured by ICP-OES (iCAP 6000 series, Thermo, Germany) and ICP-MS (7500ce, Agilent, Japan) [1]. Total phenolic compounds content (TPC) and total flavonoid compounds content (TFC) was determined applying Folin-Ciocalteau modified method [4] and modified method by Pallab et al., 2013 [5], respectively. The ability to terminate $ABTS^{\bullet+}$ cation-radical (expressed as Trolox equivalents – TEAC), concentration of individual flavonoids (catechin, epicatechin, rutin, quercetin and resveratrol) and colour characteristics were determined as previously described in Tobolkova et al., 2014 [6]. Phenolic acids were quantified according to Horacek et al., 2019 [1].

2.3. Isotope analysis

A detailed methods description is found in Horacek et al., 2019 [1]. Distillation was done using automated distillation control systems (ADCS).

Isotope ratio mass spectrometry (IRMS): Carbon isotopes were determined using an elemental analyser connected to an isotope ratio mass spectrometer (IRMS). Oxygen isotope ratios of the samples were measured by equilibration method and the equilibrated CO_2-gas is transferred into an IRMS. Isotope results are expressed in the conventional δ-notation in ‰ with respect to the V-SMOW (Vienna-Standard Mean Ocean Water) and with respect to the V-PDB (Vienna-PeeDee Belemnite) standards for oxygen and carbon, respectively. The extended uncertainty of measurements of $\delta^{13}C$ and $\delta^{18}O$ were better than ± 0.5 and ± 1.0‰, respectively, for all laboratories. For quality control and comparability of the results identical or comparable certified standards and reference materials were analysed together with the wine samples. Distillates obtained from ADCS were further analysed on ethanol content by Karl-Fischer titration, and measured against TMU (trimethylurea) as internal standard by ^{2}H-NMR spectroscopy (SNIF-NMR). Extended uncertainty is better than ± 1 ppm on D/HI, and ± 1.8 ppm on D/HII.

2.4. Statistical analysis

Methods of multivariate statistics, allowing the reduction of multi-dimensional and correlated data to only a few dimensions, were performed to compare, distinguish and discriminate the wine samples according to their origin, vintage and type (red vs. white). Principal component analysis (PCA), principal component factoring (PCF) and canonical discriminant analysis (CDA) were used in order to define the most appropriate variables and to interpret and visualise of differences between compared wine samples. Statistical elaboration enabling multivariate presentation, visualisation and classification of wine samples was performed by statistical package Unistat v.6.0 (Unistat, Ltd., London, United Kingdom).

3. Results and discussion

The current study aims to unveil the possibilities for physico-chemical analyses for the differentiation and correct classification of wine from different sides of (and close to) a border, specifically the Austrian-Czech-Slovak border region. First preliminary results, published in our previous work last year [1] identified certain parameters that enabled a differentiation of geographic origin. However, the SO_2 content, identified as the most relevant parameter, turned out to be a parameter dominated by lab influences and thus not suitable for discrimination, as also further potential parameters, e.g. K concentrations. Other parameters enabling differentiation in 2016 were less potent in 2017, e.g. Cu concentrations. The latter parameter is currently tentatively identified as parameter dominantly influenced by agricultural practice and further investigations have to confirm/test if consistently different practices are applied on the different sides of the border.

3.1. Statistical evaluation of physico-chemical characteristics, TPC, TFC, TEAC and colour characteristics

As the principal component analysis is indifferent to any factor (vintage, type, origin), the obtained trends indicate a close link between the origin of samples and their characteristics.

Differentiation of wine samples according to the origin resulted in 85.56% of correctly classified samples (Fig. 1) by canonical discriminant analysis with chromaticity, TPC and TFC as the most segregating markers. Results of CDA of wine samples differentiation according to the selected criteria are shown in Table 1.

3.2. Statistical evaluation of 35 experimental parameters

As follows from results of PCA, a complete differentiation of wines according to the country of origin was not achieved.
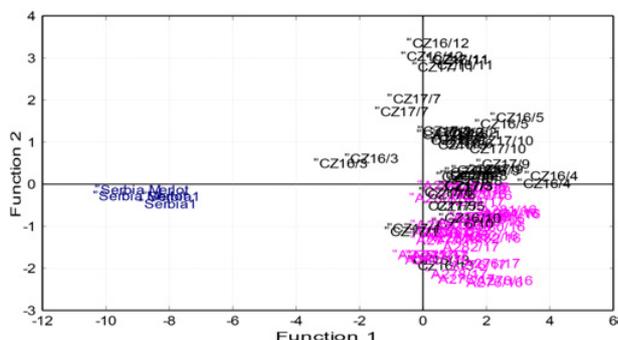
Figure 1. Discrimination analysis of wine samples according to the country of origin (without respect to type and vintage) based on 12 experimental characteristics.

Table 1. Results of canonical discrimination analysis of wine samples under study based on the following discrimination parameters: alcohol content, total acidity, volatile acidity, pH, TPC, TFC, TEAC, L*, a*, b*, chromaticity and hue angle.

Criterion	Correctly classified (%)	Number of misclassified samples	The most important parameters
Type (red vs. white)	97.78	2	TPC, a*, chromaticity, TEAC
Origin (AT vs. CZ vs. SRB)	85.56	13	chromaticity, TPC, TFC
Vintage (2016 vs. 2017)	87.78	11	a*, chromativity, TPC
Origin + vintage	83.33	2	TPC, a*, chromiticity, TEAC

Table 2. Results of canonical discrimination analysis of wine samples under study based on the following discrimination parameters: Be, B, Ba, Ca, Na, Al, P, S, Si, V, Cr, Mn, Fe, Mg, Co, Ni, Cu, Zn, Sr, glu+fru, DH1ETH, DH2ETH, δ^{13}C, δ^{18}O, alcohol content, total acidity, volatile acidity, pH, TEAC, TPC, TFC, L*, a*, b*, chromaticity and hue angle.

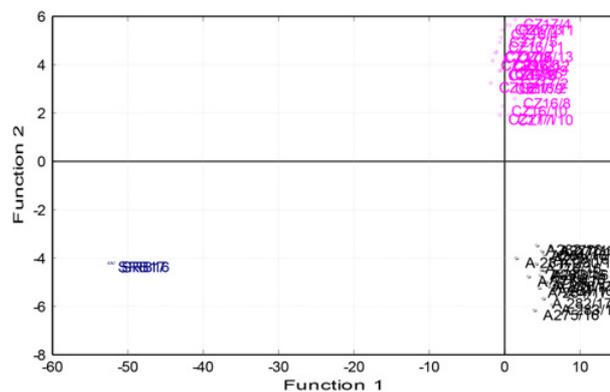| Criterion	Correctly classified (%)	Number of misclassified samples	The most important parameters
Type (red vs. white)	100	0	Chromaticity, a*, alcohol content, P, Fe, δ^{13}C
Origin (AT vs. CZ vs. SRB)	100	0	TPC, a*, alcohol content, Be, V
Vintage (2016 vs. 2017)	100	0	Chromaticity, a*, TPC, alcohol content, Be, Al, V, glu + fru
Origin + vintage	100	0	Chromaticity, a*, TPC, alcohol content, a*, Ca, glu + fru

Figure 2. Discrimination analysis of wine samples according to the country of origin (without respect to type and vintage) based on 35 experimental characteristics.

First three PCs explained cumulatively 57.50% of the total variance of data set of 35 experimental characteristics. As the most important characteristics obtained in the 1st principal component, total polyphenols, total flavonoids, TEAC, a* and chromaticity, while in the 2nd, concentration of Be, V, Cr and Co, and in the 3rd component, concentration of Na, Si and DH2ETH were recognized. Taking into consideration eigenvalue >1, eight principal components which explained 83.70% of total variance of dataset must be constructed.

Factor analysis is applied to explain the covariances or correlations between the variables. It identified strong positive correlation between antioxidant characteristics, colour value a* and chromaticity. It also found inverse correlations between L* and b* values and/or between pH and total acidity.

As follows from Table 2 summarizing results of the CDA, 100% correct classifications of wine samples according to the selected criteria were achieved for CZ, AT and Serbian samples (for SLK currently no results for 2017 are available; Table 2, Fig. 2).

Absolute classification of wine samples according to country of origin is apparent from Fig. 2. As the most important discriminators, total polyphenols, a*, alcohol content and concentration of Be and V were identified. However, as already shown last year [1] the evaluation for geographic origin does not differentiate at all between red and white wine samples.

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Confirmation by testing with commercial samples as well as further vintages still is required and research on the mechanisms and processes behind the individual discriminating parameters continues to identify the parameters which do dominantly represent environmental influences and/or differing agricultural practices and thus can be reliably used for wine provenance.

Still, besides our small dataset and currently just two vintages investigated, the results are surprisingly good and promising, as complete differentiation is achieved for AT and CZ samples despite significant differences in analyses between the two vintages.

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