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Differentiation of wine commercial samples by using fluorescence spectroscopy and Multivariate Analysis

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Abstract: Steady state fluorescence spectroscopy in combination with Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) for spectral analysis has been applied to differentiate commercial samples of wines available on the market. We have chosen the wine trade marks from the two Serbian producers that contain only one wine type, since such approach is a starting step for further analyses of the more complex samples containing different wine types. We also studied changes of the emission spectra of these samples during seven days after opening of the wine bottle. The emission spectra were recorded in the wavelength range 275 - 500 nm, after excitation in the range 255 - 300 nm. The spectra of the wine samples obtained from the same producer are very similar, i.e. contain the same components in the similar ratios. The changes of spectral components at 315 nm and 430 nm were the basis for differentiation of the wine samples from the two producers, as well as for estimation of

the wine stability in time after the bottle opening. The results indicate that this may be a useful approach in fingerprinting wines from various producers, as well as in screening the stability of wine.

Key words: wine types, riesling, emission spectra, Multivariate Curve Resolution, phenolic compounds.

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Introduction

Fluorescence is non-destructive, sensitive and fast method for analysis of fluorescent compounds contained in very low amounts (nanomolar concentrations) in the samples. It can be used for structural or concentration studies, in analytical or diagnostic purposes (Valeur 2001). We have been developing methods for measurement and analysis of emission spectra of compounds of various complexity, in the mixtures (Kalauzi *et al.* 2007) or as macromolecules composed of different kinds of monomers, such as proteins or polyphenols (Djikanović *et al.* 2012, Radotić *et al.* 2006, Savić *et al.* 2013).

The advantage of fluorescence measurement is that it does not require preceding processing of the sample, or physical separation of its components, like in the case of HPLC.

The most fluorescent molecules in wines are of phenolic nature, such as phenolic acids, stilbenes, anthocyanins, flavanols and tannins. The nature and amounts of these molecules differ from one grape variety to another, as well as depend on wine processing and ageing. Besides, wines contain some other emitters, such as proteins (Sádecká and Tóthová 2007).

The fluorescence spectra, in combination with appropriate statistical methods, may provide useful fingerprints in food analysis (Sádecká and Tóthová 2007). During the last few years application of chemometrics and developments of spectrofluorometers boosted the potential of using fluorescence in food research. Food products contain numerous intrinsic fluorophores and thus are appropriate for fluorescence spectroscopy investigations (Dion *et al.* 2008). This method in combination with chemometrics has been used to differentiate brandies from wine distillates (Sádecká *et al.* 2009) , or identity of wines from a geografic region (Yin *et al.*, 2009). Quantification of certain ingredients in wines may also be achieved by using fluorescence spectroscopy (Molina-Garcia *et al.* 2011, Sádecká and Tóthová 2012, Vidal-Carou *et al.* 1989).

The aim of this paper is to study if steady state fluorescence spectroscopy, in combination with multivariate statistical methods can be used, as a quick and non-destructive screening method, for distinguishing between commercial samples of wines available on the market. We have chosen the wine trade marks

that contain only one wine type, since such approach is a starting step for further analyses of the more complex samples containing different wine types. We used for the experiments the wine trade marks that contain riesling wine samples from the two trade marks, originated from the two wine producers in Serbia and purchased in the supermarkets. We have chosen one of the most popular white wine types and the two renowned wine producers in Serbia. We also studied changes of the emission spectra of these samples during seven days after opening of the wine bottle. We intended to see whether it is possible to distinguish wine samples on the basis of the above designations.

Materials and methods

Samples. The studies were performed on 4 wines from two different region of Serbia. All samples were white wines, sort of grapes "Riesling", 2012 vintage and purchased from the local supermarkets. Two wines were from the region of Vojvodina - producer "Vršački vinogradi" (Italijanski riesling, Banatski riesling) and the two were from Central Serbia - producer "Vino župa" (Riesling, Graševina).

All measurements were made on the day when the bottles of wine were opened and two and seven days after opening bottles. The samples were stored in the dark at room temperature until the day of analysis, diluted with water (1:49 v/v) and measured.

Fluorescence spectroscopy. Fluorescence spectra were recorded using a Fluorolog-3 spectrofluorimeter (Jobin Yvon Horiba, Paris, France) equipped with a 450 W xenon lamp and a photomultiplier tube. The slits for the excitation and emission beams were fixed at 4 nm and 2 nm, respectively. The samples placed in 10 mm (optical path) quartz cell (volume 1,5 ml).

Fluorescence emission spectra were recorded from 275 nm to 500 nm, for the excitation wavelengths varied in the 255 - 300 nm range, with 5 nm step. Fluorescence measurements were done in triplicate for each sample.

Statistical analysis of data. The intensities of the emission spectra were normalized to the scale 0 – 1. In the analysis we used 12 matrices (4 wine types x 3 time points) that corresponded to the wine samples. Each matrix was analysed by using MCR-ALS[10] which extracted the number of components, as well as their emission and excitation profiles.

Results and Discussion

Fig. 1. presents overlaid emission spectra obtained for various excitation wavelengths, of the two different riesling samples, Riesling and Graševina from

the producer Vino Župa, as well as Italianski riesling and Banatski riesling from the producer Vršački vinogradi. Fig. 2. shows the emission spectra of pure components obtained by application of the MCR-ALS method in analysis of the series of emission spectra from Fig. 1. The three components with the peaks at 315 nm, 370 nm and 430 nm were found in all samples. The shape of these components was the same for all 12 matrices (samples). It is obvious that the spectra of the wine samples obtained from the same producer are very similar, i.e. contain the same components in the similar ratios (Fig. 1). This is confirmed by the MCR-ALS analysis (Fig. 2). The corresponding loadings (excitation profiles) of the components from Fig. 2. are shown in Fig. 3. The loadings of the individual components for the samples from the same producer change in a similar way with changing excitation wavelength. This additionally confirms high similarity of the riesling wines from the two trade marks produced by the same producer. However, there is difference between the wine samples from the trade marks produced by different producer (Fig. 1, 3), although the position of the spectral components is the same as for the other producer (Fig. 2). The 315 nm component is significantly higher in the wine spectra of both Riesling and Graševina from Vino Župa, in comparison to both the Banatski riesling and Italijanski riesling samples from Vršački vinogradi (Fig. 1). This peak is more expressed in the case of Riesling wine. This difference is related to the higher content of the compound(s) emitting at 315 nm in the Vino Župa riesling wines than in the Vršački vinogradi riesling wines. The corresponding loading of the 315 nm component (Fig. 2, 3) changes in a different way with the excitation wavelengths in the samples of these two producers, attaining maximum more abruptly for the Riesling wine than for the others. Also, the 430 nm maximum is higher for both wine samples from the producer Vršački vinogradi than for the samples from Vino Župa. This is also obvious through the change of the corresponding loading of this component (Fig. 3), which increases more abruptly and attains higher maximum (3.3. rel. u.) for the Vršački vinogradi producer, while its rise is slower and maximum is lower (1.5 rel. u.) for the Vino Župa. This is due to a higher amount of the corresponding phenolic compounds emitting in this spectral region, with 430 nm maximum (Fernández *et al.* 2000, Lang *et al.* 1991).
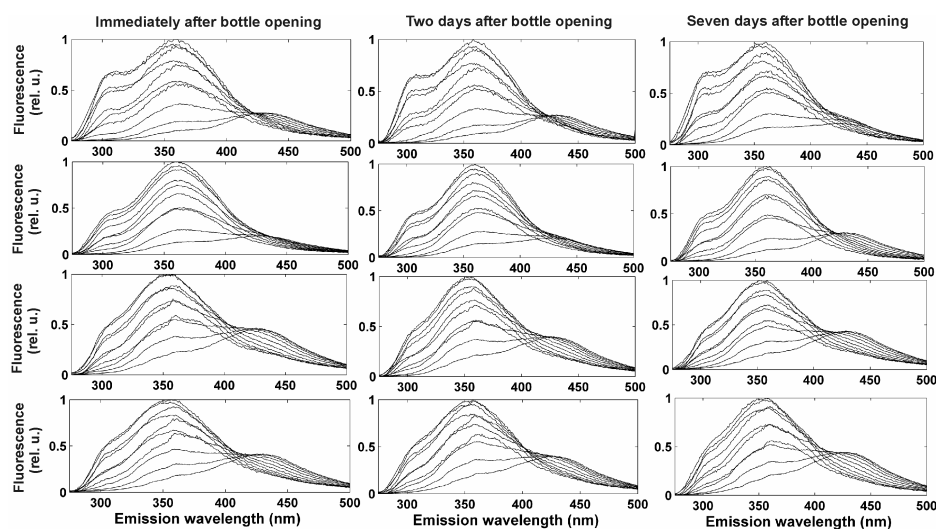
Figure 1. Emission spectra of the riesling wine samples from the four trade marks, Riesling and Graševina from the producer Vino Župa, and Italijanski riesling and Banatski riesling from the producer Vršački Vinogradi, from top to bottom, respectively. For each sample the emission spectra were recorded for the excitation wavelengths varied in the 255 - 300 nm range, with the 5 nm step.
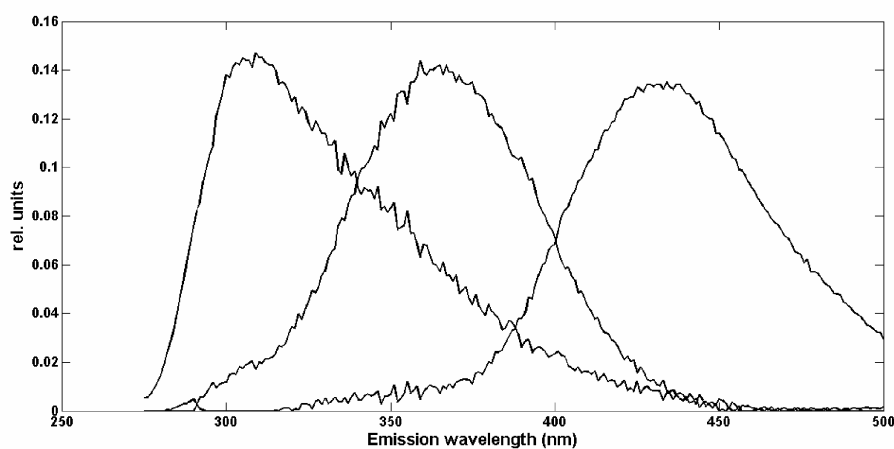

Figure 2. Emission spectra of the pure components obtained by applying the MCR-ALS method in the analysis of the emission spectra of the riesling wine samples from the Figure 1. The components have the same maxima positions and the same shape for all 12 samples.

One can also notice that, for both wine producers, there are no changes of the spectral parameters after two days of opening of the wine bottle for all trade marks (Fig. 1). However, in the case of the producer Vino Župa, for the Riesling

trade mark there is a decrease in the 430 nm spectral component after seven days of bottle opening, and its loading changed accordingly (Fig. 3), with slower increase and attaining lower maximum (1.5 rel. u.) than for the sample after two days of bottle opening (2 rel. u.). Also there was an increase in the same component for the Graševina wine after the same time period, and a corresponding change of its loading occurs, being more abrupt and with higher maximum (2 rel. u.) than for the sample two days after bottle opening (1.5 rel. u). These changes indicate that certain phenolic structures emitting at 430 nm changed possibly by oxidation processes after the bottle opening. The wine producers add certain additives that have preserving role, such as sulfuric compounds (Plaza *et al.* 2013). The spectral changes recorded in time after bottle opening may be an indicator of presence of higher concentrations of preserving additives in wine.
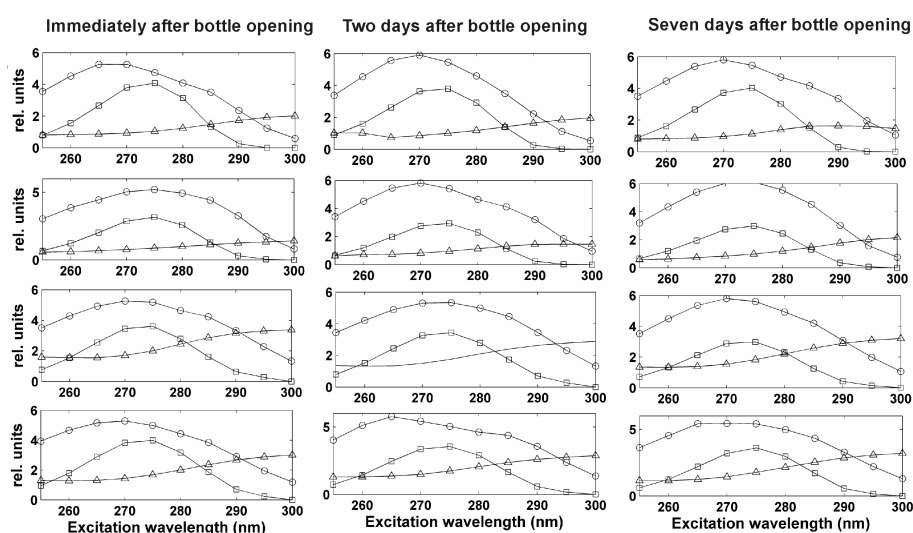

Figure 3. The excitation spectra profiles (loadings) corresponding to the spectral components from the Figure 2, obtained by the MCR-ALS method. The curves marked with the symbols (□), (○), (Δ) correspond to the components in Figure 2 with the maxima at 315 nm, 370 nm and 430 nm, respectively. From top to bottom: the four trade marks, Riesling and Graševina from the producer Vino Župa, and Italijanski riesling and Banatski riesling from the producer Vršački Vinogradi.

The 315 nm peak may be related to the flavonoid type of compounds of the catechin type (Shumow and Bodor 2011). The 370 nm maximum may originate from certain types of phenolic compounds such as gallic acid or syringic acid (Sikorska *et al.* 2012). The 430 nm maximum is related to the phenolic compounds of the type of chlorogenic acid, caffeic acid, coumarins, stilbenes (Fernández *et al.* 2000, Lang *et al.* 1991).

The presented type of analysis has potential in screening many wine samples, from different producers, since it may give quick results, without detailed analysis of the content of the corresponding wines. This may be very useful approach in fingerprinting wines from various producers, as well as in screening the compositional stability of wine.

Conclusion

These results show that fluorescence method in combination with the appropriate statistical analysis may be a tool that has potential in differentiation between the samples of the trade marks containing the same wine type, but originating from different producers. The results also show that this method may be used for simple and quick tracking changes in wine composition, caused by the oxidation processes in time after the bottle opening.

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References

Djikanović D., Simonović J., Savić A. 2012. Structural Differences Between Lignin Model Polymers Synthesized from Various Monomers. *Journal of Polymers and Environment*, **20**: 607-617.

Fernández Izquierdo M.E. ,Quesada Granados J., Villalón Mir. M., López Martinez M.C. 2000. Comparison of methods for determining coumarins in distilled beverages. *Food Chemistry,* **70**: 251-258.

Kalauzi A., Mutavdžić D., Djikanović D. 2007. Application of asymmetric model in analysis of fluorescence spectra of biologically important molecules. *Journal of Fluorescence,* **17(3)**: 319-329.

Lang M., Stober F., Lichtenthaler H.K. 1991. Fluorescence emission spectra of plant leaves and plant constituents. *Radiation and Environmental Biophysics,* **30**: 333–347.

Luykx Dion M.A.M., Ruth van Saskia M. 2008. An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry,* **107(2)**: 897–911.

Mendieta J., Diaz-Cruz M.S., Esteban M., Tauler R. 1998.: Multivariate Curve Resolution: A Possible Tool in the Detection of Intermediate Structures in Protein Folding. *Biophysical Journal,* **74(6)**: 2876–2888.

Molina-Garcia L., Ruiz-Medina A., Luisa Fernandez-de Cordova M. 2011. An automatic optosensing device for the simultaneous determination of resveratrol and piceid in wines. *Analytica Chimica Acta,* **689**: 226–233.

Plaza A., Romero J., Silva W. 2013. Extraction and quantification of SO2 content in wines using a hollow fiber contactor. *Food Science and Technology International,* **0(0)**: 1–10.

Radotić K., Kalauzi A., Djikanović D. 2006. Component analysis of the fluorescence spectra of a lignin model compound. *Journal of Photochemistry and Photobiology B,* **83**: 1-10.

Sádecká J., Tóthová J. 2007. Fluorescence Spectroscopy and Chemometrics in the Food Classification - A Review. *Czech Journal of Food Sciences,* **25**: 159-173.

Sádecká J., Tóthová J. 2012. Spectrofluorimetric determination of ellagic acid in brandy. *Food Chemistry,* **135**: 893-897.

Sádecká J., Tóthová J., Májek P. 2009. Classification of brandies and wine distillates using front face fluorescence spectroscopy. *Food Chemistry,* **117**: 491-498.

Savić A., Kardos R., Nyitrai M., Radotić K. 2013. Decomposition of complex fluorescence spectra containing components with close emission maxima positions and similar quantum yields. *Journal of Fluorescence,* **23**: 605-610.

Shumow L., Bodor A. 2011. An industry consensus study on an HPLC fluorescence method for the determination of (±)-catechin and (±)-epicatechin in cocoa and chocolate products. *Chemistry Central Journal,* **5(39)**: 1-7.

Sikorska E., Khmelinskii I., Sikorski M. 2012. Analysis of Olive Oils by Fluorescence Spectroscopy, in Methods and Applications. *Olive Oil - Constituents, Quality, Health Properties and Bioconversions*, ISBN: 978-953-307-921-9, InTech ahead of print : 63-88.

Valeur B. 2001. *Molecular fluorescence. Principles and applications.* Germany, Weinheim: John Wiley – VCH.

Vidal-Carou M.C., Isla-Gavin M.J., Marine-Font A., Codony-Salcedo R. 1989. Histamine and tyramine in natural sparkling wine, vermouth, cider, and vinegar. *Journal of Food Composition and Analysis,* **2**: 210-218.

Yin C., Li H., Ding C., Wang H. 2009. Preliminary investigation on variety, brewery and vintage of wines using three-dimensional fluorescence spectroscopy. *Food Science and Technology Research,* **15**: 27 – 38.

Razlikovanje komercijalnih uzoraka vina korišćenjem fluorescentne spektroskopije i multivarijacione analize

- originalni naučni rad -

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Rezime

Fluorescentna spektroskopija u kombinaciji sa metodom Multivarijaciona Rezolucija Krivih – Naizmenični Najmanji Kvadrati (Multivariate Curve Resolution – Alternating Least Squares, MCR-ALS), je primenjena za razlikovanje komercijalnih uzoraka vina dostupnih na tržištu. Odabrali smo trgovačke marke vina od dva srpska proizvođača koje sadrže samo jednu vrstu vina, jer je ovakav pristup početni korak za kasnije analize složenijih uzoraka koji sadrže različite vrste vina. Takođe smo proučavali promene emisionih spektara ovih uzoraka u toku sedam dana posle otvaranja boce. Emisioni spektri su mereni u opsegu talasnih dužina 275 - 500 nm, posle pobuđivanja u opsegu 255 - 300 nm. Spektri uzoraka vina od istog proizvođača su veoma slični, to jest sadrže iste komponente u sličnom međusobnom odnosu. Promene komponenata spektara na 315 nm i 430 nm bile su osnova za razlikovanje uzoraka vina od dva proizvođača, kao i za procenu stabilnosti vina u vremenu posle otvaranja boce. Rezultati ukazuju da ovo može biti koristan pristup u razlikovanju vina od različitih proizvođača, kao i u praćenju stabilnosti sastava vina.

Ključne reči: tipovi vina, rizling, emisioni spektar, multivariaciona rezolucija krivih, fenolne komponente.