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METROLOGY, STANDARDIZATION, AND CONTROL OF NANOTECHNOLOGIES

Study of the Capabilities of the Fluorescence Spectroscopy Method for the Identification of Wine Varitality

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Abstract—The chemical composition of a wine depends on several factors that determine the wine's identity, including grape variety, geographic origin, biophysical environment of the vineyard, harvest conditions, and winemaking techniques. Analytical methods for varietal identification of wines are based mainly on determining the composition and content of volatile and phenolic compounds using various chromatographic methods that require highly qualified personnel and complex and expensive analytical instruments. Recently, the following aspects of wine analysis have become of paramount importance: speed, user-friendliness, and cost-effectiveness. One such method is three-dimensional fluorescence spectroscopy: a fast, noninvasive, sensitive and affordable method. The complete fluorescence landscape includes information about several fluorophores in the composition of a wine and can be considered as a characteristic "fingerprint" that will allow the identification of its varietal identity. The results of studying the use of three-dimensional fluorescence spectroscopy for the identification of wines depending on the grape variety are presented.

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INTRODUCTION

The composition of a wine depends on several factors that determine the wine's identity, including grape variety, geographic origin, biophysical environment of the vineyard, harvest conditions, and winemaking technology [1].

The analytical methods for the varietal identification of wines are based mainly on determining the composition and content of volatile and phenolic compounds using various chromatographic methods that require highly qualified personnel and complex and expensive analytical instruments [2–5].

Classical methods, in particular gas chromatography and high-performance liquid chromatography, are constantly being improved [6]. However, recently, aspects of wine analysis such as speed, user-friendliness, and cost-effectiveness have become of paramount importance [7]. One of the possible solutions in this direction may be spectroscopic methods (near and mid-IR (infrared) spectroscopy, UV (ultraviolet) spectroscopy, Raman spectroscopy, nuclear-magnetic-resonance methods (**NMR**), and fluorescence spectroscopy), which are characterized by ease of sample preparation and a high speed of analysis [8].

One of the analytical methods with high potential for the varietal identification of wines is three-dimensional fluorescence spectroscopy: a fast, noninvasive, sensitive, and affordable method that includes the sequential acquisition of excitation and/or emission spectra at several wavelengths. The advantage of this method is that all information can be obtained by simultaneously changing the excitation and emission wavelengths. The complete fluorescence landscape can be represented as a matrix consisting of graphs of excitation versus emission wavelengths. This matrix is called the excitation–emission matrix (**EEM**) [9].

EEM consists of signals derived from fluorophores (fluorescent molecules) present in wine, which vary in intensity depending on type and concentration. Due to the wide range of fluorescent compounds present in wine, a specific EEM represents an overlapping signal of the individual contributions of fluorophores.

The main fluorophores in wine are phenolic acids, stilbenes, anthocyanins, flavonoids, and tannins.

Phenolic compounds are a major group of substances that affect the organoleptic characteristics (color, taste, astringency, and softness) and quality of wine. In addition, these compounds are important for food safety due to their antioxidant and bactericidal effects [10]. The phenolic composition of wine depends, firstly, on the content of phenols in the raw material, i.e., grapes, which is influenced by the variety, year of harvest, climatic conditions during ripening, and soil type, and secondly, on the methods used in the winemaking process and conditions of aging [11].

Polyphenols are divided into two families: flavonoid and nonflavonoid compounds. The most

Table 1. Description of the wines studied

Code	Name of wine, vintage year	Manufacturer
321	Rkatsiteli, white dry, 2019	Inkerman Vintage Wine Factory LLC, manufacturer Kachinsky LLC
322	Rkatsiteli, white dry, 2020	"
323	Rkatsiteli, white dry, 2021	"
324	Aligote Crimean, white dry, 2019	Inkerman Vintage Wine Factory LLC, Crimea
325	Aligote Crimean, white dry, 2020	"
326	Aligote Crimean, white dry, 2021	"
327	Saperavi, red dry, 2019	Inkerman Vintage Wine Factory LLC, manufacturer Kachinsky LLC
328	Saperavi, red dry, 2020	"
329	Saperavi, red dry, 2021	"
330	Merlot Kachinsky, red dry, 2019	Inkerman Vintage Wine Factory LLC, Crimea
331	Merlot Kachinsky, red dry, 2020	"
332	Merlot Kachinsky, red dry, 2021	"

important nonflavonoids found in wine are phenolic acids (derivatives of benzoic or cinnamic acid) and compounds like stilbene. Phenolic acids are represented mainly by cataric, cutaric, and fertaric acids, which are usually found in the form of esters. Hydroxycinnamic esters are one of the most common groups of phenolic compounds found in grapes [12]. Stilbene-like compounds include resveratrol, its glucoside piceid, astringin, and viniferins. Among flavonoids, three subgroups are important: flavonols, flavan-3-ols, and anthocyanins. Flavonols are found in grape skins in the form of glycosides: myricetin, quercetin, kaempferol, isorhamnetin, sircitin, and laricitrin. Flavan-3-ols (monomeric catechins and polymeric proanthocyanidins) are another large family of polyphenolic compounds, consisting mainly of catechin, epicatechin, gallocatechin, epigallocatechin, and their corresponding polymers found in the skins and seeds of grapes [13].

Analysis of published data has shown that fluorescence spectrometry has recently been widely used in the study of wines, for example, for the quantitative determination of polyphenols in red wine [14], changes in the content of anthocyanin pigments during wine aging [15], when monitoring the darkening of sparkling wines [16], to determine geographical origin [17] and wine producers [18, 19].

In wine varietal identification, research has focused mainly on red-wine varieties [20]. To differentiate red wines, the obtained results were subjected to multivariate analysis, including principal-component analysis, independent-component analysis, parallel factor analysis, factorial discriminant analysis, and partial least-squares discriminant analysis [21]. In the case of white wines, a sequential projection algorithm followed by linear discriminant analysis was used to create a simple and effective model for identifying Chardonnay, Sauvignon Blanc, and Torrontes varieties [22].

Thus, 3D fluorescence spectroscopy is one of the analytical methods with high potential for the varietal identification of wines; the types and amounts of fluorescent molecules vary depending on the grape variety and maturity; processing and aging of wine also influence the composition of these compounds. The complete fluorescence landscape includes information about several fluorophores in its composition and can be considered as a characteristic fingerprint that will allow identifying the varietal identity of a wine to be discerned.

The purpose of the study is to evaluate the possibilities of identifying wines depending on the grape variety using fluorescence spectroscopy.

EXPERIMENTAL

Objects of research. In this work, six samples of commercially available white (Rkatsiteli, Aligote) and red wines (Saperavi, Merlot) were analyzed. A description of the wines is given in Table 1.

After opening the bottles, samples of the wines under study were taken into 4-mL glass bottles with Teflon lids and stored in a refrigerator at +4°C. Immediately before analysis, aliquots of the analyzed samples were centrifuged (4000 rpm, 15 min).

Fluorescence measurements. Measurements were performed on a Thermo Scientific™ Varioskan™ LUX multifunction microplate reader with a xenon lamp (Thermo Scientific™ SkanIt™ software). Wine samples with a volume of 200-μL were placed into the wells of a microplate (Thermo Scientific™ Nunc™ F96 MicroWell™ Black Polystyrene Plate (Thermo Scientific 237108)) and the spectra were recorded at a temperature of 25.5 ± 1.0°C in triplicate. The excitation and emission monochromator slits were set to

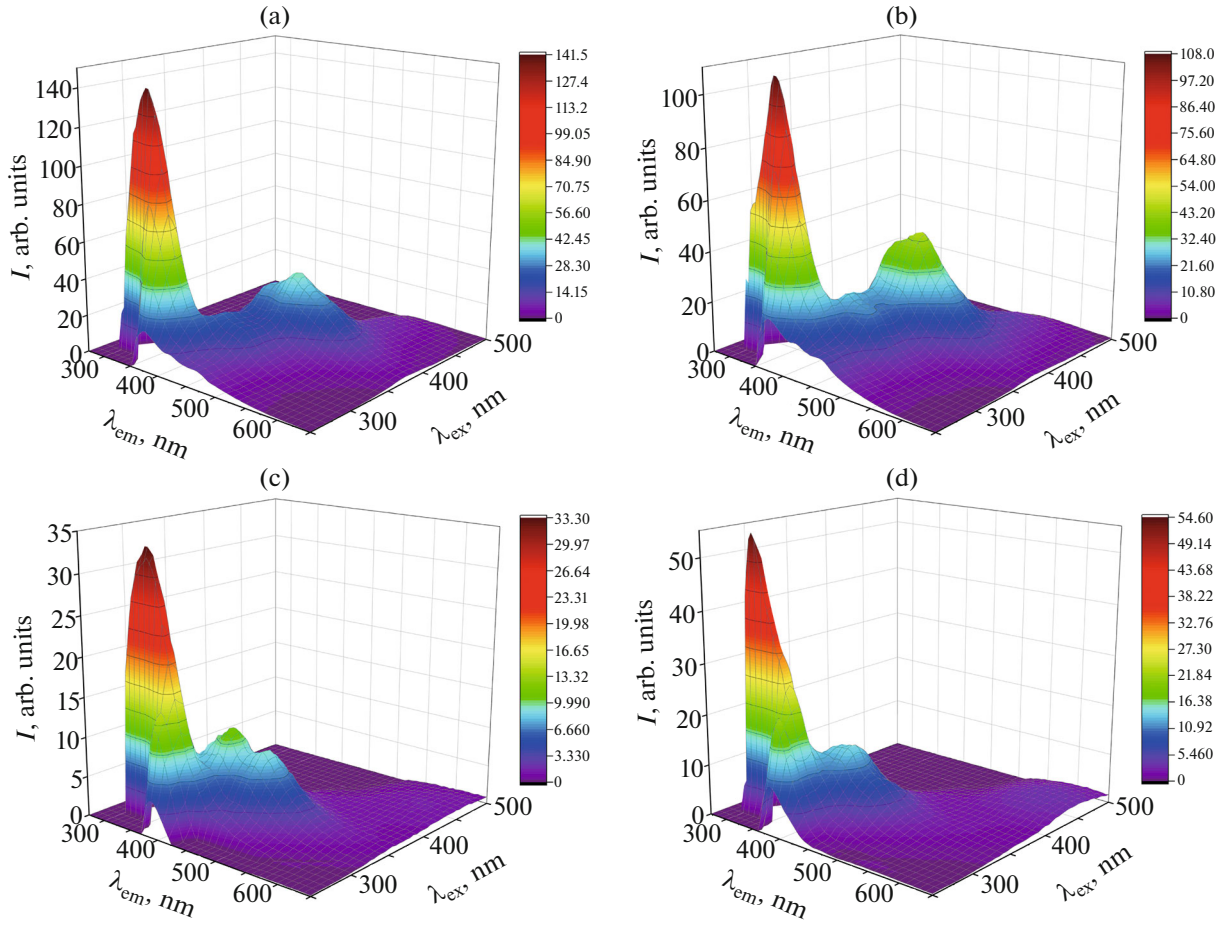

Fig. 1. 3D surfaces of the wines under study: white, Rkatsiteli (a), Aligote (b); red, Saperavi (c), Merlot (d).

5 nm. The data-acquisition speed was 500 nm/min. The wavelength range of excitation (λ_{ex}) and emission (λ_{em}) were 250–500 and 275–600 nm, respectively, with a wavelength step of 5 nm. The surfaces were recorded as multiple emission spectra. The total scanning time for one sample was ~30 min.

For each sample, the spectra were combined into one matrix, resulting in both 3D surfaces and contour maps in the Origin program (OriginLab, USA), version 9.0.

RESULTS AND DISCUSSION

Figure 1 shows the typical surfaces of each of the studied wines of different grape varieties. The EEM fluorescence surfaces of the studied samples provide an overall intensity profile across the range of excitation and emission wavelengths scanned, which can be used as a spectral fingerprint of each wine.

We note that fluorophores of different wine varieties cause a complex excitation–emission pattern and each of the studied varieties has its own unique surface shape (characterized by a specific profile), which in turn makes it possible to identify characteristic maxima and differentiate with respect to the grape variety.

According to the spectra presented in Fig. 1, there are differences in the number of peaks/shoulders between the wine varieties studied. Thus, white-wine varieties are characterized by four maxima and one shoulder, while red wines are characterized by three maxima and one shoulder. We note that in addition to differentiation in the number of peaks and the general shape of the spectra, which exists among the studied varieties, a specific imprint of each variety was noted in terms of the individual intensity of characteristic signals and their ratio depending on the year of harvest.

Figure 2 presents the EEM surfaces of wine samples as contour maps.

It is known that the 3D fluorescence spectra of standard phenolic compounds have characteristic spectral regions with maxima λ_{ex} in the range of 260–360 nm. In [11], data on the fluorescence properties ($\lambda_{ex}/\lambda_{em}$) fluorophores present in wine were summarized. For example, for phenolic acids and aldehydes in the region of 260–320/320–440 nm; flavonols in

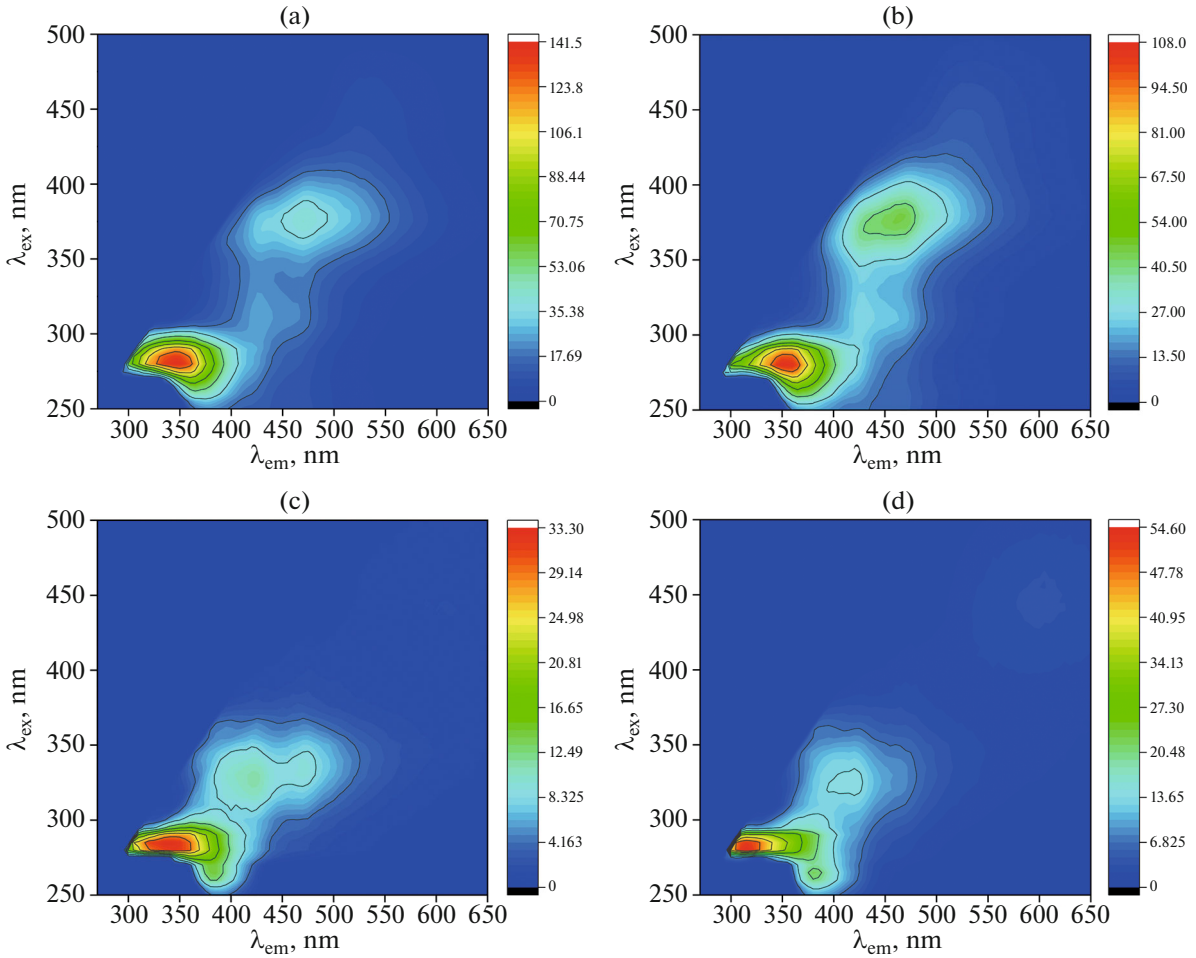

Fig. 2. Contour maps of the studied wines: white, Rkatsiteli (a), Aligote (b); red, Saperavi (c), Merlot (d).

the region of 260–270/370–420 nm; and monomeric and polymeric flavan-3-ols in the region of 280–290/310–360 nm.

A comparison of samples of white wines Rkatsiteli and Aligote revealed, on the one hand, some similarity in general forms: the presence of maxima I–III (Table 2); on the other hand, the presence of shifts in the main peaks of the maxima, which is due to existing natural differences in terms of their general composition.

When considering red wines of the Saperavi and Merlot varieties, a clear difference was revealed, namely, the presence of a peak at 265/380–390 nm for Merlot, which was absent in the analysis of all other wine varieties. Moreover, the maximum intensity of the peak in the case of Saperavi was at $\lambda_{ex}/\lambda_{em}$ 280–285/335–355, and in the case of Merlot, 280/315.

In more detail [23], methyl syringate, catechin, as well as gallic, protocatechuic, lilac, vanillic, and homogentisic acids were identified in the region of 260–315/315–345 nm. At 300–360/380–450 nm, the presence of kaempferol, as well as hydroxycinnamic acids: caffeic, caftaric, chlorogenic, *p*-coumaric, ferulic, and sinapinic. Some phenolic compounds, such as ellagic and gentisic acids, fluoresce in a wider range of the spectrum: 280–380/400–480 nm.

Variations that appear in specific pairs of $\lambda_{ex}/\lambda_{em}$ can contribute to the varietal identification of wines: variations in fluorophores in wine determine differences in molecular fingerprints, which will allow the use of these chemical markers (without necessarily identifying individual fluorophores) for wine verification.

CONCLUSIONS

Analytical methods for the varietal identification of wines are based mainly on determining the composition and content of volatile and phenolic compounds using various chromatographic methods that require highly qualified personnel and complex and expensive analytical instruments. Recently, aspects of wine analysis such as speed, user-friendliness, and cost-effectiveness have become of paramount importance.

Table 2. Characteristic maxima

Sample code	Maximum ($\lambda_{ex}/\lambda_{em}$)			Intensity, arb. units		
	I	II	III	I	II	III
White wines						
321	280/345	305/425	375/470	100	18.31	29.39
322	280/350	315/430	375/465	100	16.57	23.19
323	280/350	320/425	375/465	100	13.96	12.69
324	280/350	310/430	380/470	100	22.90	40.84
325	280/350	310/430	380/465	100	24.77	42.40
326	280/350	320/425	375/470	100	17.83	21.03
Red wines						
327	285/335	325/430	330/470	100	34.56	28.31
328	285/345	325/430		100	34.39	
329	280/355	325/425		100	24.28	
330	265/380	280/315	325/420	40.30	100	26.47
331	265/390	280/315	320/395	28.52	100	18.83
332	265/380	280/315	320/420	37.23	100	21.39

The analysis of published data showed that spectroscopic methods of analysis, due to their simplicity, high speed, and accuracy, are actively used in determining the authenticity of wines and their geographical origin. One such method is 3D fluorescence spectroscopy, a fast, noninvasive, sensitive, and available method.

To assess the possibility of classifying wines depending on the grape variety, a study of four wine varieties (Rkatsiteli, Aligote, Saperavi, and Merlot) was carried out using fluorescence excitation–emission matrix spectroscopy.

Simple visual characterization of a typical EEM surface revealed specific profiles characteristic of each variety. EEMs are unique to each individual wine and reflect its molecular fingerprint, and the features of EEM data can be used to identify wines by grape variety.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. B. Médina, M. H. Salagoïty, F. Guyon, et al., *New Analytical Approaches for Verifying the Origin of Food* (Woodhead, Cambridge, UK, 2013).
2. D. Cozzolino, H. E. Smyth, W. Cynkar, et al., Talanta **68**, 382 (2005). https://doi.org/10.1016/j.talanta.2005.08.057
3. J. Heras-Roger, C. Díaz-Romero, and J. Darias-Martín, Food Chem. **196**, 1224 (2016). https://doi.org/10.1016/j.foodchem.2015.10.085
4. A. Ziółkowska, E. Wąsowicz, and H. J. Jeleń, Food Chem. **213**, 714 (2016). https://doi.org/10.1016/j.foodchem.2016.06.120
5. V. M. Pozhidaev, A. M. Ismagulov, Ya. E. Sergeeva, et al., Nanobiotechnol. Rep. **17**, 932 (2022). https://doi.org/10.1134/s263516762206009x
6. A. Popîrdă, C. E. Luchian, V. V. Cotea, et al., Agriculture **11**, 225 (2021). https://doi.org/10.3390/agriculture11030225
7. M. Arslan, H. E. Tahir, M. Zareef, et al., Trends Food Sci. Technol. **107**, 80 (2021). https://doi.org/10.1016/j.tifs.2020.11.021
8. J. Chapman, S. Gangadoo, V. K. Truong, and D. Cozzolino, Curr. Opin. Food Sci. **28**, 67 (2019). https://doi.org/10.1016/j.cofs.2019.09.001
9. R. C. Suciu, L. Zarbo, and F. Guyon, Sci. Rep. **9**, 18250 (2019). https://doi.org/10.1038/s41598-019-54697-8

10. V. L. Singleton and P. Esau, *Phenolic Substance in Grapes and Wine and Their Significanc*e (Academic Press, New York, 1969).
11. P. Vinas, C. Lopez-Erroz, J. J. Marin-Hernandez, and M. Hernandez-Cordoba, J. Chromatogr., A **871**, 85 (2000). https://doi.org/10.1016/S0021-9673(99)01087-0
12. R. C. Rodrıguez, M. P. Aguilar, and A. Gomez, J. Sep. Sci. **29**, 2772 (2006). https://doi.org/10.1002/jssc.200600206
13. J. A. Kennedy, C. Saucier, and Y. Glories, Am. J. Enol. Viticult. **57**, 239 (2006). https://doi.org/10.5344/ajev.2006.57.3.239
14. M. Cabrera-Bañegil, M. C. Hurtado-Sánchez, T. Galeano-Díaz, and I. Durán-Merás, Food Chem. **220**, 168 (2017). https://doi.org/10.1016/j.foodchem.2016.09.152
15. G. Agati, P. Matteini, J. Oliveira, et al., J. Agric. Food Chem. **61**, 10156 (2013). https://doi.org/10.1021/jf402398a
16. S. Elcoroaristizabal, R. M. Callejón, J. M. Amigo, et al., Food Chem. **206**, 284 (2016). https://doi.org/10.1016/j.foodchem.2016.03.037
17. E. Dufour, A. Letort, A. Laguet, et al., Anal. Chim. Acta **563**, 292 (2006). https://doi.org/10.1016/j.aca.2005.11.005
18. M. Mutavdžić, D. Mutavdžić, and K. Radotić, Acta Agric. Serb. **36**, 169 (2013).
19. Y. Wan, F. Pan, and M. Shen, Spectrochim. Acta A **96**, 605 (2012). https://doi.org/10.1016/j.saa.2012.07.030
20. J. Sádecká and M. Jakubíková, J. Food Sci. Technol. **57**, 2545 (2020). https://doi.org/10.1007/s13197-020-04291-y
21. S. M. Azcarate, G. A. de Araújo, M. R. Alcaraz, et al., Food Chem. **184**, 214 (2015). https://doi.org/10.1016/j.foodchem.2015.03.081
22. D. Airado-Rodríguez, I. Durán-Merás, T. Galeano-Díaz, and J. P. Wold, J. Food Compos. Anal. **24**, 257 (2011). https://doi.org/10.1021/jf8033623
23. R. Ríos-Reina, J. M. Camiña, R. M. Callejón, and S. M. Azcarate, TrAC Trends Anal. Chem. **134**, 116121 (2021). https://doi.org/10.1016/j.trac.2020.116121