ORIGINAL PAPER

CHARACTERIZATION OF RED GRAPES SKIN EXTRACTS USING VIBRATIONAL SPECTROSCOPY AND CHEMOMETRICS

GEORGETA-CARMEN ALECU (HOLBAN)[1], RADU LUCIAN OLTEANU[2]*, CRISTIANA RADULESCU[2,3*], RALUCA MARIA STIRBESCU[2], CEZARINA NECULA[1,4], DOINA-NICOLETA BOBOACA-MIHAESCU[1]

Manuscript received: 02.04.2020; Accepted paper: 12.06.2020; Published online: 30.06.2020.

Abstract. *The study aims a chemometric-based vibrational spectroscopy investigation, Fourier transform infrared (FTIR) and Raman spectroscopy, on the hydroalcoholic extracts obtained from four variety of red grapes (skin). The multivariate analysis, by combining both principal component analysis (PCA) and linear discriminant analysis (LDA) approaches (called PC-LDA model), applied on the FTIR and Raman spectral data was performed to determine the differences in the chemical composition according to the red grapes variety and the type of vineyards (i.e. conventional or traditional and ecological), correlated also with antioxidant activity and total phenolic content values of the extracts. By applying PC-LDA model a separation between extracts from different vineyads can be observed. A well-defined differentiation based on antioxidant activity and total phenolic content could not be highlighted. Among the extracts, those obtained from ecological varieties showed the greatest similarity, indicating that these extracts have equivalent chemical composition.*

Keywords: *red grapes skin extracts, vibrational spectroscopy, multivariate analysis, PC-LDA model*

1. INTRODUCTION

Grapes contain a large variety of nutrients, such as minerals, vitamins, carbohydrates, fibers and phytochemicals. World maket, due to the increase and constant requirements for ecological products with both high quality and productivity aspects, require the application of newer and safer methods for characterization of vine genetic resources and for improvement of amelioration process of it [1]. The applied treatment can influence the plants growth but also the chemical composition [2, 3], the main differences between ecological and conventional grapes cultivation consisting in the type of periodical bio-chemical treatments.

Polyphenols are ones of the most important phytochemicals in grapes, possessing many biological activities and health-promoting benefits [3, 4]. Specific efficient extraction of phenolic compounds from the different grape parts (skin, steam, leaf and seed) may be a source of potential bio-resources for industries like pharmaceuticals and cosmetics, as well as

[1] University of Agronomic Sciences and Veterinary Medicine of Bucharest, Doctoral School, 011464 Bucharest, Romania.
[2] Valahia University of Targoviste, Institute of Multidisciplinary Research for Science and Technology, 130004 Targoviste, Romania.
[3] Valahia University of Targoviste, Faclulty of Sciences and Arts, Sciences and Advanced Technologies Department, 130004 Targoviste, Romania.
[4] Valahia University of Targoviste, Faculty of Sciences and Engineering, 140003 Alexandria, Romania.
* *Corresponding authors:* radu.olteanu@valahia.ro; radulescucristiana@yahoo.com.

in other biomedical and biotechnological applications [1, 3]. The phenolic compounds can be flavonoids or non-flavonoids, flavonoids being the most numerous in the plant kingdom [5]. The flavonoids, depending on the oxidation state of the central pyran ring, can themselves be subdivided into many subclasses such as anthocyanidins, flavones, flavonols, flavanones, flavanols and isoflavones [6]. The main subclasses of flavonoids (flavonols, flavanols and anthocyanins) present in red grapes (*Vitis vinifera* L.) can be found in the seeds and berry skins, being transferred to the wine during the fermentation process [5]. Whereas flavanols are present primarly in seeds and stems, flavonols and anthocyanins are mainly localized in the skin [5, 7]. The three main groups of non-flavonoids are represented by phenolic acids (derivatives of benzoic and cinnamic acids), stilbenes (whos main representative is resveratrol) and lignans [5, 6, 8].

The antioxidative characteristics, being most notable bioactivity of phenolic compounds from grapes [3, 9], have been widely studied, including scavenging of free radicals, inhibition of lipid oxidation, reduction of hydroperoxide formation, and so on [3, 9, 10]. The antioxidant activity of red wines is also associated with polyphenols (flavonoids, phenolic acids, stilbenes, coummarines) content, the phenolic composition varying greatly due to environmental and climate condition, grape variety, degree of ripness, soil type [8, 11]. Reknown compounds with strong antiradical / antioxidant activity, such as resveratrol, quercetin and rutin are generally found in grape skin extracts, the values of antioxidant activity being very different in the extracts from different parts of grapes, and even pomace having a notable antioxidant capacity [3, 8, 12].

Vibrational spectroscopy combined with chemometrics can lead to a good prediction of chemical composition of different plant extracts, the multivariate analysis can be used to maximize the information obtained from spectral datasets and, therefore, to evaluate different types of extracts [13, 14]. In addition between the advantages of attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) can be mentioned the scanning of a broad region of the spectrum in a short amount of time at high spectral resolution and a good signal-to-noise ratio [14-19]. Previous studies shown that appropriate application of chemometrics has provide accurate and fast characterization of plant extract based on analytical spectroscopy data that contain multidimensional information [20, 21].

This study investigated the chemical composition of four red grape skin extracts, to identify possible correlations between the vineyard type (conventional and ecological), grape varieties (Merlot, Feteasca Neagra, Pinot Noir and Muscat Hamburg) and hydroalcoholic extracts characteristics (i.e. antioxidant activity and total phenolic content). In this respect, the multivariate analysis, by combining both principal component analysis (PCA) and linear discriminant analysis (LDA) approaches (called PC-LDA model), applied on the FTIR and Raman spectral data was performed to determine the differences in the chemical composition according to the red grapes variety and the type of vineyards correlated also with antioxidant activity and total phenolic content values of the extracts.

2. MATERIALS AND METHODS

2.1. MATERIALS

All chemicals and reagents were used as purchased from commercial suppliers (MilliporeSigma, USA and Chimopar, Romania, respectively) and were of the highest available purity. Particularly, 2,2-diphenyl-1-picrylhydrazyl (DPPH), polyphenolic standards

(analytical grade; purity ≥99%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Gallic acid, Folin-Ciocaltau reagent, and ethanol (absolute solvent) were purchased from Merck (Darmstadt, Germany). Ultrapure water (Thermofisher Scientific, Germany) was used to prepare standard solutions and blanks. All other chemicals were analytical reagent grade.

2.2. METHODS

Sampling

Grapes of four varieties (Merlot, Feteasca Neagra, Pinot Noir and Muscat Hamburg) were collected from two different vineyards from Romania: conventional culture (with various pesticides treatments applied) and respectively ecological culture. Raw products of same batch were used during tests, skin being manually separated from each sampled grape variety and vineyard.

Extraction procedure

Hydroalcoholic extracts of studied grapes varieties were obtained from berry skins that were first dried at 40ºC for 48 hours, and then stored for further experiments at room temperature. The ultrasound assisted method at room temperature was applied. The extractive solutions consist in 50 % water - 50% solvent. The time of extraction was 16 minutes for each sample for a ratio crush dried berry skin: solvent = 1:10.

FTIR and Raman spectroscopy

The qualitative composition of red grapes extracts was characterized by vibrational spectroscopy, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and Raman spectroscopy. Molecular investigation of functional groups of organic compounds presents in the extracts was performed by ATR-FTIR using a Vertex 80v spectrometer (Bruker, Ettlingen, Germany), equipped with diamond ATR crystal accessory, for high refractive index bulk sample. The diamond ATR had a sampling area of approximately 0.5 mm^2, and the infrared spectra were collected at 4 cm^{-1} resolution over 128 scans. The important absorption frequencies were noted in the range of 4000–800 cm^{-1}, as well as the fingerprint region of the spectra, with a resolution of 0.2 cm^{-1} and 0.1% accuracy (transmittance). Raman spectral data were recorded with Xantus-2™ portable Raman spectrometer (Rigaku, Shibuya, Japan), with λ = 1064 nm. The samples were analyzed, without previous treatment, in triplicate, being used the average spectra.

Ultraviolet-visibile spectroscopy

Antioxidant activity (AA) and total phenolic content (TPC) were determined in freshly hydroalcoholic extracts using Evolution™ 260 Bio UV-Visible spectrophotometer (Thermo Scientific, Madison USA) equipped with glass cuvettes of 1 cm.

Total phenolic content was evaluated by the Folin-Ciocalteu colorimetric assay described in the literature [22], slightly modified. The concentrations were calculated using the calibration curve drawn before each test set, in the concentration range of 0.1–1.0 mg/mL of gallic acid (GA), used as reference. Appropriate dilutions were made as needed from grape extracts, therefore that samples absorbances fit the calibration curve range. TPC were expressed as mg GA equivalents per mL of hydroalcoholic grape extract. The antioxidant activity potential of different red grapes extracts was evaluated using 1,1-d-2-picrylhydrazyl (DPPH) method described in a previous research [23]. Analytical data were collected on triplicate samples, mean values being reported in Table 3.

Multivariate analysis

Principal component analysis, as a multivariate technique, was used to reduce a large set of variables to smaller one that still contains most of the information from the initial set. The procedure transforms a number of possibly correlated variables into a smaller number of uncorrelated variables (i.e. principal components) [14, 21, 24]. Linear discriminat analysis used as a tool for classification, dimension reduction and data vizualization, can provide interpretable classification results despite its simplicity [25, 26].

The software XLSTAT 2019.2.2.599941 version (21.2.59941 – Copyright Addinsoft 1995-2019) was used. The PCA and LDA techniques were used for multivariate analysis of the ATR-FTIR and Raman spectra in spectral range 4000-800 cm^{-1} and 1700-400 cm^{-1}, respectively.

3. RESULTS AND DISCUSSION

3.1. RESULTS

Infrared spectral assay

FTIR data for extract samples from ecological vineyard type have shown similarities concerning spectra in the wavenumber range of 4000-400 cm^{-1} (Table 1, Fig. 1b); the observed peaks were assigned to different bonds according with their chemical structures.

Table 1. Tentative assignments of significant peaks from FTIR spectra of red grapes skin extracts based on grape variety and vineyard type

Grape variety	Sample code	Vineyard type	Wavenumber [cm^{-1}] and relative intensity[a]	
Merlot	v1-C	Conventional	3293/2979/2925/2855/1742/1643/1454/1417/1383/1324/1274/1155/1085/1044/877	s/m/w/w/w/m/w/w/w/w/w/w/m/s/m
	v1-E	Ecological	3293/2979/2925/2855/1742/1643/1454/1417/1383/1324/1274/1155/1085/1044/877	s/m/w/w/w/m/w/w/w/w/w/w/m/s/m
Feteasca Neagra	v2-C	Conventional	3293/2979/2120/1643/1454/1417/1383/1324/1274/1085/1044/877	s/m/w/m/w/w/w/w/w/m/s/m
	v2-E	Ecological	3293/2979/2120/1643/1454/1417/1383/1324/1274/1085/1044/877	s/m/w/m/w/w/w/w/w/m/s/m
Pinot Noir	v3-C	Conventional	3280/2975/2935/2882/1645/1460/1415/1331/1272/1234/1130/1040/990/922/835	s/w/w/w/m/w/w/w/w/w/m/s/m/m/w
	v3-E	Ecological	3282/2976/2936/2883/1645/1459/1414/1379/1334/1292/1234/1136/1079/1040/990/922/836	s/w/w/w/m/w/w/w/w/w/w/m/m/s/m/m/m
Muscat Hamburg	v4-C	Conventional	3282/2977/2932/2884/1643/1459/1414/1380/1334/1234/1136/1075/1040/990/922/837	s/w/w/w/m/w/w/w/w/w/w/m/m/s/m/m/w
	v4-E	Ecological	3282/2977/2932/2884/1643/1459/1414/1380/1334/1234/1136/1075/1040/990/922/837	s/w/w/w/m/w/w/w/w/w/w/m/m/s/m/m/w

[a]*(s) strong; (m) medium; (w) weak.*

In the spectral region 3300-2100 cm^{-1}, the spectral characteristics indicate a differentiation of the extracts into two categories corresponding to the Merlot (v1-E) and Feteasca Neagra (v2-E) and Pinot Noir (v3-E) and Muscat Hamburg (v4-E) varieties, respectively. The differentiation appears especially for the peaks recorded at 3293, 3282 and 2884 cm^{-1}. The spectral bands in the range 3500-3100 cm^{-1} can be attributed to the cumulative streching vibrations of the -OH groups, a characteristic aspect of polyphenolic extracts [13, 27- 29]. Usually in this spectral range polyphenolic extracts have vibration bands similar to acids; however, the amount of vibrational contributions of the -OH groups is actually recorded. The spectral band located at approximately 2979 cm^{-1} can be assigned with the solvent (ethanol) being associated with the stretching vibrations of the -OH groups [30]. In the range 2932-2925 cm^{-1} the bands present in the FTIR spectra can be associated with the stretching vibrations CH, CH_2 and CH_3 due to carbohydrates; however, the spectral range 3500-3100 cm^{-1} doesnt show obvious aspects to identify the nature of the extracts [13]. Generally, in the spectral range 3500-3200 cm^{-1} in the case of vegetal tissues the bands are associated with O-H and N-H stretching vibrations from carbohydrates, proteins, alcohols and phenolic compounds [31]. The peak at 2855 cm^{-1} can be associated with CH_2 symmetrical stretching vibration (mainly lipids and reduced contributions due to proteins and carbohydrates) due to the presence of aliphatic compounds in the cuticle (cutin, waxes) [5] and that of 1742 cm^{-1} due to stretching vibration C=O in saturated esters [32].

In the spectral range 1650-1200 cm^{-1}, the presence of the peaks common to all extracts at 1644, 1456, 1415, 1380 and 1330 cm^{-1} can be noticed. Similar to the first spectral domain analyzed (3300-2100 cm^{-1}) there is also a differentiation between the varieties Merlot (v1-E) and Feteasca Neagra (v2-E) and respectively Pinot Noir (v3-E) and Muscat Hamburg (v4-E) relative to the peaks recorded at 1274 cm^{-1} and 1234 cm^{-1}, respectively; the peak at 1234 cm^{-1} can be associated with the bending vibrations of methyl groups [1]. The spectral bands

associated with the aromatic streching vibrations C=C-C appear in the range 1611-1444 cm^{-1} and those assigned with the C-O streching vibrations in the range 1368-1157 cm^{-1} and 1031-1023 cm^{-1} [13, 27, 28, 32-34]. The medium intensity peak at 1644 cm^{-1} can be associated with the aromatic C=C streching vibrations due to condensed tannins [13] as well to the C=O streching vibrations and the presence of unsaturated bonds in flavonoid structures [29, 31, 35]; the presence of this peak in all extracts suggests the presence of both flavones and flavanones [35].

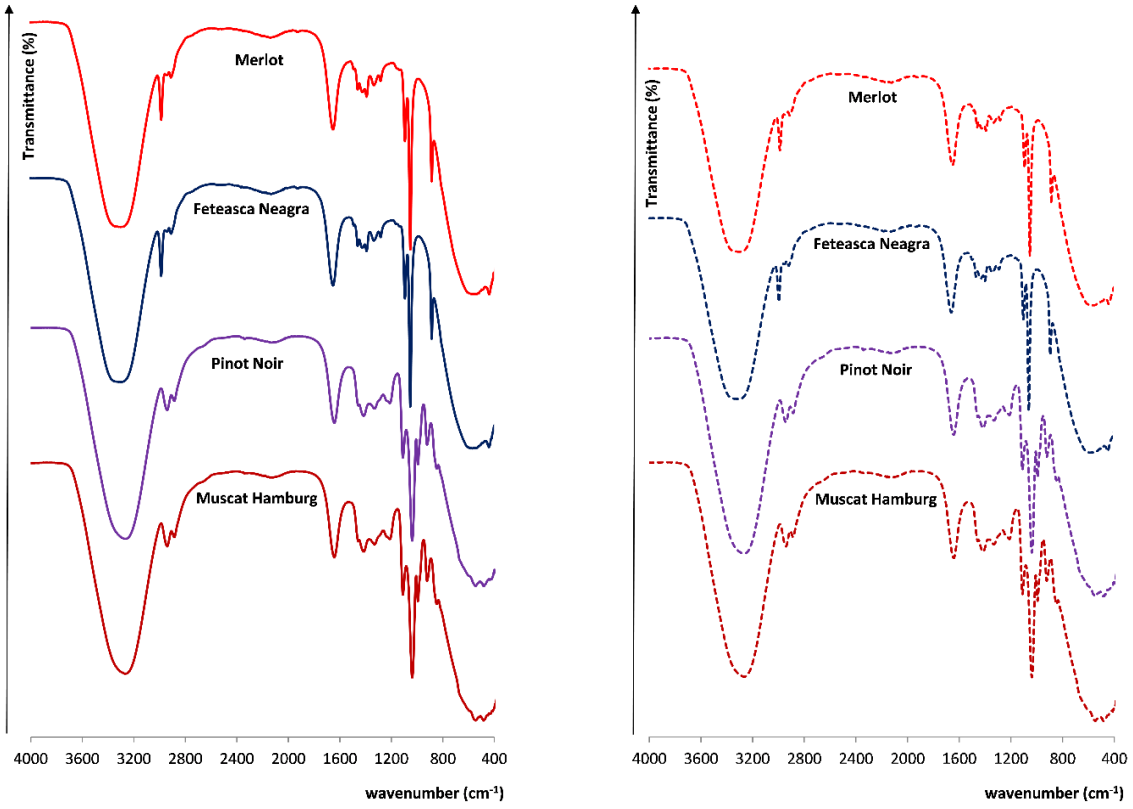

Figure 1. Overlaps of Fourier transform infrared (FTIR) spectra for investigated extracts obtained from different vineyard: (a) conventional and (b) ecological.

Low intensity bands from 1456 cm^{-1} and 1415 cm^{-1} can be associated with C-H bending vibrations of CH_2 and CH_3 groups (polysaccharides associated with cell walls, lipids and proteins) [31], C=C-C stretching vibrations associated with the aromatic nucleus [36] and respectively O-H bending vibrations (polysaccharides associated with cell walls, alcohols and carboxylic acids) [31]; the spectral band at 1456 cm^{-1} can be due to the C-H bending vibrations of the CH_2 and CH_3 groups and the bending vibrations associated with the aromatic cycles (flavonoids) [30]. Spectral bands from 1380 cm^{-1} and 1234 cm^{-1} can be associated with symmetrical C-H bending vibrations of CH_2 and CH_3 groups (polysaccharides associated with cell walls, lipids and proteins) [31], O-H planar deformation from polyphenolic compounds [36] and C-O stretching vibrations, respectively [31].

The bands present in the spectral range 1134-1068 cm^{-1} are associated to the aromatic C-H planar deformation [27, 32, 34] and tertiary (1136 cm^{-1}) or secondary (1085 cm^{-1}) alcohols [30]. In the spectral range 923-773 cm^{-1} the medium intensity spectral bands can be associated with out-of-plane aromatic C-H bending vibrations [13]. Spectral peaks from 1155 cm^{-1} and 1085 cm^{-1} may be due to symmetrical stretching vibrations of CH_2, O-H or C-O associated with different groups (cell wall-associated polysaccharides) and C-O deformation vibrations (secondary alcohols, aliphatic esters), respectively [31, 37]. Stretching vibrations (polysaccharides from cell walls) due to O-H and C-OH groups can be associated with the

peak present at 1044 cm^{-1} [37]. The peaks present in the FTIR spectra at 990, 922 and 877 cm^{-1} can be associated with C-O stretching vibrations (cell walls), C-H deformation vibrations and C-O stretching vibrations (monosaccharides) [31] and C-C respectively [30]. The differentiation of the extracts into two categories corresponding to the varieties Merlot (v1-E) and Feteasca Neagra (v2-E) and Pinot Noir (v3-E) and Muscat Hamburg (v4-E), respectively, is much more evident in this spectral range (1150-830 cm^{-1}). For the first two extracts mentioned above, the peaks present at 1085 cm^{-1} and 877 cm^{-1} are highlighted, while the spectra of v3-E and v4-E extracts show distinct peaks at 1136, 1079, 990, 922 and 837 cm^{-1}. It is also noted the presence of the intense peak located at about 1044 cm^{-1} in the spectrum of all investigated extracts.

The FTIR spectra recorded for extracts obtained from red grape varieties in conventional culture (Table 1, Fig. 1a) contain spectral characteristics in the range 3300-2100 cm^{-1} that allow a differentiation into two categories, the first combining Merlot (v1-C) and Feteasca Neagra (v2-C) extracts and the second one, Pinot Noir (v3-C) and Muscat Hamburg (v4-C) extracts. There are revealed peaks of high intensity at 3293 cm^{-1} and 3282 cm^{-1} and low intensity at 2884 cm^{-1}. It is also observed the presence of a common peak to all extracts at 2979 cm^{-1} as well as the appearance of low intensity peaks at 2935, 2855 and 2120 cm^{-1}. In the spectral range 1650-1200 cm^{-1} there are no significant features for investigated extracts, being present common low intensity peaks at 1644, 1456, 1415 and 1330 cm^{-1}. The differentiation of the extracts into two categories corresponding to the varieties Merlot (v1-C), Feteasca Neagra (v2-C) and Pinot Noir (v3-C), Muscat Hamburg (v4-C), respectively, is more evident in the 1150-830 cm^{-1} spectral range. In the case of the first two extracts, peaks present at 1085 cm^{-1} and 877 cm^{-1} are highlighted, while the spectra of v3-C and v4-C extracts show distinct peaks at 1136, 990, 922 and 837 cm^{-1}; the intense peak located at approximately 1044 cm^{-1} in the spectrum of all investigated extracts was also noted.

The analysis of the FTIR spectral characteristics based on the vineyard type, ecological and conventional, indicates similarities in the case of all investigated extracts (relative to the position and intensity of the recorded peaks). However, there are also specific features in the case of Pinot Noir extracts (v3-E and v3-C) in the spectral ranges 1650-1200 cm^{-1} and 1150-830 cm^{-1} relative to the presence / absence of peaks in the FTIR spectrum of those two extracts.

Raman data for extract samples from both conventional (Table 2, Fig. 2a) and ecological (Table 2, Fig. 2b) vineyard type have been analysed in a similar manner as FTIR data, concerning spectral features in the 1650-400 cm^{-1} spectral range; the observed peaks were assigned to different bonds according with their chemical structures.

In the spectral range 1650-1200 cm^{-1} (Raman spectra of the ecological extracts), the Merlot extract (v1-E) shows distinct spectral bands at 1475, 1452, 1393 and 1270 cm^{-1}. The rest of the extracts (v2-E, v3-E and v4-E) have similar spectral characteristics. The peak from 1294 cm^{-1} can be associated with CH_2 twisting vibrations (aliphatic compounds in the skin cuticle) [5]. The peak at 1634 cm^{-1} associated with aromatic C=C stretching vibrations suggests the presence of anthocyanins in the extract [37] and the intense peak at 1332 cm^{-1}, related with O-H bending vibrations of the C-OH group in the aromatic cycle [38], may be due to the presence of flavonols (quercetin) [37].

Table 2. Tentative assignments of significant peaks from Raman spectra of red grapes skin extracts based on grape variety and vineyard type.

Grape variety	Sample code	Vineyard type	Raman shift [cm^{-1}] and relative intensity[a]	
Merlot	v1-C	Conventional	434/501/749/880/1047/1088/1270/1393/1452/ 1475/	m/w/w/s/m/m/m/w/ s/w/
	v1-E	Ecological	434/501/749/880/1047/1088/1270/1393/1452/ 1475/	m/w/w/s/m/m/m/w/ s/w/
Feteasca Neagra	v2-C	Conventional	434/520/650/705/802/837/923/998/1039/1080/ 1129/1231/1294/1309/1332/1460/1634	m/s/w/w/m/s/m/m/ w/s/w/m/m/m/s/w
	v2-E	Ecological	434/520/650/705/802/837/923/998/1039/1080/ 1129/1231/1294/1309/1332/1460/1634	m/s/w/w/m/s/m/m/ w/s/w/m/m/m/s/w
Pinot Noir	v3-C	Conventional	434/482/668/846/914/981/1047/1088/1263/1301/ 1467/1627	m/s/m/s/m/w/s/w/m /w/s/w
	v3-E	Ecological	434/520/650/705/802/837/923/998/1039/1080/ 1129/1231/1294/1309/1332/1460/1634	m/s/w/w/m/s/m/m/ w/s/w/m/m/m/s/w
Muscat Hamburg	v4-C	Conventional	434/520/650/705/802/837/923/998/1039/1080/ 1129/1231/1294/1309/1332/1460/1634	m/s/w/w/m/s/m/m/ w/s/w/m/m/m/s/w
	v4-E	Ecological	434/520/650/705/802/837/923/998/1039/1080/1129/ 1231/1294/1309/1332/1460/1634	m/s/w/w/m/s/m/m/ w/s/w/m/m/m/s/w

[a](s) strong; (m) medium; (w) weak.

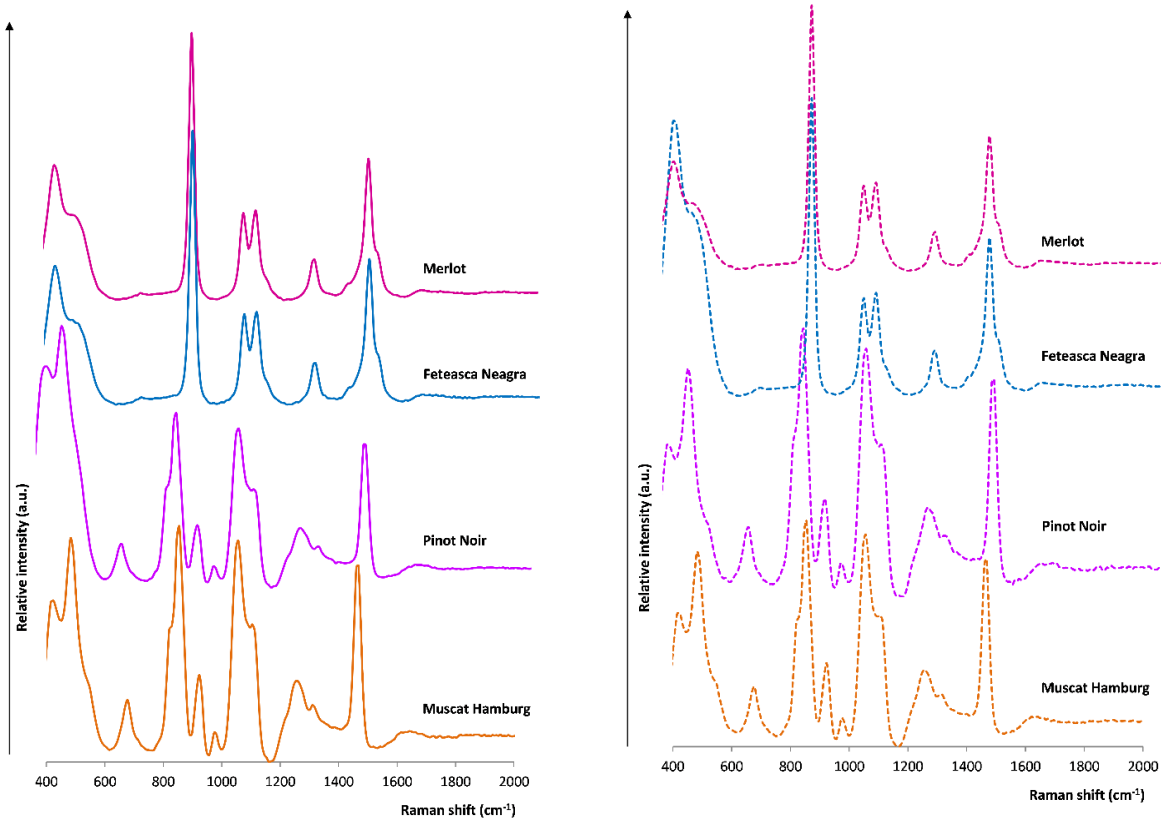

Figure 2. Overlaps of Raman spectra for investigated extracts obtained from different vineyard: (a) conventional and (b) ecological.

The specific spectral features of v1-E extract can be observed also in the spectral range 1150-830 cm^{-1}; distinct peaks from the rest of the extracts at 1088, 1047 and 880 cm^{-1}. The rest of the extracts (v2-E, v3-E and v4-E) have similar spectral characteristics with peaks at 1129, 1080, 1039, 998, 923 and 837 cm^{-1}. The 1129 cm^{-1} spectral band can be related with C-C, C-O, and C-O-C stretching vibrations due to polysaccharides [5]. The presence of flavonoids is also suggested by the existence of the 998 cm^{-1} peak related with C-C stretching vibrations in the aromatic ring [37]. Similar to the two Raman spectral regions mentioned above, in the spectral range 830-400 cm^{-1}, v1-E extract is differentiated from the rest of the extracts by distinct peaks at 749 and 501 cm^{-1}. The rest of the extracts (v2-E, v3-E and v4-E) have similar spectral characteristics with peaks of vary intensity, situated at 802, 705, 650 and 520 cm^{-1}; the spectral bands in the range 800-700 cm^{-1} can be related with aromatic C-H out-of-plane bending vibrations due to the aromatic cycle [37].

In the spectral range 1650-1200 cm^{-1} there are distinct peaks of variable intensity specific to the extracts obtained from conventional vineyard (Table 2, Fig. 2b): v1-C (1475, 1452, 1393 and 1270 cm^{-1}), v2-C (1332 and 1309 cm^{-1}), v3-C (1627, 1467, 1301 and 1263 cm^{-1}); in the Raman spectrum of the v4-C extract, there are instead a series of common peakss with those of the v2-C extract located at 1634, 1460, 1294 and 1231 cm^{-1}. The peak at 1231 cm^{-1} can be due to aromatic C-C stretching vibrations (aromatic cycle associated with flavones) [37]. The differences between the spectra of the extracts from varieties in conventional culture (v1-C, v2-C, v3-C and v4-C) in the spectral range 1150-830 cm^{-1} are less obvious being present distinct peaks for v1-C (880 cm^{-1}) and v3-C (981, 914 and 846 cm^{-1}) extracts; the two extracts also show common spectral bands located at 1088 and 1047 cm^{-1}. The v2-C and v4-C extracts have similar spectral characteristics highlighted by the presence of variable intensity bands at 1129, 1080, 1039, 998, 923 and 837 cm^{-1}.

In the Raman spectra (spectral range 830-300 cm^{-1}) of v1-C and v3-C extracts are present distinct spectral bands at 749, 501 and 325 cm^{-1} and at 668 and 482 cm^{-1}, respectively. The v2-C and v4-C extracts have similar spectral characteristics highlighted by the presence of low and medium intensity bands at 802, 705, 650 and 520 cm^{-1}. The peaks from 520 cm^{-1} and 501 cm^{-1} can be related with the deformation vibrations of aromatic cycle in flavonoids and the C-C deformation vibrations, respectively [37].

The Raman spectra of hydroalcoholic extracts (conventional type) from red grape skin, present some distinct features for Merlot (v1-C) and Pinot Noir (v3-C) extracts over the entire spectral range as opposed to Feteasca Neagra (v2-C) and Muscat Hamburg (v4-C) extracts with similar spectral characteristics relative to the position and intensity of the spectral bands. The Raman spectral characteristics based on the vineyard, ecological and conventional, indicates similarities for the investigated extracts (relative to the position and intensity of recorded peaks) excepting Pinot Noir extracts (v3-E and v3-C) for which there are differences on the whole investigated spectral domain related to the presence / absence of peaks in the Raman spectrum of the two extracts.

The large data sets, generated from both FTIR and Raman spectroscopy, in wich essential information may not be readily evident, can be more accurate investigated by multivariate analysis. Multivariate analysis provides a means of quantifying constituents that are involved in complex matrices interactions without eliminating matrix interferences [39].

Antioxidant activity and total phenolic content

Table 3 shows the values obtained for antioxidant activity (AA) and total phenolic content (TPC) for the investigated hydroalcoholic extracts. By comparing the AA of hydroalcoholic extracts based on their origin, conventional versus ecological, it was found

that the grape skin extract of Pinot Noir variety had a higher antioxidant activity for the ecological grapes. Merlot, Feteasca Neagra and Muscat Hamburg varieties showed a higher antioxidant activity in grape extracts from conventional vineyard.

Table 3. Antioxidant activity and total phenolic content of red grapes skin extracts based on grape variety and vineyard type.

Grape variety	Sample code	Vineyard type	Antioxidant activity [%]	Total phenolic content [mgGA/mL]
Merlot	v1-C	Conventional	20.0	1.484
	v1-E	Ecological	17.1	1.263
Feteasca Neagra	v2-C	Conventional	22.5	1.288
	v2-E	Ecological	17.6	1.087
Pinot Noir	v3-C	Conventional	19.4	1.099
	v3-E	Ecological	30.4	1.262
Muscat Hamburg	v4-C	Conventional	21.8	0.905
	v4-E	Ecological	16.9	1.130

Total phenolic content values obtained also vary, Merlot and Feteasca Neagra having a higher TPC content for conventional vineyard when compared with Pinot Noir and Muscat Hamburg with higher values for ecological vineyard. It is noticeable that the values of AA and TPC for Muscat Hamburg extracts are not directy correlated like for the rest of the varieties when compared by vineyard type.

It is important to point out that in the extracts there could be different compounds with antioxidant capacity apart from polyphenols, as well as polyphenols or other compounds with a scant or null antioxidant activity [40].

Multivariate analysis

The decomposition of the spectral FTIR and Raman data through PCA indicates, based on the accumulated variability (the sum of percentage of variability explained by that principal component and the preceding one), that with first two principal components (PCs), 87.30% (i.e., FTIR data) and respectively 75.52% (i.e., Raman data) of the total variability of the data studied was included.

In Fig. 3a shows the graph of the PC1 scores (57.13%) versus those of PC2 (30.17%) based on FTIR data. In the score plot, the samples (hydroalcoholic extracts) are arranged in space relative to each other according to their PCs scores. The samples with similar scores will occupy similar position whilst those with dissimilar scores will be positioned some distance away, thus allowing clusters to be identified [14, 41]. From the plot it shows that PC1 separates v3 and v4 extracts (conventional vineyard type) having positive scores, from the rest ones (negative scores). PC2 allow differentiation between grapes varieties into two clusters, first containing v1 and v2 varieties (positive scores) and second one v3 and v4 (negative scores). It can notice also that due to PC2, a grouping based on vineyard type especially for v3 and v4 extracts.

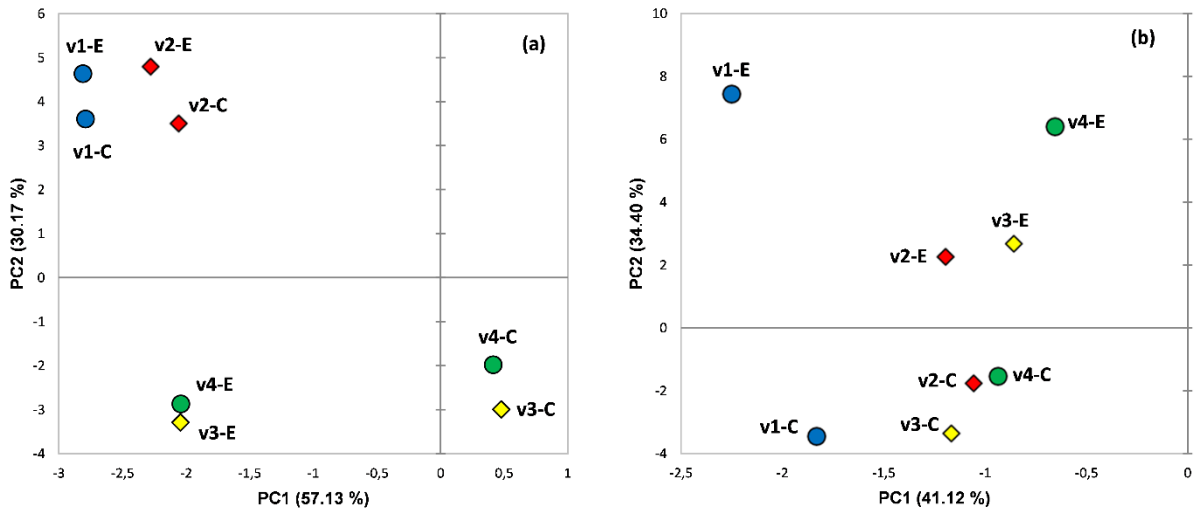

Figure 3. PCA score plots for FTIR (a) and Raman data (b)

The plots of the decomposition of the Raman spectral data through PCA for the first two PCs (75.52% of the total variability of the data studied accumulated) are illustrated in Fig. 3b. Figure 3b shows the graph of the PC1 scores (41.12%) versus those of PC2 (34.40%). From the plot it is show that PC2 separates ecological extracts (positive score) from the conventional (negative scores). It can be observed a clustering between v2-E, v3-E and v4-E who are grouped excepting v1-E, allowing the distinction of v1-E from the rest of ecological extracts. From the conventional type extracts v1-C and v3-C are distinct from the the v2-C and v4-C who are clusterd tight together. Comparing same plots (PC1 versus PC2) for FTIR and Raman data (Fig. 3) can be seen that PC2 is clustering mainly ecological and conventional extracts v3 and v4 (FTIR data – negative for v3-E, v3-E / v3-C, v4-C positive scores, Raman data – positive for v3-E, v3-E / v3-C, v4-C negative scores); this feature suggest once more FTIR and Raman data complementarity.

The degree of influence that an observation has on a component is measured by the fraction of the inertia carried by this component that is due to the observation (called the contribution of this observation, usually displayed by software). In this respect, an observation may have a high contribution to a component, and yet have a negligible contribution to another component [14]. Although interpretation of a PCA insists on observations that contribute much to the inertia (of a principal component, or of the Principal Plane) and observations that are well represented on a principal component, or on the Principal Plane (squared cosine close to 1), these two concepts are not equivalent.

For outlier detection multidimensional tests were applied; they are used to compare samples described by several variables: Mahalanobis distance (the Bonferroni correction was used for the alpha significance level set to 5%), Wilk's lambda (Rao's approximation), Box and Kullback's tests (for testing the equality of the within-groups covariance matrices). Based on results of the PCA plot (FTIR and Raman data), the tests were performed assigning four groups based on grape varieties (two extracts in each group). Tests on averages identify the difference: the test of the Wilks' lambda concludes that there is a significant difference between the groups' means (at least one of the means vector is different from another, as the computed p-value was lower than the significance level alpha).

By using PCA on spectral data was possible to identify some important structural information in FTIR and Raman datasets by obtaining patterns which compactly represent the data. Often, interpretation of the complex biochemical information obtained through vibrational spectroscopic techniques requires further data analysis using supervised procedures [14, 28, 32, 43].

After PCA was applied on the original FTIR and Raman datasets the first two principal components scores were retained for further analysis by combining both PCA and linear discriminant analysis (LDA) approaches (PC-LDA model); the model improves the efficiency of classification as it automatically finds the most diagnostically significant features [23, 43]. PC-LDA was applied onto all extracts; the significance level was set at 5% and stepwise (forward) selection method was used (threshold value at 0.05 to enter and respectively at 0.10 to be removed of the model). The two Box's tests (Chi-squared asymptotic approximation and Fisher's F asymptotic approximation) confirm that was needed to reject the hypothesis that the covariance matrices are equal between the groups.

Table 4. Prior and posterior classification of the grape skin extracts using PC-LDA (FTIR data).

Observation / Extract	***Prior***	***Posterior***	***Membership probabilities***	
			Pr(conventional)	Pr(ecological)
v1-C	conventional	conventional	1.000	0.000
v1-E	ecological	ecological	0.000	1.000
v2-C	conventional	conventional	1.000	0.000
v2-E	ecological	ecological	0,000	1.000
v3-C	conventional	conventional	1.000	0.000
v3-E*	ecological	ecological	0.000	1.000
v4-C	conventional	conventional	1.000	0.000
v4-E	ecological	ecological	0.000	1.000
*validation set				

Table 5. Prior and posterior classification of the grape skin extracts using PC-LDA (Raman data).

Observation / Extract	***Prior***	***Posterior***	***Membership probabilities***	
			Pr(conventional)	Pr(ecological)
v1-C	conventional	conventional	1.000	0.000
v1-E	ecological	ecological	0.000	1.000
v2-C	conventional	conventional	1.000	0.000
v2-E	ecological	ecological	0.000	1.000
v3-C	conventional	conventional	1.000	0.000
v3-E*	ecological	ecological	0.000	1.000
v4-C	conventional	conventional	1.000	0.000
v4-E	ecological	ecological	0.000	1.000
*validation set				

Table 4 (FTIR data) and Table 5 (Raman data) lists for each observation (grape extract) the probability to belong to each group; the probabilities are posterior probabilities that consider the prior probabilities through Bayes formula. As it can be noticed all the extracts, according to the type of vineyard (conventional and ecological), have not been reclassified. By representing the observations on the factor axes (the variance represented with the first factor was 93.32% for FTIR and respectively 73.98% for Raman data) it allows to confirm that the extracts are discriminated on the factor axes (Fig. 4).

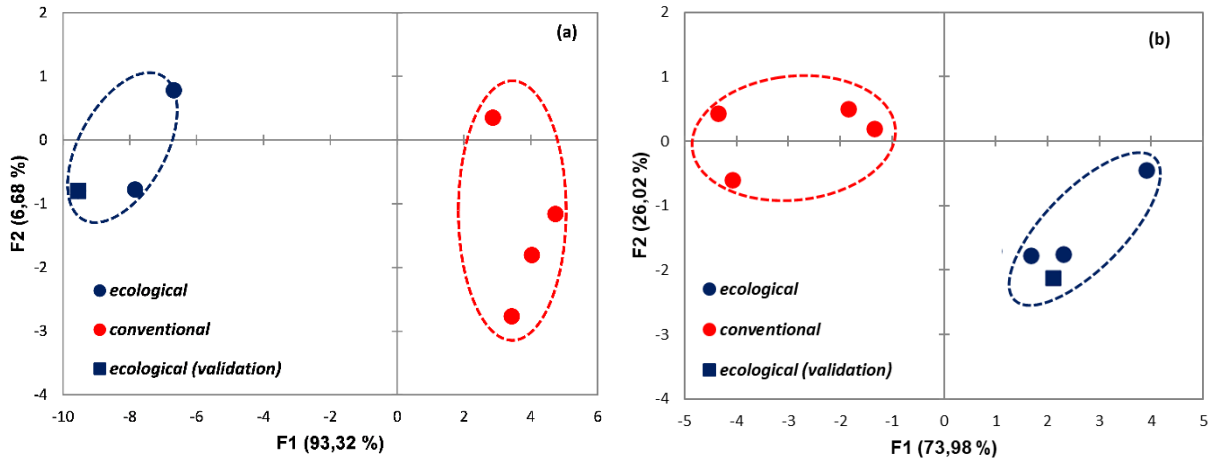

Figure 3. PC-LDA score plots for FTIR (a) and Raman data (b).

The confusion matrices based on FTIR (Table 6) and Raman data (Table 7), also called classification tables, summarizes the reclassification of the observations / extracts and allows to see that are well classified.

Table 6. Confusion matrix for the estimation sample (FTIR data).

From / to	*Conventional*	*Ecological*	*Total*	*% correct*
Conventional	4	0	4	100.00
Ecological	0	4	4	100.00
Total	4	4	8	100.00

Table 7. Confusion matrix for the estimation sample (Raman data).

From / to	*Conventional*	*Ecological*	*Total*	*% correct*
Conventional	4	0	4	100.00
Ecological	0	4	4	100.00
Total	4	4	8	100.00

Cross-validation allows to see what would be the prediction for a given observation / extract if it is left out of the estimation sample (leave-one-out method): the parameter optimisation is performed on 7 of the 8 observations and then the performance of the tuned algorithm is tested on the 8^{th} observation (in this step, the 8^{th} observation is the test set and the other nine are the training data for optimising the free parameters of the algorithm); the process is repeated 10 times, each time leaving out a different observation to use as the single test case.

As can be seen (Tables 8 and 9) the same two observations for both FTIR and Raman datasets (v3-C and v3-E) are miss-classified (either the corresponding solvent allow extraction of same main compounds for both extracts or some information necessary to discriminate the extracts is not available). Also as is pointed out by other studies [13, 39, 44] although fundamental absorptions or vibrations are visualized as intense bands or peaks, the infrared spectrum is complex due the presence of three types of bands: weak overtone, combination and, difference bands [39].

Table 8. Cross-validation (FTIR data): prior and posterior classification and membership probabilities.

Observation / Extract	*Prior*	*Posterior*	*Conventional*	*Ecological*
v1-C	conventional	conventional	1.000	0.000
v1-E	ecological	ecological	0.000	1.000
v2-C	conventional	conventional	1.000	0.000
v2-E	ecological	ecological	0.000	1.000
v3-C	conventional	ecological	0.000	1.000
v3-E	ecological	conventional	1.000	0.000
v4-C	conventional	conventional	1.000	0.000
v4-E	ecological	conventional	1.000	0.000

Table 9. Cross-validation (Raman data): prior and posterior classification and membership probabilities

Observation / Extract	*Prior*	*Posterior*	*Conventional*	*Ecological*
v1-C	conventional	conventional	1.000	0.000
v1-E	ecological	ecological	0.000	1.000
v2-C	conventional	conventional	1.000	0.000
v2-E	ecological	ecological	0.000	1.000
v3-C	conventional	ecological	0.000	1.000
v3-E	ecological	conventional	1.000	0.000
v4-C	conventional	conventional	1.000	0.000
v4-E	ecological	conventional	1.000	0.000

4. CONCLUSIONS

In this study, *Vitis vinifera* L. berry skin extracts obtained from different varieties (Merlot, Feteasca Neagra, Pinot Noir and Muscat Hamburg) and two types of vineyards (conventional and ecological), were analyzed by FTIR and Raman spectroscopy combined with multivariate analyses. ATR-FTIR and Raman spectroscopic techniques are nondestructive, clean and fast without supplementary reagents and pre-treatment of the samples, and were able in differentiation of the extracts, providing structural information on molecular features of a large range of compunds. By applying PC-LDA model a separation between extracts from different vineyads can be observed. A well-defined differentiation based on antioxidant activity and total phenolic content could not be highlighted. Among the extracts, those obtained from ecological varieties showed the greatest similarity, indicating that these extracts have equivalent chemical composition. The constant interest in the biological activities of ecological grape and grape by-products contribute to their valorization as a source of bioactive phytochemicals with potential application in cosmetic, pharmaceutical, and food industries.

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