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ORIGINAL ARTICLE

Study of the impact of vine cultivation technology on the Feteasca Neagra wine phenolic composition and antioxidant properties

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Abstract In this work a comparative assessment was performed on individual and total polyphenols and biochemical properties of some Feteasca Neagra red wines obtained from grapes cultivated with different farming technologies (organic vs. conventional). The effect of a 30% cluster thinning treatment in both organic and conventional vineyard, compared to control plots with no thinning, was also monitored. The wines were obtained during two vintages, one with more favourable climatic conditions and one less favourable, in the period 2010–2019. Our results indicate that by applying a 30% cluster thinning treatment in the vineyard it is possible to increase the concentration of total and individual polyphenols of the resulted Feteasca Neagra wines. Furthermore, the differences observed between the phenolic profiles of wines from conventionally and organically produced grapes showed that organic Feteasca Neagra wines have higher phenolic compounds concentrations and antioxidant properties, with some important individual phenols accumulating in larger quantities than in the case of conventional wines. The beneficial effect of the organic cultivation is more evident in years characterised by less favourable climatic conditions. The statistical analysis tools applied to the analytical data allowed a good discrimination of experimental wine variants according to the vine cultivation technology (organic vs. conventional, with and without cluster thinning) and vintage. Thus, the results indicated that the applied cultivation technologies, especially when both organic cultivation and cluster thinning are combined, can greatly improve the polyphenolic content of Feteasca Neagra wines. The absolute value of the increase in polyphenol concentration was higher in favourable years, but the relative increase, in percentages, as compared to control wines, was higher in less favourable years. The combination of both viticultural practices may be thus exploited in order to obtain wines with higher polyphenolic content, which leads to better structure, better ageing potential, enhanced nutritional and antioxidant properties.

Keywords Red wines · Phenolic compounds · Antioxidant activity · Organic versus conventional · Cluster thinning

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Introduction

Wine is a widely consumed beverage, with thousands of years of tradition. Over time, by applying specific vineyard and winemaking technologies, the quality of wine substantially increased. Generally, wine quality depends on its chemical composition, and in the specific case of red wines quality is strongly related to the type and amount of

polyphenolic compounds, which play specific roles (Prajitna et al. 2007; Villamor and Ross 2013), such as improving the colour intensity and stability, the structure, and the mouthfeel of the wines, as well as their ageing potential. Moreover, due to their potential beneficial effects on human health, raising the concentration of these compounds is highly desirable and researched. Several authors have demonstrated that wine-derived phenolic compounds have major implications on human health (Antoce and Stockley 2019), with proven effects on reducing all-cause-mortality risk (Grosso et al. 2017), on prevention or alleviation of chronic diseases such as cardiovascular disease (Teissedre et al. 2018) or diabetes (Luz et al. 2018), on improving cognitive function (Mehlig et al. 2008) and so on. Many mechanisms are involved in the beneficial health effects of polyphenols, some of them being based on their antioxidant activity (Yoo et al. 2010). Moderate consumption of red wine contributes to the increase of the antioxidant defence status of the human organism and lowers the oxidative stress (Košmerl et al. 2013). The rise in consumer awareness regarding the food-health relationship encouraged the development of products rich in antioxidants, including wine with higher amounts of polyphenols.

Although the biosynthesis of polyphenols is complex and predominantly dependent on genetic expression in plants (in our case in grapevine), the quantity and types of phenolic compounds present in wine can be influenced by three main factors: *(i)* the nature of the raw material, including grape cultivar, grape maturation level at harvest time, vine cultivation technology, soil and climatic conditions (Obreque-Slier et al. 2010; Cravero et al. 2012; Giuffrè 2013), *(ii)* the winemaking process (Coletta et al. 2014) and *(iii)* phenolic compounds evolution during wine ageing (González-Neves et al. 2012).

Optimal synthesis and accumulation of the polyphenols during the grape ripening phase, as a prerequisite for wines rich in polyphenols, can be modulated by various vine cultivation practices, such as cluster thinning, pruning techniques, green harvesting, berry removal, leaf removal, irrigation and fertilization management, as well as organic or biodynamic cultivation techniques (Mulero et al. 2010; Cañón et al. 2014; Karoglan et al. 2014).

Organic agriculture has attracted an increasing interest for food production throughout the world and one key reason for this interest is the assumption that organic food consumption is beneficial to health (Johansson et al. 2014). In the case of wine, although clear legislation for organic wine is now in force and organic wine is available commercially, it is more difficult than in the case of other food products to get certification and to convince the consumers of the product quality (Antoce 2019). Nevertheless, there are several studies (Mulero et al. 2010; Vrček et al. 2011; Bunea et al. 2012; Tassoni et al. 2013; Garaguso and Nardini 2015) on the influence of organic cultivation on the secondary metabolites in grapes and wines. In such works the phenolic content, antioxidant activity, as well as biogenic amines and metal contents were determined in wines from different local or international grape cultivars, from white Pignoletto, Traminac, Chardonnay to red Sangiovese, Cabernet-Sauvignon, Merlot, Cabernet Franc, Plavac Mali varieties.

Several studies investigated the impact of cluster thinning in vineyard on phenolic compounds in wines, especially anthocyanins, flavonoids, tannins and stilbens (resveratrol). Removal of some clusters, one month after blooming, has a beneficial effect on the phenolic maturity of Merlot and Syrah red wine grape varieties, increasing the phenolic composition of wines as indicated by increases in total anthocyanins, total phenolic compounds and total resveratrol content (Bubola et al. 2011; Villango et al. 2015). Advanced maturity and crop thinning decreased acidity and increased anthocyanins and phenolic compounds in Cabernet Sauvignon (Petrie and Clingeleffer 2006). By decreasing yield per vine, wine colour and phenolic compounds content significantly increased, along with the levels of metabolites which contribute to the wine aroma and flavour (King et al. 2015). Previously, we have also shown that a 30% cluster thinning had beneficial compositional effects on the grapes of Feteasca Neagra and Cabernet Sauvignon, as compared to controls without cluster thinning (Artem et al. 2015a), without significantly affecting the yield (1.57 ± 0.6 vs. 2.06 ± 0.5 kg/vine for Feteasca Neagra, 1.67 ± 0.5 vs. 2.22 ± 0.7 kg/vine for Cabernet Sauvignon).

Our previous studies regarding the effect of 30% cluster thinning (Artem et al. 2015a) and organic cultivation system (Artem et al. 2015b) were performed only for the grapes thus obtained. In contrast, the objective of the present study was to show the influence of these viticultural practices on the wines produced from such grapes, by assessing the polyphenolic composition (total and individual polyphenols, total and individual anthocyanins, total flavonoids contents) and the antioxidant activity of the wines of Feteasca Neagra. By using multivariate data analysis, the possibility of differentiating between wines obtained by different cultivation practices through the detection of specific markers was also evaluated.

Materials and methods

Vineyard site and experimental design

The research was performed in Murfatlar, Dobrogea region, Romania — a vineyard with favourable climate and soil conditions for quality wine. Detailed presentation of the vineyard, location and soil characteristics is included in a previous paper (Artem et al. 2015a).

The variety used is the autochthonous Feteasca Neagra (*Vitis Vinifiera L.*) cultivated in organic and conventional plantations established in 2007. The organic and conventional plantations are located close to each other, therefore differences in climate and soil quality could be considered negligible. The Feteasca Neagra variety was certified for organic production by the authorizing organism ICEA Romania (an entity mandated by the Ministry of Agriculture and Rural Development of Romania), after undergoing a three years period of conversion.

The experimental design included blocks for two variants (organic and conventional cultivation systems) and two vine treatments (no cluster thinning and 30% cluster thinning), with three repetitions for each, resulting in 4 experimental variants and 12 samples in total. In case of the organic cultivation system all applied operations respected the technological steps and rules as imposed by Regulation (EC) No 834/2007 concerning organic production and labelling. The organic system included 8 annual treatments using only products approved for organic cultivation (copper hydroxide, sulfur, atraBot traps, algae extracts), while the conventional system included 7 annual treatments based on synthetic herbicides and pesticides (such as glyphosate, cymoxanil, dithiocarbamates, triadimenol, phthalimides, iprodione, alpha-cypermetrin, kresoxim-methyl).

The 30% cluster thinning treatment was performed manually at the beginning of veraison and its effect was evaluated against the control with no thinning.

Harvesting and winemaking

The experiment was performed during two harvest years, one more favourable (2014) and one less favourable (2013) for viticulture in the region of Murfatlar. The favorability of a year depends on many climatic factors, but for the reason of simplicity, the main parameter selected and correlated with polyphenol accumulation was the total number of hours of sunshine in the period July–September, when the grapes are ripening and attain maturity. Thus, in the period of 2010–2019, the most favourable years, with more than 800 h our sunshine, were 2010, 2011, 2012, **2014**, 2015, 2016 and the less favorable years, with less than 800 h of sunshine were **2013**, 2017, 2018 and 2019. Other important climatic parameters for these years, such as rainfall and temperatures, are included in Table 1. The studied area is characterised by a favourable climate for grape cultivation, with moderate temperatures (average from 20.3–29.6 °C), long periods of sunshine (687–956 h) and low rainfall (57–283 mm) occurring in summer, between July and September. No irrigation was applied to the area during the course of this study.

Even though fluctuations may occur from one vintage to another, we consider that the results obtained and the tendencies observed for the years 2013 and 2014 are representative for a less favourable and more favourable year, respectively. Based on the studies and forecasts regarding the climate change in Romania we expect minimal effects in the next few years. A study performed in Bucharest (Bucur et al. 2019) for a period of 20 years showed that the evolution of the average temperatures in the viticulture growing season over the entire period 1961–2018 led to an increase of only + 0.75 °C in the recent period (1998–2018) as compared to the reference period 1961–1997.

As the quality of wines is strongly influenced by the grapes quality it is very important to harvest at optimal time, when the grapes reach phenolic maturity. Thus, the harvest time was slightly different each year, as grape maturation depended on the climatic conditions of the two years included in the study: in 2013 Feteasca Neagra was harvested on September 12, while in 2014 harvesting was done on September 16 (Artem et al. 2015b). The grapes were hand picked, collected in plastic containers and transported in the same day to the winemaking facility.

The vinification process was carried out in batches of 50 kg in the microvinification department, by applying the classic technology for obtaining quality dry red wines, including fermentation and maceration on skins (5–8 days). In order to end the maceration process at the maximum extraction level, samples were analysed daily until the total polyphenols and total anthocyanins remained constant. To prevent the development of unwanted microorganisms, as well as oxidation, 50 mg L^{-1} of SO_2 was added during both conventional and organic vinification.

Chemicals

For the determination of total polyphenols composition and antioxidant activity, Folin–Ciocalteau phenol reagent, radical scavenging assay reagents 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich. All reagents for the determination of phenolic content by HPLC and UV–VIS measurements (anhydrous

sodium carbonate, sodium acetate, methanol, 96% ethanol, acetonitrile, formic acid, hydrochloric and orthophosphoric acids) were of analytical grade and were obtained from Merck (Darmstadt, Germany). Ultra-pure water, produced by a Milli-Q Millipore system (Bedford, MA, USA), was used for the preparation of aqueous solutions and HPLC mobile phases. Analytical standards (gallic acid, syringic acid, *p*-coumaric acid, (+)-catechin, (−)-epicatechin, resveratrol, rutin, quercetin and malvidin-3-O-glucoside), were selected based on their frequency of occurrence in wines and their availability as commercial standards, and purchased from Sigma-Aldrich (Steinheim, Germany) at a ≥ 99% purity (HPLC grade).

Analytical investigations of wines

HPLC–PDA analysis of phenolic compounds

Determination of individual polyphenols was carried out by reversed phase—high performance liquid chromatography (RP-HPLC) using a Thermo Finnigan Surveyor Plus HPLC System (Thermo Fisher Scientific Inc., San Jose, USA) equipped with a Surveyor Photodiode Array Detector (PDA), Surveyor autosampler, Surveyor LC Pump (Quaternary gradient) and Chrome Quest Chromatography Workstation. Wine samples were previously degassed in ultrasonic bath and directly analyzed, without any previous treatment with the exception of filtration through 0.45 µm PTFE filters. The separation of gallic acid, syringic acid, *p*-coumaric acid, (+)-catechin, (−)-epicatechin, resveratrol, rutin and quercetin was done on an Accuacore PFP (2.6 µm, 100 × 2.1 mm) column, using increasing gradient eluents based on water/ formic acid/ acetonitrile, the method being presented in detail in Marinas et al. (2014). These phenolic compounds separated from the wine samples were identified by their retention times and UV spectra, based on similarity with those of the corresponding standards. The concentrations of investigated compounds found in wine were calculated as mg L^{-1} using external calibration curves, which were obtained for each compound. Calibration curves revealed good linearity, with R^2 coefficients higher than 0.995. LODs ranged from 0.09–0.21 mg L^{-1}, whereas LOQs ranged from 0.20–0.76 mg L^{-1}. For each quantified phenolic compound, the recovery rates were between 74 and 78% for phenolic acids, 76–88% for flavonoids and 94% for *t*-resveratrol, while the precision values were < 5%. The anthocyanins were separated on an Aquasil C18 column (5 µm, 250 × 4,6 mm), in accordance to the method MA-E-AS315-11 recommended by the International Organisation of Vine and Wine (OIV) and included in the Compendium of International Methods of Analysis-OIV (OIV 2008). Nine major anthocyanins, most important for colour in wines, were analyzed, namely: 3-O-monoglucosides of delphynidin (De), cyanidin (Cy), petunidin (Pt), peonidin (Pe) and malvidin (Mv), as well as acylated and coumaroylated glucosides of peonidin (Pea and Pec) and malvidin (Mva and Mvc). Malvidin-3-O-glucoside (Mv) (oenin) was identified using a standard, while the other anthocyanins were identified based on the elution order in accordance to the OIV method. The results for anthocyanins were reported based on the calibration curve of malvidin-3-O-glucoside (Mv) and expressed as mg L^{-1}. Other details and examples of chromatograms can be found in Geana et al. (2011, 2015). All HPLC analyses were performed in triplicate and data are given as means with standard deviations. Blank solution and control samples were analyzed in order to monitor performance related to variable factors or random error.

Total polyphenol composition

The polyphenol composition (total polyphenols, total anthocyanins and total flavonoids) was determined by spectrophotometric methods using UV–VIS spectrophotometer Helios Alpha (Thermo Spectronic, Great Britain) and Specord 250 Plus UV–VIS spectrophotometer using a 1 cm path length glass cuvettes. The determinations were conducted in triplicate and results were reported as means with standard deviations.

Total polyphenols (mg GAE L^{-1}) were determined by the Folin–Ciocalteau method, based on the ability of the wine phenolic compounds to get oxidized with the Folin–Ciocalteau reagent, using the method described by Singleton et al. (1999), with some modification. The resulting blue colour has a maximum absorbance at 675 nm, the absorbance being proportional to the amount of total phenolic compounds. Values were expressed as mg gallic acid equivalents (GAE) per L of wine.

The total anthocyanins (mg L^{-1}) were determined by the method Ribéreau-Gayon-Stonestreet, based on the change of anthocyanins' colour depending on pH; it consists in measuring the 520 nm absorbance variation in the colour of anthocyanins at pH 0.6 and 3.5 against distilled water (Ribéreau-Gayon et al. 2017). The total anthocyanins content was expressed as mg L^{-1} of wine, calculated based on the standard curve generated by serial dilution of the standard (anthocyanins from *Vitis Vinifera*), covering the concentration range 0–375 mg L^{-1}.

The total flavonoids content of wines was determined according to the method described by Hosu et al. (2014), by treating 0.5 mL of wine with 0.4 mL of 25 g L^{-1} $AlCl_3$ solution, 0.5 mL of 100 g L^{-1} CH_3COONa solution and 4 mL distilled water. After 15 min, the absorbance of the mixture was measured at 430 nm. Total flavonoid content expressed in mg rutin L^{-1} of wine was calculated using the

Table 1 Main climate parameters for the Murfatlar region during grape ripening

Year	Period	Temperature			Precipitation L/m^2)	Sunshine duration/ h	Number of days with rainfall > 10 L/m^2
		T_{med}/ °C	T_{min}/ °C	T_{max}/ °C			
2010	Veraison (July)	25.4	38.0	16.0	211.5	330.8	4 (2, 8, 9, 25 Jul.)
	Intermediate (August)	29.6	36.4	17.2	1.2	319.1	0
	Ripening (September)	23.2	30.6	11.5	49.3	204.9	1 (30 Aug.)
2011	Veraison (July)	26.6	37.0	15.6	85.7	308.7	3 (12,026,30 Jul.)
	Intermediate (August)	25.0	37.0	17.0	8.0	338.0	0
	Ripening (September)	22.5	34.0	11.0	5.0	288.4	0
2012	Veraison (July)	28.0	38.2	13.1	33.2	314.6	1 (11 Jul)
	Intermediate (August)	26.1	39.8	10.7	22.0	345.6	0
	Ripening (September)	20.7	36.0	7.8	5.6	288.2	0
2013	Veraison (July)	27.2	18.9	32.1	158.8	307.0	4 (4, 16, 30, 31 Jul.)
	Intermediate (August)	27.3	18.8	32.6	52.0	286.5	3 (4, 12, 29 Aug.)
	Ripening (September)	20.3	13.8	25.6	72.6	144.9	3 (14, 29, 30 Sept.)
2014	Veraison (July)	26.6	13.4	36.0	98.8	306.6	4 (4,5,17, 20 Jul.)
	Intermediate (August)	27.1	11.9	38.6	32.3	308.1	1 (17 Aug.)
	Ripening (September)	21.0	13.9	27.2	31.2	211.5	2 (23, 26 Sept.)
2015	Veraison (July)	28.3	14.8	40.9	44.0	324.1	1 (31 Jul.)
	Intermediate (August)	27.3	14.3	38.9	52.9	290.7	1 (18 Aug.)
	Ripening (September)	23.1	11.0	36.1	10.0	319.0	0
2016	Veraison (July)	28.2	13.4	38.1	2.2	358.9	0
	Intermediate (August)	26.9	10.3	38.7	20.8	308.5	1 (18 Aug.)
	Ripening (September)	21.9	7.2	36.3	33.6	288.8	1 (19 Sept.)
2017	Veraison (July)	27.1	11.3	39.2	103.2	269.5	2 (4, 28 Jul.)
	Intermediate (August)	27.8	10.9	39.8	8.2	273.3	0
	Ripening (September)	23.0	10.0	38.3	1.8	183.8	0
2018	Veraison (July)	26.4	14.6	35.0	110.8	196.0	4 (5, 22, 24, 27 Jul.)
	Intermediate (August)	26.9	14.2	37.4	46.4	280.9	1 (7 Aug.)
	Ripening (September)	21.7	2.0	35.9	4.2	212.7	0
2019	Veraison (July)	26.7	14.4	38.9	17.0	245.6	0
	Intermediate (August)	24.4	14.5	39.0	8.1	251.7	0
	Ripening (September)	22.8	7.0	36.1	36.9	189.7	2 (20, 24 Sept.)

calibration curve obtained for rutin in the 0–125 mg L^{-1} concentration range.

Antioxidant activity—DPPH method

The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the antioxidant activity of wine, according to the method described by Hosu et al. (2014). The decrease of absorbance of the radical is proportional to the concentration and antioxidant activity of the sample analysed. Absorbance measurements are transformed to antioxidant activity using Trolox as reference, in the concentration range of 50–1000 µmol L^{-1}. The results were expressed as mmol L^{-1} Trolox equivalents.

Data processing and statistical analysis

Each chemical parameter was measured in triplicate. The obtained data were expressed as mean values with standard deviations and were processed according to the wine sample category. Data were examined separately by taking into account the year and treatment applied in the vineyard, using one-way analysis of variance (ANOVA). The Duncan test was used to compare the means and discriminate the wine category ($p \leq 0.05$).

Principal Component Analysis (PCA) was performed in order to examine the possible grouping of samples according to the wine category on the basis of phenolic composition (individual polyphenols and anthocyanins, total polyphenols, total anthocyanins and total flavonoids) and antioxidant activity (DPPH method). Linear discriminant analysis (LDA) was used in order to identify markers with high discriminating power. All the mathematical and statistical analyses were performed using Microsoft Excel 2010 and XLSTAT Addinsoft version 15.5.03.3707.

Results and discussions

Determination of individual polyphenols in organic or conventional wine samples

The amounts of individual polyphenolic compounds vary considerably in different types of wine, depending on the grape variety, vintage and treatments applied in the vineyard (Villaño et al. 2006). Even if genotype differences cause a large variation of secondary metabolites within each variety, a small but systematic effect is induced by the growth conditions (organic vs. conventional culture, cluster thinning vs. no thinning etc.), which can still significantly affect the average levels, but the magnitude of the differences is very difficult to predict. It was demonstrated that the level of secondary metabolites with defensive role in organic plants is consistently higher in organically cultured crops than in conventional ones (Vrček et al. 2011). Even though it is unlikely to find important differences between organic and conventional plant products as far as the contents of proteins, carbohydrates, minerals and vitamins are concerned, it is possible to find differences in many defence-related secondary metabolites, but further investigations are warranted (Brandt and Mølgaard 2001). Also, more research is needed to reliably determine the relationships between agricultural practices, such as, but not exclusively, cluster thinning and the synthesis and accumulation of phytochemicals in specific crops.

In this regard, our results too confirm a variation in the phenolic content among the wine samples obtained in different years with different cultural practices. The contents of the individual polyphenols in Feteasca Neagra wines obtained from grapes cultivated in organic or conventional system, with or without 30% cluster thinning, in two representative harvest years, are reported in Table 2. The experiments were performed in 2013 and 2014, which are representative for a favourable year (2014) and a less favourable year (2013) in the period 2010–2019 (Table 1) as regards the average climatic conditions.

Wines produced with 30% cluster thinning treatment were generally expected to have higher total and individual polyphenolic concentrations as compared to the control, irrespective of the harvest year, considering that other authors have also proven this effect in their studies. General increases in wine polyphenols and anthocyanins were previously observed by Di Profio et al. (2011) in Merlot and Cabernet Sauvignon wines, obtained from grapes for which cluster thinning was applied. More recently, Mawdsley et al. (2019) have reported in Pinot noir grapes an increase in berry anthocyanins and total phenolics by 43% and 87% in 2017 and by 103% and 140% in 2018 for thinning to one cluster per shoot as compared to the non-thinned control. The timing of the thinning applied appeared to induce no significant differences. On the other hand, the cluster removal has been observed by other authors (Karoglan et al. 2014) to produce only a small effect on Cabernet Sauvignon wine composition, which may be due to an already low load before the application of cluster thinning treatment. Our experiments too showed that for Feteasca Neagra variety total polyphenols (Table 3) and some of the measured specific polyphenols significantly increased in the variants produced with cluster thinning, as compared to non-thinning cultivation systems (organic or conventional), irrespective of the harvest year. Increased concentrations were consistently observed for the variants with 30% cluster thinning for the most valuable polyphenols—epicatechin, rutin (quercetin-3-O-rutinoside), quercetin and resveratrol—for both organically or conventionally cultivated grapes. The concentrations

determined for the organic cultivation with cluster thinning as compared to the control with conventional cultivation and no cluster thinning increased by 174% for epicatechin, 160% for rutin, 360% for quercetin, 41% for resveratrol in 2013 and by 127%, 57%, 100% and 63% in 2014, respectively. Among anthocyanins a significant increase (by 242% in 2013 and 148% in 2014) was recorded for delphinidin, a compound valued for its antitumoral properties (Wang and Stoner 2008).

The organic cultivation proved to be beneficial for Feteasca Neagra, higher values being recorded for many polyphenols as compared to the similar variants produced by conventional cultivation, irrespective of whether the cluster thinning treatment was applied or not. However, these values depended also on the vintage, which means that climatic conditions can greatly influence the phenolic profile, exceeding the effect of organic cultivation. The climatic conditions in 2014 were more favourable for obtaining a rich harvest, with high quality wines, being warmer and dry compared to 2013, due to a greater number of sunny days, over 800 h in July–September (Table 1). A significant increase of the levels of epicatechin, rutin (in 2013 only), quercetin, *t*-resveratrol, De, Pt (2014 only), Mv (2014 only), as well as acylated and coumaroylated malvidin derivatives (Mva and Mvc) was observed in organic system cultivation, while the concentration of gallic acid, syringic acid, catechin, quercetin, Cy, Pe, Pea and Pec were relatively little influenced by any treatments in the vineyard. All of the individual anthocyanins, with the exception of Cy which remained relatively constant, were however higher in the wines of 2014, irrespective of the treatments applied in the vineyard, showing thus that the colour pigments accumulation is more dependent on weather conditions rather than on other viticultural practices (Table 2). Pt increased in organic cluster thinning samples by 71% in 2013 and by 174% in 2014, Pe increased by 96% in 2014 and Mv by 110% in 2014. Considering the harvest year, the individual polyphenolic compounds show very high statistically significant differences ($p \leq 0.001$) or extremely significant differences ($p \leq 0.0001$), for gallic acid, syringic acid, catechin, quercetin, resveratrol, Pt, Pe, Mv and Pec. The tendency to get higher polyphenol concentrations in organically produced grapes can have many explanations related to biotic or abiotic stress. The compounds which showed significant concentration increase under the organic cultivation system (such as epicatechin, rutin, quercetin, *t*-resveratrol, De, Pt, Mv and its acylated and coumaroylated derivatives can be considered as markers to follow when trying to determine the enhancement of Feteasca Neagra wine phenolic profile by this type of cultural system.

When considering both harvest year and treatments applied in the vineyard, almost all investigated phenolic compounds show extremely significant differences from one experimental variant to another, proof of the difficulty to predict the influence of a certain treatment when weather conditions are not also accounted for (Table 2). However, it is to be expected that in rainy years (like 2013) total and specific polyphenol accumulation will be lower than in the sunny years (like 2014).

To clearly show the overall effect of the treatments in the vineyard and production year and to prove that polyphenol accumulation responded positively to the organic cultivation system especially when coupled with cluster thinning, instead of looking only at some particular individual phenolic compounds, it is better to evaluate the total polyphenols, as well as some of the major components (total flavonoid and total anthocyanin concentrations). Total polyphenols, along with the antioxidant activity in wines, are better indicators than individual specific phenols in determining the possible health benefits of wine.

Determination of total phenolic compounds and antioxidant activity in organic and conventional wine samples

As already shown by many authors the polyphenolic potential of the grapes is significantly influenced by variety, climatic conditions, by treatments such as cluster thinning, culture system (organic vs. conventional) and so on. We too have previously shown that embracing new vineyard practices, such as 30% cluster thinning and organic cultivation systems, may lead to a measurable increase of the quality of grapes, expressed by general phenolic compound related parameters (Artem et al. 2015b).

The results obtained in this study for total polyphenols, total anthocyanins, total flavonoids contents and antioxidant activity of Feteasca Neagra red wines are summarised in Table 3, which includes the wines obtained from organically or conventionally grown grapes, with or without cluster thinning, in two vintages in Murfatlar vineyard. Different letters indicate significant differences (95% confidence) between wines. Considering the harvest year, the statistical analysis for total polyphenols, total anthocyanins and antioxidant activity parameters showed that the p value was ≤ 0.0001, which means that extremely significant differences are observable from one vintage to another for these parameters, while total flavonoids content showed only significant differences ($p \leq 0.05$).

On the contrary, when considering the overall applied treatments (organic cultivation, conventional cultivation, 30% cluster thinning or no thinning), ANOVA analysis for total polyphenols, flavonoids and antioxidant activity parameters showed no significant differences. The only significant difference clearly appears for the total

Table 2 The effect of different treatments applied in the vineyard on individual phenolic compounds of Feteasca Neagra wines obtained in two vintages representative for the period 2010–2019

Compound (mg L^{-1})	Vintage 2013				Vintage 2014				ANOVA		
	Org C	Org CT	Conv C	Conv CT	Org C	Org CT	Conv C	Conv CT	Year (Y)	Treatment (T)	Yx T
Gallic acid	10.02 ± 2.30^{a}	10.94 ± 2.50^{a}	7.87 ± 1.70^{a}	7.92 ± 1.20^{a}	21.97 ± 2.80^{c}	23.42 ± 2.40^{c}	29.72 ± 3.20^{b}	35.15 ± 2.60^{a}	****	ns	****
Syringic acid	1.19 ± 0.23^{a}	1.15 ± 0.25^{a}	1.05 ± 0.27^{a}	1.05 ± 0.22^{a}	3.38 ± 0.8^{a}	3.19 ± 0.60^{a}	3.62 ± 0.90^{a}	2.97 ± 0.201^{a}	****	ns	****
p-coumaric acid	2.90 ± 0.70^{a}	3.09 ± 0.90^{a}	3.56 ± 0.74^{a}	3.66 ± 0.60^{a}	1.79 ± 0.30^{b}	2.43 ± 0.51^{b}	3.40 ± 0.30^{a}	3.96 ± 0.60^{a}	ns	**	**
Catechin	7.43 ± 1.20^{a}	8.23 ± 1.30^{a}	9.63 ± 1.50^{a}	10.17 ± 1.70^{a}	17.63 ± 2.10^{b}	19.47 ± 2.80^{b}	16.54 ± 3.20^{b}	24.47 ± 2.30^{a}	****	ns	****
Epicatechin	8.73 ± 1.60^{b}	14.43 ± 2.1^{a}	5.27 ± 1.80^{c}	12.63 ± 2.20^{a}	6.59 ± 1.20^{c}	17.10 ± 2.70^{a}	7.52 ± 1.80^{c}	12.50 ± 2.40^{b}	ns	****	****
Rutin	1.68 ± 0.06^{b}	2.06 ± 0.08^{a}	0.12 ± 0.05^{b}	0.24 ± 0.09b	0.33 ± 0.03^{d}	0.77 ± 0.05^{a}	0.49 ± 0.02^{c}	0.68 ± 0.05^{b}	ns	**	****
Quercetin	0.13 ± 0.05^{ab}	0.23 ± 0.19^{a}	0.05 ± 0.02^{b}	0.06 ± 0.04^{b}	0.28 ± 0.06^{a}	0.32 ± 0.05^{a}	0.16 ± 0.07^{b}	0.38 ± 0.03^{a}	***	*	****
t-resveratrol	0.88 ± 0.03^{b}	1.06 ± 0.09^{a}	0.75 ± 0.05^{b}	1.08 ± 0.10^{a}	1.74 ± 0.09^{b}	2.14 ± 0.10^{a}	1.31 ± 0.07^{c}	1.40 ± 0.06^{c}	****	ns	****
De	0.67 ± 0.22^{b}	1.71 ± 0.63^{a}	0.50 ± 0.32^{b}	0.69 ± 0.37^{b}	1.75 ± 0.27^{b}	2.48 ± 0.33^{a}	1.00 ± 0.18^{c}	1.80 ± 0.36^{b}	**	**	****
Cy	0.25 ± 0.11^{b}	0.59 ± 0.26^{a}	0.38 ± 0.10^{ab}	0.40 ± 0.13^{ab}	0.27 ± 0.10^{a}	0.29 ± 0.04^{a}	0.31 ± 0.09^{a}	0.37 ± 0.11^{a}	ns	ns	ns
Pt	0.92 ± 0.55^{a}	1.71 ± 0.38^{a}	1.00 ± 0.30^{a}	1.16 ± 0.52^{a}	4.26 ± 1.172^{ab}	5.93 ± 1.20^{a}	2.16 ± 1.06^{b}	3.90 ± 1.18^{ab}	****	ns	****
Pe	0.26 ± 0.11^{a}	0.39 ± 0.22^{a}	0.39 ± 0.22^{a}	0.43 ± 0.15^{a}	3.38 ± 0.52^{b}	4.64 ± 0.66^{a}	2.36 ± 0.30^{b}	5.19 ± 0.76^{a}	****	ns	****
Mv	18.91 ± 4.30^{a}	20.73 ± 2.60^{a}	16.08 ± 2.90^{a}	18.94 ± 3.20^{a}	32.75 ± 6.30^{ab}	43.29 ± 7.50^{a}	20.57 ± 6.20^{a}	31.56 ± 5.80^{ab}	****	**	****
Pea	0.35 ± 0.10^{b}	0.35 ± 0.18^{b}	0.82 ± 0.26^{a}	0.74 ± 0.29^{ab}	0.51 ± 0.21^{a}	0.60 ± 0.16^{a}	0.51 ± 0.20^{a}	0.48 ± 0.18^{a}	ns	ns	ns
Mva	5.65 ± 1.62^{a}	6.96 ± 1.35^{a}	0.77 ± 0.28^{b}	0.84 ± 0.33^{b}	1.34 ± 0.30^{ab}	1.78 ± 0.35^{a}	1.04 ± 0.27^{b}	1.25 ± 0.38^{ab}	*	ns	****
Pec	0.34 ± 0.20^{a}	0.37 ± 0.199^{a}	0.30 ± 0.18^{a}	0.33 ± 0.10^{a}	0.86 ± 0.21^{ab}	1.16 ± 0.32^{a}	0.54 ± 0.16^{b}	0.83 ± 0.20^{ab}	****	ns	****
Mvc	2.40 ± 0.30^{a}	1.47 ± 0.10^{b}	1.15 ± 0.10^{b}	1.31 ± 0.16^{b}	1.88 ± 0.33^{b}	2.53 ± 0.38^{a}	1.14 ± 0.27^{c}	1.66 ± 0.29^{bc}	ns	***	****

Org–organic system, Conv–conventional system, CT–30% cluster thinning, C–control; Means (n = 3) ± standard deviation followed by different lowercase letters in the line differ significantly according to the Duncan's multiple range test for year (Y), treatment (T) and both, year and treatment (Y × T): ns: $p > 0.05$ (not significant), *$p \leq 0.05$ (significant), **$p \leq 0.01$ (highly significant), ***$p \leq 0.001$ (very highly significant) and ****$p \leq 0.0001$ (extremely significant)

Tabel 3 The effect of the two types of vineyard treatments on phenolic compounds content and antioxidant activity of Feteasca Neagra wines obtained in two vintages representative for the period 2010–2019

Harvest year	Treatment	Parameters			
		Total polyphenols (mg L^{-1})	Total anthocyanins (mg L^{-1})	Total flavonoids (mg L^{-1} rutin)	Antioxidant activity(μmoli L^{-1} Trolox)
2013	Org C	1293 ± 12^{b}	484 ± 6^{c}	90.2 ± 2.0^{ab}	5986 ± 279^{b}
	Org CT	1529 ± 14^{a}	654 ± 5^{a}	96.8 ± 2.9^{a}	7379 ± 168^{a}
	Conv C	1009 ± 12^{c}	416 ± 6^{d}	83.2 ± 4.1^{b}	5028 ± 396^{c}
	Conv CT	1336 ± 13^{b}	523 ± 10^{b}	92.8 ± 4.1^{a}	6458 ± 261^{b}
2014	Org C	849 ± 13^{c}	333 ± 4^{b}	85.4 ± 3.4^{a}	4358 ± 235^{b}
	Org CT	943 ± 13^{a}	408 ± 5^{a}	94.6 ± 4.6^{a}	4913 ± 190^{a}
	Conv C	894 ± 16^{bc}	286 ± 6^{c}	75.2 ± 5.7^{a}	4286 ± 178^{b}
	Conv CT	930 ± 15^{ab}	352 ± 4^{b}	81.0 ± 6.2^{a}	4565 ± 211^{ab}
ANOVA	*Year (Y)*	****	****	*	****
	Treatment (T)	ns	*	ns	ns
	Y x T	****	****	ns	****

Org–organic system, Conv–conventional system, CT–30% cluster thinning, C–control; Means (n = 3) ± standard deviation followed by different lowercase letters in the column differ significantly according to the Duncan's multiple range test (95% confidence) for year (Y), treatment (T) and both, year and treatment (Y × T): ns: $p > 0.05$ (not significant), *$p \leq 0.05$ (significant), **$p \leq 0.01$ (highly significant), ***$p \leq 0.001$ (very highly significant) and ****$p \leq 0.0001$ (extremely significant)

anthocyanins in the case of Feteasca Neagra experimental wine variants, cluster thinning being the treatment which induces this effect, by increasing the total colour pigment content. However, when comparing the means for each treatment type performed in the vineyard, by applying the Duncan post-hoc test, it is clear that cluster thinning increases the concentrations of the polyphenols, the effect being more visible for the vintage 2013, which was more affected by rain. Thus, in 2013 cluster thinning increased the concentration determined in wines for total polyphenols by 18% in organic and by 32% in conventionally cultivated grapes, for total anthocyanins by 35% in organic and by 26% in conventionally cultivated grapes and for antioxidant activity by 24% in organic and by 28% in conventionally cultivated grapes. In 2014, the values were 11%, 4%, 22%, 23%, 13% and 6%, respectively.

When we consider both harvest year and vineyard treatments, we observe again, as in the case of the vintage influence, that total polyphenols, total anthocyanins and antioxidant activity show extremely significant differences between experimental variants, while flavonoids presented non-significant differences. It is clear that the vintage year is more decisive in the polyphenol accumulation than any treatment, but, in any specific vintage, cluster thinning helps every time by increasing the total anthocyanin content. A slighter, but also significant positive effect is induced by organic cultivation, which also increases the total polyphenols and anthocyanin content. Thus, in 2013, organic cultivation increased total polyphenols and total anthocyanins by 28% and 16%, respectively, in non-cluster-thinning variants and by 14% and 25% in cluster thinning variants.

Directly correlated to these total polyphenol and anthocyanin increases, induced independently by cluster thinning and by organic cultivation, the antioxidant activity of investigated wines, which can be viewed as a measure of the wine quality, also displayed increases. Higher values as compared to those of control samples for both total anthocyanins and antioxidant activity were determined in Feteasca Neagra for 2013 vintage, meaning that the application of treatments such as cluster thinning or organic cultivation have more important effects in less climatic favourable years.

Discriminant analysis

Principal Component Analysis (PCA) was used to provide graphical presentation of the differences between the Feteasca Neagra wines obtained by different treatments (organic vs. conventional, with or without 30% cluster thinning) and different harvest years. The parameters used for the statistical analysis were major individual anthocyanins, phenolic acids, flavanols, flavonols, stilbens, as well as total polyphenols (TP), total anthocyanins (TA), total flavonoids (TF) and antioxidant activity (AA). As presented in Fig. 1, PCA revealed

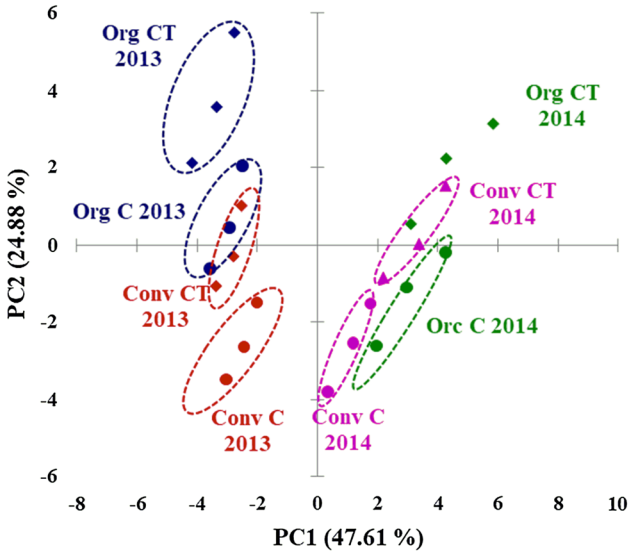

Fig. 1 Principal component analysis (PCA) plot showing separation of experimental wine variants in accordance to the treatments in the vineyard and the vintage

acceptable discrimination based on two principal components accounting for 72.5% of the total variability (PC1–47.61% and PC2–24.88%), the samples being grouped in accordance to the vine treatment and vintage. The first principal component separated the groups in accordance to the vintage, samples from the less favourable vintage (2013) being grouped in the left part of the diagram, while the samples from the favourable vintage (2014) gathered in the right part. This means that the major influence is determined by the vintage and not the cultural practices, but even the vintage itself is not a very strong influential factor, as PC1 accounts for only 47.61% of the total variability. The second principal component mainly discriminated Feteasca Neagra wines in accordance to viticultural practices, conventional Feteasca Neagra wines being grouped in the lower part of the diagram, while the organic ones were placed in the upper part. This discrimination is not as clear as in the case of the vintage, as it is also influenced by the cluster thinning practice, also included in the same variability represented by a total of 24.88% (PC2). It shows, however, that there are observable differences between conventional and organic wines (Fig. 1).

The PCA analysis shows that the wines from organic grapes can be differentiated from those obtained with conventionally cultivated grapes, with better discrimination results for wines obtained in harvest year 2013. The best discrimination was observed for wines produced in different harvest years, demonstrating by this analysis too that wine polyphenolic composition was affected by the different weather conditions of each year.

In order to identify markers with high discriminating power LDA was also used. The most significant variables for the discrimination of wines (with p-values < 0.0001) correlated with the applied cultivation technology and were: gallic acid, rutin, *t*-resveratrol, quercetin, Mvc, TP and TA. As presented in Fig. 2a, the samples were correctly discriminated according to the cultivation technology and year, 99.8% of the variability being included in discriminant factors 1 and 2. Best discrimination is attained for wines produced in the less favourable year, 2013, when the influence of the organic viticultural system was greater.

For the Feteasca Neagra experimental wines variants, the most discriminant phenolic compounds are rutin, Mva and Pea, their content having the highest influence on the discriminant factor 1 (F1 = 65.82%).

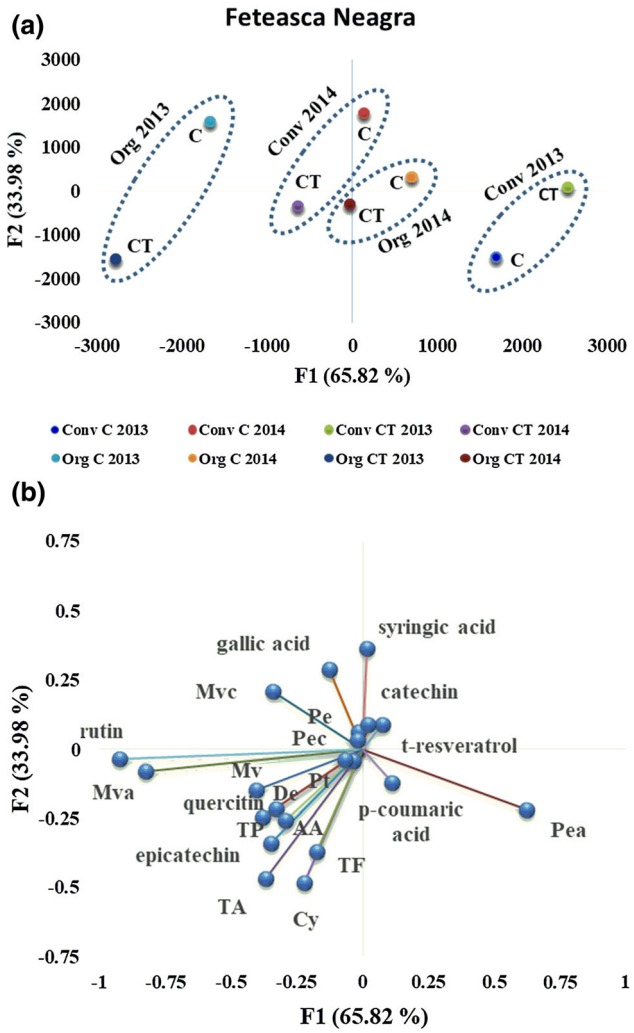

Fig. 2 **a** Linear discriminant analysis for Feteasca Neagra experimental wines variants based on individual phenolic compounds profiles, total polyphenols (TP), total anthocyanins (TA), total flavonoids (TF) and antioxidant activity (AA) for two harvest years; **b** influence of the analysed parameters on the discriminant factors of Feteasca Neagra wine variants

Syringic acid, catechin, *t*-resveratrol and *p*-coumaric acid are mainly included in the discriminant factor 2, which is expressing 33.98% of the total variability. The total polyphenol content (TP), anthocyanins content (TA), total flavanoids content (TF), antioxidant activity, epicatechin, quercetin, gallic acid and most of the monomeric anthocyanins have a more complex influence, being included in both F1 and F2 discriminant factors (Fig. 2b).

Such markers, especially those influencing preponderantly either F1 or F2 should be validated in further studies and may be useful for future attempts to discriminate wines from organic or conventional grapes.

Conclusion

The present study indicates that viticultural practices can be used to improve the phenolic quality of red wines. For Feteasca Neagra variety positive differences in the phenolic composition and biochemical properties are demonstrated to be induced by both organic cultivation and cluster thinning in the vineyard. Generally, wines from organically grown grapes display a higher phenolic concentration as compared to those from conventionally grown grapes, the effect increasing when cluster thinning is used in addition to organic cultivation.

Organic cultivation increased the total polyphenols of Feteasca Neagra by 28% in the less favourable year (2013) and by 5% in the favourable year (2014), total antocyanins rose by 16% in both 2013 and 2014 and total antioxidant activity went up by 19% in 2013 and by 2% in 2014, as compared to conventional cultivation. When 30% cluster thinning was also performed in addition to organic cultivation, as compared to conventional cultivation without cluster thinning, the total phenols of Feteasca Neagra increased by 52% in 2013 and by 5% in 2014, total antocyanins increased by 57% in 2013 and by 42% in 2014 and total antioxidant activity increased by 47% 2013 and by 14% in 2014.

These results show that the practice of organic cultivation combined with cluster thinning can lead to clearly better results in any vintage. However, the improvement in phenolic composition and wine antioxidant quality is especially important in years with less favourable climatic conditions, when impressively higher values are obtained as compared to the conventional grape cultivation in the same year.

Phenolic compounds such as *p*-coumaric acid, rutin, *t*-resveratrol, Mva, Mvc, showing significant differences in organically cultivated grapes, should be further investigated as markers of the organic viticultural systems.

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Declarations

Conflict of interest none.

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