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Evolution of phenolic profile of white wines treated with enzymes

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ABSTRACT

The aim of this study is to monitor the evolution of the principal phenolic compounds throughout the fermentation stage of white wines treated with different enzymes. The effect of five commercial enzymes on the evolution of the phenolic profile during the alcoholic fermentation of white wines obtained from Fetească regală and Sauvignon blanc varieties was evaluated. Physicochemical properties of resulted wine samples have been analyzed according to OIV standards and regulations. The evolution of the principal phenolic compounds was carried out using HPLC method. Enzymatic treatments did not significantly affect the physicochemical composition of the obtained wines. The analyzed samples showed different variations on the phenolic compound content, depending on the type of added enzyme and grape variety. The statistical analysis confirms that enzymes significantly contributed to the enrichment of the wines with phenolic compounds, especially with *p*-coumaric, gentisic, caftaric, and protocatechuic acids.

1. Introduction

Wine's general quality and chemical composition are strongly connected to the raw material state, alcoholic fermentation and applied treatments (Losada, Andrés, Cacho, Revilla, & López, 2011). Consumers' requirements focus on high quality products that provide safe and nutritional characteristics. As other food sectors, wine producers are introducing various technological amendments based on biotechnological resources. Numerous constituents of wine are influenced, at diverse phases of winemaking, by biochemical transformations that are catalyzed by specific enzymes. For example, enzymes participate in the oxidation of grape phenolic compounds, in the constitution of volatile substances during pre-fermentative actions and in the conversion of odorless precursors into odor-active components through fermentation (Ugliano, 2009). Understanding the important effects of enzymes during the winemaking process can help in the progress of operative strategies for optimizing wine processing. Grape berries and wine yeasts represent the major sources of enzymes involved in the various biochemical transformations that take place during winemaking. However, typical winemaking parameters such as high sugar and ethanol contents, low pH levels and high quantities of polyphenols can potentially inhibit the activity of enzymes, frequently during synergistic interactions which result in augmented inhibitory effects. Since the grape's enzymes are neither efficient nor sufficient under winemaking conditions, the wine-maker can boost their activity by using commercial products as supplements, usually obtained by fermenting pure cultures of selected microorganisms (Ugliano, 2009).

Initially used in the fruit juice industry in the 1950s to increase juice yield and improve clarification process, commercial enzymes were adopted by the winemaking industry worldwide since 1970s. The main advantage of using enzymes in winemaking is mainly due to their specificity of action (without producing unwanted secondary compounds), giving producers the ability to obtain constant quality throughout harvests. Commercial enzymes are usually blends of products with different activities, such as glycosidases, glucanases, pectinases and proteases (Claus & Mojsov, 2018). These products participate in diverse biotransformation reactions and have a positive contribution to wine quality during all winemaking stages and aging period (improving clarification and filtration process of musts and wines, increasing their stability, improving the phenolic and volatile profile and wines' color (Armada, Fernández, & Falqué, 2010). Also, commercial enzyme preparations are eco-friendly and have great economic benefits (significant reduction of energy consumption or time saving) (Claus & Mojsov, 2018).

Commercial β-glycosidases are ussualy specific for the extraction of aroma compounds in wines. Zhu, Du, and Li (2014) confirm a significant increase in the total quantity of volatiles in wines compared to the controls by adding *β*-glycosidases. de Andrades, Graebin, Ayub,

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Fernandez-Lafuente, and Rodrigues (2019) evaluate the effects of β-glucosidases activities of nine commercial enzymatic preparations on their behavior towards pH, thermal stability, kinetic parameters and glucose tolerance.

The commercial enzymes based on pectinase are usually heterogeneous mixtures of polygalacturonases, pectin lyases and pectin methylesterases activities. These products hydrolyse the pecto-cellulosic cell walls of the grapes skin improving juice yield. Besides, it's generating an increase in flocculation speed of the must before the alcoholic fermentation by breaking down pectins and macromolecules to smaller components, thus removing matter in suspension (favoring the clarification of juice and wine) (Ugliano, 2009). Different authors obtained a better extraction of color compounds, phenolics and proanthocyanidins by using different pectinase enzymes preparation (Osete-Alcaraz, Bautista-Ortín, Ortega-Regules, & Gómez-Plaza, 2019; Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2012). Ma et al. (2016) proved a synergic effect of ultrasound and pectinase on pectin hydrolysis kinetics.

Grapes (*Vitis* sp) are among the most consumed fruits in world, whether processed or not, while being a particular rich source of phenolic compounds. Phenolic compounds are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables, and other plants. Since phenolic compounds are mostly located in the solid parts of berries (skin and seeds), a high proportion is transferred during the pressing operation into the juice and remains blocked there after the alcoholic fermentation. Wine phenolic content depends on grape variety and maturation stage, climatic conditions, soil, winemaking protocols that influence their extraction rate (Lorrain, Ky, Pechamat, & Teissedre, 2013) and chemical reactions which take place during fermentation (including enzymatic and non-enzymatic oxidation) but also aging (Lorrain et al., 2013; Preserova, Ranc, Milde, Kubistova, & Stavek, 2015). Besides that, vine pruning and training system and phytosanitary conditions of the grapes can also affect their phenolic composition. Trellising has a significant influence on grape maturity that, in turn, affects the phenolic content of grapes and wines (Cortell & Kennedy, 2006; Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007; Nikfardjam, Laszlo, & Dietrich, 2006; Pastor del Rio & Kennedy, 2006).

Phenolic compounds have antioxidant, antibacterial, anti-carcinogenic and antiviral properties that apparently protect consumers from various diseases such as cardiovascular disease (Frankel, German, Kinsella, Parks, & Kanner, 1993; Lorrain et al., 2013). Recent studies have confirmed the antibacterial role of phenolic compounds extracted from plants in the dental field and can be used at a reasonable cost to prepare remedies for oral cavity hygiene (Spinei, 2015). In particular, the antioxidant activity of the phenolic compounds and their protective effect against oxidative stress may be an indicator of their potential health benefit. They work as catalysts of oxidation reactions or as promoters of some enzymes. Therefore, their analysis and quantification are of great interest (Nogueira et al., 2008).

Phenols are a large group of compounds that share certain chemical characteristics that can be further separated to two main groups: non-flavonoid and flavonoid compounds. Between flavonoid compounds, the most important groups are flavanols (also reffered as catechins), flavonols and anthocyanins. Non-flavonoids contain phenolic acids and their esters (with tartaric acid) and are differentiated by substitution of their benzene ring (Gómez-Alonso et al., 2007).

Hydroxycinnamic acids constitute the main non-flavonoid phenolic compounds usually found in white wines (*p*-coumaric, caffeic, ferulic and sinapic acids are some of the most referenced compounds) and mostly affect wine color. These compounds are mostly located in the grape pulp (Garrido & Borges, 2013; Kallithraka, Salacha, & Tzourou, 2009) and their presence in white wines increases the browning potential. It is well known that time of skin contact and pressure parameters can significantly affect phenolic content of must and wine

Cinnamic acids are related to the wine browning process and constitute precursors of volatile phenolic compounds. Caftaric and fertaric acids exist in their *trans*- isomeric form, originating from the pulp of the grapes and, during grape pressing, being rapidly released into the must. Although white wines possess a lower concentration of phenolic constituents compared to the red ones, they contain, in turn, high amounts of caftaric acid (Kammerer & Carle, 2009).

For this experiment, a Romanian autochthonous and an international grape variety were chosen (Feteasca regală and Sauvignon blanc, respectively). Feteasca regală is known to all Romanian wine makers due to its high concentration of phenolic compounds and thus, such research is of interest. Sauvignon blanc was chosen as it is one of the most appreciated wines worldwide, but its behaviour in Romanian vineyards and cellars has not been discussed from the point of view of phenolics and the influence of enzymes on their evolution.

Although many studies established the role of phenolics in winemaking technology, there has always been a challenge due to the complexity and diversity of these compounds in wines. The novelty of this work consists in the study of the influence of enzymatic preparations inoculated before the alcoholic fermentation, in must, as most studies analyse the use of enzymatic preparations during different phases of the wine making process. This work contributes to the enrichment and consolidation of specialized literature regarding the influence of some commercial enzymes on different bioactive compounds, increasing the array of wine assortment and optimization of winemaking process.

This work aimed to identify the main phenolic compounds and monitor their evolution throughout the alcoholic fermentation stage of white wines treated with different enzymes. The results were compared with other recent studies on similar products.

2. Materials and methods

2.1. Grapes and winemaking procedures

Sauvignon blanc and Feteasca regală grapes were harvested in 2018 at full maturity from Iași vineyard and processed by the classic method for obtaining white wines. The must was divided into six aliquots in containers of 50 L. *Saccharomyces* yeast (*Levulia® esperide,* AEB) at a dose of 20 g/hL and 30 g/hL yeast nutrient (FERMOPLUS® CH, AEB), both dissolved in must, were inoculated in each container. Different commercial enzymes based on pectolytic and β-glycosidase activities were added to musts before alcoholic fermentation: Endozym Thiol®, AEB – V1; Endozym® β-Split, AEB – V2; Zymovarietal® aroma G, SODINAL – V3; Endozym® Ice, AEB – V4; Zimarome®, BSG WINE – V5 and no enzyme – V6), at a dose of 3 g/hL for powder products (Endozym® β-Split, Zymovarietal® aroma G, Zimarome®) and 3 mL/hL for the liquid ones (Endozym Thiol®, Endozym® Ice). All these commercial enzyme preparations were obtained from microorganisms cultivated on substrates under conditions that optimize their production and facilitate their purification. The administrated doses were in line with the producer's instructions and current European legislation. The fermentation was carried out at 16–18 °C for about three weeks and samples were constantly collected every three days and kept at −20 °C until the time of analysis. When the alcoholic fermentation ended, the wines were racked, sulphated (1.5 mL/L SO_2 6%) and filtered through 0.45 μm sterile membrane filters. All wine samples were bottled, stored under controlled conditions (constant temperature 8 °C, dark, stable humidity 70–80%) and analyzed after approximately 3 months.

2.2. Standard physicochemical parameters

Were determined according to the International Organization of Vine and Wine Compendium methods of analysis (OIV, 2019). Each sample was analyzed in triplicate for: total acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), alcoholic strength (% vol.), pH, density, total sugar (g/L), free and total sulfur dioxide (mg/L), malic acid

Table 1
Physicochemical parameters of resulted wines.

Var	TA g/L $C_4H_6O_6$	pH	AC % vol.	MA g/L	VA g/L $C_2H_4O_2$	ρ	TS g/L	LA g/L	Free SO_2 mg/L	Total SO_2 mg/L	TDE g/L	NE g/L
Feteasca regală												
V1	4.2 ± 0.08	3.28 ± 0.05	12.8 ± 0.29	2.7 ± 0.28	0.13 ± 0.00	0.9908 ± 0.15	1.8 ± 0.00	0.0 ± 0.00	23 ± 0.16	56 ± 0.25	19.6 ± 0.05	17.8 ± 0.22
V2	4.2 ± 0.01	3.36 ± 0.00	12.7 ± 0.00	2.9 ± 0.32	0.00 ± 0.01	0.9909 ± 0.45	1.7 ± 0.10	0.1 ± 0.00	23 ± 014	56 ± 0.05	19.6 ± 0.10	17.9 ± 0.35
V3	4.2 ± 0.10	3.3 ± 0.05	12.8 ± 0.29	2.9 ± 0.07	0.08 ± 0.05	0.9907 ± 0.00	1.6 ± 0.07	0.2 ± 0.26	26 ± 0.45	59 ± 0.35	19.3 ± 0.15	17.7 ± 0.12
V4	4.2 ± 0.14	3.28 ± 0.00	12.7 ± 0.53	2.8 ± 0.00	0.14 ± 0.07	0.9907 ± 0.07	1.4 ± 0.25	0.2 ± 0.06	26 ± 0.21	59 ± 0.55	19 ± 0.20	17.6 ± 0.14
V5	4.3 ± 0.00	3.27 ± 0.01	12.7 ± 0.30	2.8 ± 0.00	0.10 ± 0.00	0.9907 ± 0.16	1.3 ± 0.19	0.2 ± 0.00	23 ± 0.55	59 ± 0.10	19 ± 0.10	17.7 ± 0.22
V6	4.2 ± 0.15	3.28 ± 0.45	12.8 ± 0.08	2.9 ± 0.19	0.12 ± 0.21	0.9907 ± 0.00	1.4 ± 0.08	0.3 ± 0.00	26 ± 0.17	56 ± 0.05	19.3 ± 0.07	17.9 ± 0.16
Sauvignon blanc												
V1	3.3 ± 0.00	3.40 ± 0.06	16.2 ± 0.35	0.5 ± 0.10	0.29 ± 0.15	0.9911 ± 0.05	2.2 ± 0.03	1.3 ± 0.00	15 ± 0.55	61 ± 0.23	30.2 ± 0.35	28 ± 0.08
V2	3.2 ± 0.00	3.42 ± 0.08	16.2 ± 0.03	0.3 ± 0.05	0.29 ± 0.20	0.9910 ± 0.05	1.9 ± 0.10	1.6 ± 0.00	18 ± 0.40	61 ± 0.14	30.0 ± 0.28	28.1 ± 0.05
V3	3.3 ± 0.01	3.42 ± 0.05	16.2 ± 0.10	0.3 ± 0.15	0.28 ± 0.15	0.9911 ± 0.00	2.1 ± 0.23	1.6 ± 0.15	18 ± 0.20	59 ± 0.08	30.2 ± 0.17	28.1 ± 0.18
V4	3.2 ± 0.05	3.42 ± 0.05	16.2 ± 0.05	0.3 ± 0.45	0.29 ± 0.05	0.9912 ± 0.00	2.4 ± 0.00	1.5 ± 0.00	15 ± 0.10	61 ± 0.06	30.5 ± 0.08	28.1 ± 0.12
V5	3.3 ± 0.01	3.42 ± 0.02	16.2 ± 0.00	0.3 ± 0.08	0.30 ± 0.35	0.9914 ± 0.03	2.6 ± 0.02	1.4 ± 0.05	18 ± 0.32	64 ± 0.16	30.0 ± 0.18	28.2 ± 0.05
V6	3.3 ± 0.00	3.44 ± 0.03	16.2 ± 0.00	0.3 ± 0.05	0.32 ± 0.25	0.9914 ± 0.05	2.7 ± 0.07	1.6 ± 0.05	18 ± 0.30	60 ± 0.20	30.1 ± 0.08	28.2 ± 0.03

Values are means of triplicate determination (n = 3) ± S.D; Var –analyzed variant; TA – total acidity; MA – malic acid; VA – volatile acidity; ρ – density; TS – total sugars; LA – lactic acid; TDE – total dry extract; NE – non-reductive extract.

(g/L), lactic acid (g/L), total dry extract (g/L) and non-reducing extract (g/L).

2.3. The quantification of polyphenols

was performed using an Agilent 1100 HPLC Series system (Agilent, SUA) equipped with an auto-sampler G1311A type and a reversed-phase Zorbax SB-C18 analytical column (100 × 3.0 mm, 3.5 μm particles) for the separation of the analytes. The column was operated at 40 °C in a G1316A oven. For the elution, a degasser (G1322A) and a binary gradient pump (G1311A) were used. The isocratic elution was performed using a mixture of 1 mM ammonium acetate/acetonitrile (73/27, v/v). The flow rate was 1 mL/min and the injection volume 5 μL. All solvents were filtered through 0.5 μm (Sartorius) filters and degassed in an ultrasonic bath. The detection of *trans*- and *cis*- resveratrol was performed with an Agilent Ion Trap VL mass spectrometer (Agilent, USA), operated with an atmospheric pressure chemical ionization (APCI) ion source in negative mode. The nitrogen was used as nebulizing and dry gas. The APCI heater was fixed at 350 °C, the nebulizer pressure – 60 PSI, dry gas flow − 5 L/min and heated at 250 °C. The mass spectrometer operated in multiple reactions monitoring mode and was set to monitor the transition m/z 227 → m/z 185. Chromatographic and mass spectrometric data acquisition were processed using Chemstation software (Agilent Technologies, Palo Alto, CA, USA), version B.01.03 and LC/MSD Trap Control (Bruker Daltonik, GmbH, Bremen, Germany), version 5.3, while data processing was performed using LC/MSD Data Analysis and Quant Analysis software (Bruker Daltonik, GmbH, Bremen, Germany), version 1.7. The determinations were performed in triplicate.

2.4. Chemicals and samples preparation

Standard solutions and reagents were of HPLC grade and all chemicals were of analytical grade (> 99%), purchased from Merck KgaA, Germany. In this study, 9 standards of the phenolic compounds were used, namely caftaric acid (from Dalton, USA), gentisic acid, ferulic acid, (from Roth, Germany), *para*-coumaric acid, chlorogenic acid, caffeic acid, ferulic acid, syringic acid, gallic acid (from Sigma, Germany) and *trans*-resveratrol (Sigma-Aldrich Chemie GmbH, Munich, Germany).

For the resveratrol assay, a methanol solution (10 mg/mL) of the *trans*-resveratrol was prepared and kept in controlled conditions of light and temperature (at 4 °C and protected from daylight). At the moment of analyses, the methanol solution was appropriately diluted with bi-distilled water. *Cis*-resveratrol was prepared from a standard solution of *trans*-resveratrol after 10 min of irradiation with a UV lamp (254 nm). In the beginning, two working solutions of *trans*-resveratrol with a concentration of 4.9 μg/mL were prepared. The first one was necessary to obtain the calibration curve of *trans*-resveratrol, in a range between 10.47 and 837.86 μg/mL (n = 7). The second working solution was irradiated using UV light and similar dilutions were made as for *trans*-resveratrol. The residual (non-converted) *trans*-resveratrol content was monitored by chromatography. The final concentration of *cis*-resveratrol was calculated as the difference between the concentration of *trans*-resveratrol before and after irradiation, respectively. When comparing the concentrations of *trans*-resveratrol with and without irradiation, the conversion yield of *trans*-resveratrol to *cis*-resveratrol, after 10 min of irradiation was found to be approximately 90%. This way a calibration curve was constructed for *cis*-resveratrol in the range of 9.12–730.14 μg/mL.

Wine aliquots were injected into the chromatographic system without any sample pretreatment step. Determinations were performed in triplicate.

Table 2
Evolution of phenolic content during alcoholic fermentation in Fetească regală wines.

Fetească regală	V1 Concentrations (μg/mL)	V2	V3	V4	V5	V6
I						
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd
P-coumaric acid	nd	nd	nd	nd	nd	nd
Ferulic acid	0.35 ± 0.10	0.35 ± 0.15	0.35 ± 0.12	0.25 ± 0.10	0.35 ± 0.16	0.32 ± 0.10
Gallic acid	0.28 ± 0.10	0.26 ± 0.11	0.20 ± 0.20	0.25 ± 0.05	0.26 ± 0.10	0.24 ± 0.15
Protocatechuic acid	13.40 ± 0.125	13.35 ± 0.25	12.24 ± 0.04	12.36 ± 0.05	11.8 ± 0.10	6.68 ± 0.05
Chlorogenic acid	nd	nd	nd	nd	nd	nd
Trans-resveratrol	nd	nd	nd	nd	nd	nd
Cis-resveratrol	0.23 ± 0.25	0.61 ± 0.50	0.21 ± 0.01	0.30 ± 0.10	0.16 ± 0.15	0.19 ± 0.15
II						
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	0.51 ± 0.07	0.72 ± 0.10	0.74 ± 0.15	0.50 ± 0.15	0.49 ± 0.10	0.36 ± 0.04
P-Coumaric acid	0.03 ± 0.05	0.07 ± 0.25	0.09 ± 0.15	0.07 ± 0.15	0.09 ± 0.15	nd
Ferulic acid	0.29 ± 0.10	0.32 ± 0.20	0.38 ± 0.15	0.35 ± 0.15	0.35 ± 0.15	0.28 ± 0.15
Gallic acid	0.32 ± 0.15	0.54 ± 0.10	0.65 ± 0.15	0.54 ± 0.15	0.45 ± 0.15	0.39 ± 0.15
Protocatechuic acid	13.7 ± 0.01	13.4 ± 0.07	21.64 ± 0.15	11.71 ± 0.15	11.76 ± 0.15	7.64 ± 0.15
Chlorogenic acid	nd	nd	nd	nd	nd	nd
Trans-resveratrol	0.76 ± 0.15	0.65 ± 0.18	0.61 ± 0.04	0.73 ± 0.15	0.60 ± 0.10	0.47 ± 0.15
Cis-resveratrol	2.75 ± 0.05	2.59 ± 0.05	2.43 ± 0.15	2.70 ± 0.21	0.16 ± 0.40	2.19 ± 0.15
III						
Caftaric acid	16.73 ± 0.10	nd	nd	nd	nd	nd
Caffeic acid	0.15 ± 0.15	0.8 ± 0.20	0.81 ± 0.18	0.57 ± 0.20	0.5 ± 0.15	0.45 ± 0.35
P-Coumaric Acid	0.45 ± 0.01	0.06 ± 0.015	nd	nd	0.06 ± 0.10	0.00 ± 0.05
Ferulic acid	nd	0.23 ± 0.10	0.27 ± 0.08	0.24 ± 0.15	0.25 ± 0.105	0.23 ± 0.10
Gallic acid	0.7 ± 0.08	0.79 ± 0.05	0.68 ± 0.17	0.77 ± 0.10	0.66 ± 0.15	0.55 ± 0.10
Protocatechuic acid	13.32 ± 0.17	13.52 ± 0.19	12.76 ± 0.21	12.10 ± 0.15	11.88 ± 0.15	10.67 ± 0.15
Chlorogenic acid	nd	nd	nd	nd	nd	nd
Trans-resveratrol	nd	1.16 ± 0.40	1.04 ± 0.15	1.06 ± 0.15	1.28 ± 0.15	1.18 ± 0.15
Cis-resveratrol	nd	3.11 ± 0.27	3.04 ± 0.25	3.03 ± 0.57	3.32 ± 0.14	3.60 ± 0.27
IV						
Caftaric acid	18.59 ± 0.31	nd	nd	nd	nd	nd
Caffeic acid	0.83 ± 0.24	0.89 ± 0.10	0.73 ± 0.15	0.57 ± 0.16	0.51 ± 0.19	0.39 ± 0.13
P-coumaric acid	0.49 ± 0.42	nd	nd	nd	0.03 ± 0.15	nd
Ferulic acid	0.53 ± 1.10	0.23 ± 0.80	0.22 ± 1.25	0.25 ± 2.38	0.25 ± 0.97	0.20 ± 0.45
Gallic acid	0.67 ± 0.64	0.75 ± 0.13	0.66 ± 0.15	0.72 ± 1.10	0.68 ± 1.15	0.51 ± 0.15
Protocatechuic acid	12.93 ± 2.15	13.31 ± 1.10	12.46 ± 0.75	11.78 ± 0.90	11.63 ± 1.15	8.53 ± 1.10
Chlorogenic acid	1.28 ± 0.64	2.00 ± 0.25	0.97 ± 0.25	0.53 ± 0.35	0.78 ± 0.15	0.46 ± 0.10
Trans-resveratrol	0.86 ± 0.17	1.39 ± 0.14	1.22 ± 0.20	1.28 ± 0.25	1.46 ± 0.28	1.17 ± 0.80
Cis-resveratrol	0.27 ± 0.14	3.16 ± 0.22	3.38 ± 0.44	3.50 ± 0.37	3.76 ± 0.34	3.63 ± 0.30
V						
Caftaric acid	7.81 ± 0.43^{a}	nd	nd	nd	nd	nd
Caffeic acid	3.28 ± 0.17^{b}	1.17 ± 0.48^{b}	1.07 ± 0.10^{b}	0.61 ± 0.82^{b}	0.70 ± 0.77^{b}	0.52 ± 0.15^{b}
P-coumaric acid	$0.28 \pm 0.25^{a,b,c}$	$0.17 \pm 0.37^{a,b,c}$	$0.15 \pm 0.15^{a,b,c}$	$0.07 \pm 0.28^{a,b,c}$	$0.26 \pm 0.19^{a,b,c}$	$0.06 \pm 0.15^{a,b,c}$
Ferulic acid	$0.46 \pm 1.76^{b,c}$	$0.41 \pm 0.26^{b,c}$	$0.41 \pm 0.43^{b,c}$	$0.41 \pm 1.21^{b,c}$	$0.43 \pm 0.37^{b,c}$	$0.33 \pm 0.43^{b,c}$
Gallic acid	$1.00 \pm 1.49^{b,c}$	$0.88 \pm 0.72^{b,c}$	$0.85 \pm 0.28^{b,c}$	$0.78 \pm 0.15^{b,c}$	$0.87 \pm 0.38^{b,c}$	$0.64 \pm 0.34^{b,c}$
Protocatechuic acid	11.04 ± 1.23^{a}	11.04 ± 1.15^{a}	10.01 ± 0.75^{a}	10.02 ± 0.45^{a}	10.01 ± 1.25^{a}	9.24 ± 0.72^{a}
Chlorogenic acid	$2.10 \pm 0.19^{b,c}$	$3.01 \pm 0.17^{b,c}$	$1.21 \pm 0.08^{b,c}$	$0.77 \pm 0.03^{b,c}$	$0.94 \pm 0.15^{b,c}$	$0.80 \pm 0.03^{b,c}$
Trans-resveratrol	$2.12 \pm 0.85^{b,c}$	$1.43 \pm 0.45^{b,c}$	$1.13 \pm 0.40^{b,c}$	$1.00 \pm 0.15^{b,c}$	$1.33 \pm 0.34^{b,c}$	$0.29 \pm 0.49^{b,c}$
Cis-resveratrol	$2.42 \pm 0.25^{b,c}$	$3.42 \pm 0.18^{b,c}$	$3.83 \pm 1.15^{b,c}$	$3.81 \pm 0.75^{b,c}$	$3.91 \pm 1.13^{b,c}$	$3.81 \pm 0.15^{b,c}$

Values are means of triplicate determination (n = 3) ± S.D; nd – not detected or bellow detection limit.
I, II, III, IV – stage of alcoholic fermentation (day 1, 4, 7, and 10); V – wine.
The superscript symbol in wines samples indicates that the factors (a - administrated enzymes, b - sample collecting stage and c - grape variety) with a P-value < 0.05 have a statistically significant effect on the parameter at the 95.0% confidence level The superscript letters n.s. indicates that the factor does not have a statistically significant influence.

2.5. Statistical analysis

All determinations were run in triplicate and values were averaged. Statistical analysis including ANOVA test was performed using the PC software package STATGRAPHICS 18®. Enzymes treatment, grape variety and stage of fermentation were considered as qualitative variables.

3. Results and discussions

3.1. Effects of enzymatic pre-treatment on basic parameters of resulted wine

Table 1 presents the values of the physicochemical parameters of wines. It is known that the alcoholic strength of wine is directly proportional to the total sugar content of the grapes. The obtained wines were dry ones with over 12.7% vol. on Fetească regală and 16.2% vol. on Sauvignon blanc samples.

No major differences were registered in total and volatile acidity of analyzed wines, which means that enzymes have no influence on these indicators. Their content in wine depends on grapes' variety,

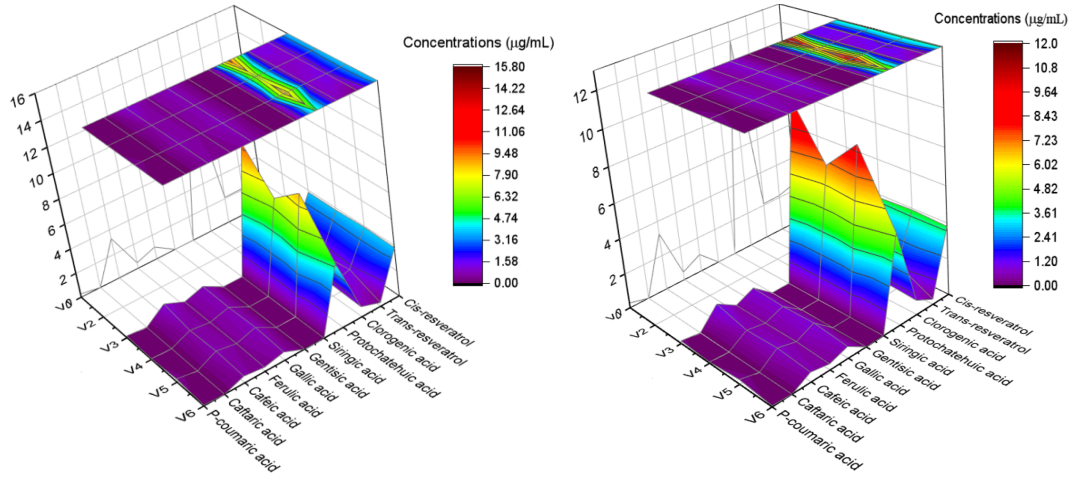

Fig. 1. Phenolic profile of analyzed wines (Sauvignon blanc – left; Fetească regală – right).

maturation, climate, winemaking practices, wine storage and pH values.

The total dry extract values ranged between 19 and 19.6 g/L for Fetească regală wine and 30–30.5 g/L in Sauvignon blanc samples. The content of non-reductive extract of most Romanian wines varies between 13 and 35 g/L, according to grape variety, health conditions, applied technology and wine treatments (Cotea, 1985). The respective values of non-reductive dry extract ranged between 17.6 and 17.9 g/L for Fetească regală samples and 28–28.2 g/L in case of Sauvignon blanc wines.

Lactic acid is usually formed during alcoholic fermentation by the transformation of carbohydrates and under the action of yeast. In young wines, lactic acid is found in small quantities, usually up to 0.5 g/L (Cotea, 1985). The experimental samples do not exceed 0.3 g/L in Fetească regală wines and 1.3–1.6 g/L in Sauvignon blanc, respectively. Higher lactic acid values may indicate the onset of malolactic fermentation or degradation of carbohydrates, tartaric acid and glycerol (Cotea, 1985).

The presented data showed only a minor influence of the enzymes on physicochemical parameters.

3.2. Effects of enzymatic pre-treatment on the evolution of phenolic compounds

Phenolic compounds are generally biosynthesised by the shikimate pathway, from which they are produced using intermediates of carbohydrate metabolism. Protocatechuic and gallic acids are synthesized from 3-dehydroshikimic acid by direct aromatization reaction, although these are also formed from benzoic and cinnamic acid. Phenylalanine represents the metabolic source for the synthesis of cinnamic, p-coumaric, caffeic and ferulic acid. Syringic acid descends from benzoic acid, generally derived from corresponding cinnamic acid derivatives through the enzymatic reactions of β-oxidation. The increase of *p*-coumaric acid concentration can be explained by a release during enzyme catalyzed degradation of acylated anthocyanins as a result of different esterase activity. The decrease in the phenolic acids could be explained by different reactions that involve anthocyanins during the fermentation stage. Resveratrol is usually produced in grapevine tissue as an active protection strategy against diseases. On the other hand, these compounds may result because of the activity of extracellular enzymes released by the pathogenic agent in an attempt to remove undesirable toxic components (Srinivasulu, Ramgopal, Ramanjaneyulu, Anuradha, & Suresh Kumar, 2018).

Table 2 shows the results of monitoring phenolic compounds of Fetească regală samples during alcoholic fermentation.

Ferulic and gallic acid originate from the grapes and are being transferred to the must during pressing. Ferulic acid diminishes its concentration from the middle of the fermentation, followed by a new increase at the end of this process. After alcoholic fermentation, ferulic acid presented the highest value in V1 wine (treated with Endozym Thiol®, AEB), followed by V5 (Zimarome®, BSG WINE) and the lowest in V6 (no enzyme).

Gallic acid can also results from the hydrolysis of gallic esters that take place during fermentation. The concentrations of this compound showed various fluctuations depending on the grape variety, the applied treatment or the stage of fermentation. In wine, the highest level (1.00 μg/mL) of this component registered in V1 (treated with Endozym Thiol®, AEB), followed by V2, with 0.88 μg/mL (Endozym® β-Split, AEB), while the lowest was found in V6 (control sample). Soto Vázquez, Río Segade, and Orriols Fernández (2010) also reported a significant increase of gallic acid in wine produced using enzymes and tannins.

Caffeic, *p*-coumaric and caftaric acids appear during alcoholic fermentation, resulting from various chemical reactions and have different fluctuations during the fermentation stage. Significant differences in wines can be observed depending on the type of administrated enzymes. For example, caftaric acid was not detected in wines, except V1 (treated with Endozym Thiol®, AEB). Thus, the enzymatic preparation administered in this variant had a major influence on the extraction and enrichment in this compound.

Protocatechuic acid was identified to be the dominant phenolic acid and its highest concentration was detected in V1 wine. Also, V1 sample was distinguished by high concentrations of protocatechuic and *p*-coumaric acids after alcoholic fermentation, compared to the control sample, where they showed the lowest levels. V2 (Endozym® β-Split, AEB) and V5 (Zimarome®, BSG WINE) samples were remarked for their high levels in chlorogenic acid and *cis*-resveratrol, respectively.

Its levels in wine are usually influenced by winemaking practices, temperature conditions, sunshine hours and *Botrytis* infection of grapes (Malovaná, García Montelongo, Pérez, & Rodríguez-Delgado, 2001; Varelis, Melton, & Shahidi, 2018). The amounts of resveratrol in analyzed samples showed different variations during the alcoholic fermentation, depending on the times of sample collection, grape variety and inoculated enzymes. All stabilized wines contained more *cis*- resveratrol than its *trans*-isomer.

Major differences can be observed on the samples treated with enzymes compared to control variants. The majority of identified phenolic compounds showed increasing concentrations in wines treated with enzymes compared to control samples. The phenolic profile of analyzed wines can be observed in Fig. 1. Table 3 presents the results of monitoring the phenolic compounds of Sauvignon blanc samples during the alcoholic fermentation (See Table 4).

p-coumaric and syringic acid originate from the grapes and

Table 3
Evolution of phenolic content during alcoholic fermentation in Sauvignon blanc wines.

Sauvignon blanc	V1 Concentrations (μg/mL)	V2	V3	V4	V5	V6
I						
p-Coumaric acid	0.21 ± 0.15	0.36 ± 0.15	0.33 ± 0.15	nd	0.19 ± 0.15	0.16 ± 0.15
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd
Gallic acid	0.16 ± 0.15	0.16 ± 0.15	0.20 ± 0.15	0.17 ± 0.15	0.17 ± 0.15	0.2 ± 0.15
Gentisic acid	nd	nd	nd	nd	nd	nd
Syringic acid	0.20 ± 0.15	0.35 ± 0.15	0.32 ± 0.15	nd	0.10 ± 0.15	0.16 ± 0.15
Protocatechuic acid	10.74 ± 0.15	11.21 ± 0.15	12.17 ± 0.15	10.32 ± 0.15	10.73 ± 0.15	10.13 ± 0.15
Trans-resveratrol	nd	0.19 ± 0.15	nd	nd	nd	nd
Cis-resveratrol	nd	nd	nd	nd	nd	nd
II						
p-Coumaric acid	0.43 ± 0.15	0.42 ± 0.15	0.46 ± 0.15	0.39 ± 0.15	0.33 ± 0.15	0.17 ± 0.15
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd
Gallic acid	0.17 ± 0.15	0.18 ± 0.15	0.21 ± 0.15	0.17 ± 0.15	0.17 ± 0.15	0.2 ± 0.15
Gentisic acid	0.17 ± 0.15	nd	0.17 ± 0.15	nd	nd	nd
Syringic acid	0.43 ± 0.15	0.41 ± 0.15	0.47 ± 0.15	0.39 ± 0.15	0.33 ± 0.15	0.17 ± 0.15
Syringic acid	0.43 ± 0.15	0.41 ± 0.15	0.47 ± 0.15	0.39 ± 0.15	0.33 ± 0.15	0.17 ± 0.15
Protocatechuic acid	11.82 ± 0.15	10.44 ± 0.15	10.18 ± 0.15	11.16 ± 0.15	10.12 ± 0.15	9.17 ± 0.15
Trans-resveratrol	0.19 ± 0.15	0.12 ± 0.15	nd	nd	nd	nd
Cis-resveratrol	nd	nd	nd	nd	nd	nd
III						
P-coumaric acid	0.53 ± 0.15	0.51 ± 0.15	0.48 ± 0.15	0.43 ± 0.15	0.42 ± 0.15	0.24 ± 0.15
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd
Gallic acid	0.22 ± 0.15	0.20 ± 0.15	0.20 ± 0.15	0.21 ± 0.15	0.18 ± 0.15	0.25 ± 0.15
Gentisic acid	0.19 ± 0.15	0.09 ± 0.15	0.18 ± 0.15	0.07 ± 0.15	0.09 ± 0.15	nd
Syringic acid	0.53 ± 0.15	0.51 ± 0.15	0.49 ± 0.15	0.44 ± 0.15	0.43 ± 0.15	0.24 ± 0.15
Protocatechuic acid	12.62 ± 0.15	11.64 ± 0.15	9.29 ± 0.15	12.15 ± 0.15	10.35 ± 0.15	9.56 ± 0.15
Trans-resveratrol	nd	nd	nd	nd	nd	nd
Cis-resveratrol	nd	nd	nd	nd	nd	nd
IV						
P-coumaric acid	0.42 ± 0.15	0.39 ± 0.15	0.39 ± 0.15	0.31 ± 0.15	0.38 ± 0.15	0.13 ± 0.15
Caftaric acid	nd	nd	nd	nd	nd	nd
Caffeic acid	nd	nd	nd	nd	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd
Gallic acid	0.33 ± 0.15	0.25 ± 0.15	0.28 ± 0.15	0.23 ± 0.15	0.19 ± 0.15	0.28 ± 0.15
Gentisic acid	0.18 ± 0.15	0.12 ± 0.15	0.26 ± 0.15	0.12 ± 0.15	0.07 ± 0.15	0.05 ± 0.15
Syringic acid	0.42 ± 0.15	0.39 ± 0.15	0.37 ± 0.15	0.32 ± 0.15	0.39 ± 0.15	0.13 ± 0.15
Protocatechuic acid	12.75 ± 0.15	11.79 ± 0.15	9.49 ± 0.15	12.69 ± 0.15	10.28 ± 0.15	10.06 ± 0.15
Trans-resveratrol	0.10 ± 0.15	0.40 ± 0.15	nd	0.2 ± 0.15	nd	nd
Cis-resveratrol	nd	nd	nd	nd	nd	nd
V						
P-coumaric acid	0.36 ± 0.15 ^{a,b,c}	0.33 ± 0.15 ^{a,b,c}	0.34 ± 0.15 ^{a,b,c}	0.24 ± 0.15 ^{a,b,c}	0.39 ± 0.15 ^{a,b,c}	0.12 ± 0.15 ^{a,b,c}
caftaric acid	7.34 ± 0.15^{a}	nd	2.69 ± 0.15^{a}	6.93 ± 0.15^{a}	7.23 ± 0.15^{a}	9.80 ± 0.15^{a}
Caffeic acid	3.02 ± 0.15^{b}	4.95 ± 0.15^{b}	4.49 ± 0.15^{b}	2.33 ± 0.15^{b}	2.55 ± 0.15^{b}	1.14 ± 0.15^{b}
Ferulic acid	0.35 ± 0.15^{b,c}	0.37 ± 0.15^{b,c}	0.34 ± 0.15^{b,c}	0.26 ± 0.15^{b,c}	0.32 ± 0.15^{b,c}	0.18 ± 0.15^{b,c}
Gallic acid	0.35 ± 0.15^{b,c}	0.22 ± 0.15^{b,c}	0.25 ± 0.15^{b,c}	0.27 ± 0.15^{b,c}	0.17 ± 0.15^{b,c}	0.28 ± 0.15^{b,c}
Gentisic acid	0.24 ± 0.1^{a,b,c}	0.26 ± 0.15^{a,b,c}	0.30 ± 0.15^{a,b,c}	0.16 ± 0.15^{a,b,c}	0.12 ± 0.15^{a,b,c}	0.10 ± 0.15^{a,b,c}
Syringic acid	0.36 ± 0.15^{a,b,c}	0.30 ± 0.15^{a,b,c}	0.34 ± 0.15^{a,b,c}	0.23 ± 0.15^{a,b,c}	0.38 ± 0.15^{a,b,c}	0.13 ± 0.15^{a,b,c}
Protocatechuic acid	13.75 ± 0.15^{a}	12.64 ± 0.15^{a}	9.99 ± 0.15^{a}	12.89 ± 0.15^{a}	10.68 ± 0.15^{a}	10.47 ± 0.15^{a}
Trans-resveratrol	2.39 ± 0.15^{b,c}	2.50 ± 0.15^{b,c}	2.35 ± 0.15^{b,c}	2.20 ± 0.15^{b,c}	2.39 ± 0.15^{b,c}	2.22 ± 0.15^{b,c}
Cis-resveratrol	2.96 ± 0.15^{b,c}	2.55 ± 0.15^{b,c}	2.92 ± 0.15^{b,c}	2.80 ± 0.15^{b,c}	3.21 ± 0.15^{b,c}	2.77 ± 0.15^{b,c}

Values are means of triplicate determination (n = 3) ± S.D; nd – not detected or bellow detection limit.
I, II, III, IV – stage of alcoholic fermentation (day 1, 4, 7 and 10); V – wine sample.
The superscript symbol in wines samples indicates that the factors (a - administrated enzymes, b - sample collecting stage and c - grape variety) with a P-value < 0.05 have a statistically significant effect on the parameter at the 95.0% confidence level. The superscript letters n.s. indicates that the factor does not have a statistically significant influence.

registered different fluctuations depending on the type of administrated enzymes. *p*-coumaric levels in wines varied from 0.39 μg/mL in V5 (Zimarome®, BSG WINE) to 0.12 μg/mL in the control sample.

V5 wine (Zimarome®, BSG WINE) registered the high levels of syringic acid (0.38 μg/mL) but the lowest content in gallic acid (0.17 μg/mL).

Caftaric, caffeic and ferulic acids appear after the alcoholic fermentation. V2 sample (treated with Endozym® β-Split, AEB) showed the highest content in caffeic and ferulic acids (4.95 μg/mL and 0.37 μg/mL, respectively), while the V6 (control sample) registered the lowest concentration of these elements.

Gentisic acid was formed in the middle of the fermentation stage, registering different fluctuations. This compound presented the highest level (0.3 μg/mL) in V3 sample (Zymovarietal® aroma G, SODINAL),

Table 4
The analysis of variance of the identified phenolic compouds.

Phenolic compound	Source main effects	Sum of squares	Df	Mean square	F-ratio	P-value
P-coumaric acid	A:Administrated enzymes	0.3055	5	0.0611	6.55	**0.0001***
	B: Fermentation stage	0.1563	4	0.0391	4.19	**0.0054****
	C:Grape variety	0.9428	1	0.9428	101.07	**0.0000***
	RESIDUAL	0.4570	49	0.0093		
	TOTAL (CORRECTED)	1.8616	59			
Caffeic acid	A:Administrated enzymes	2.6493	5	0.5299	0.97	0.4475 ns
	B: Fermentation stage	36.7299	4	9.1825	16.75	**0.0000***
	C:Grape variety	0.0062	1	0.0062	0.01	0.9155 ns
	RESIDUAL	26.8620	49	0.5482		
	TOTAL (CORRECTED)	66.2474	59			
Caftaric acid	A:Administrated enzymes	175.9320	5	35.1865	3.03	**0.0184*****
	B: Fermentation stage	98.5843	4	24.6461	2.12	0.0920 ns
	C:Grape variety	1.3936	1	1.3936	0.12	0.7305 ns
	RESIDUAL	568.8370	49	11.6089		
	TOTAL (CORRECTED)	844.7470	59			
Chlorogenic acid	A:Administrated enzymes	1.0789	5	0.2158	1.01	0.4224 ns
	B: Fermentation stage	5.8447	4	1.4612	6.83	**0.0002***
	C:Grape variety	3.6784	1	3.6784	17.21	**0.0001***
	RESIDUAL	10.4759	49	0.2138		
	TOTAL (CORRECTED)	21.0777	59			
Ferulic acid	A:Administrated enzymes	0.0156	5	0.0031	0.56	0.7317 ns
	B. Fermentation stage	0.4670	4	0.1168	20.81	**0.0000***
	C:Grape variety	0.9358	1	0.9358	166.82	**0.0000***
	RESIDUAL	0.2749	49	0.0056		
	TOTAL (CORRECTED)	1.6933	59			
Gallic acid	A:Administrated enzymes	0.0425	5	0.0085	0.66	0.6588 ns
	B: Fermentation stage	0.7802	4	0.1951	15.04	**0.0000***
	C:Grape variety	2.0598	1	2.0598	158.81	**0.0000***
	RESIDUAL	0.6355	49	0.0130		
	TOTAL (CORRECTED)	3.51802	59			
Gentisic acid	A:Administrated enzymes	0.0442	5	0.0088	2.86	**0.0242***
	B: Fermentation stage	0.0677	4	0.0169	5.48	**0.0010****
	C:Grape variety	0.1458	1	0.1458	47.2	**0.0000***
	RESIDUAL	0.1514	49	0.0031		
	TOTAL (CORRECTED)	0.4091	59			
Protocatechuic acid	A:Administrated enzymes	116.0440	5	23.2088	6.14	**0.0002***
	B: Fermentation stage	36.0518	4	9.0129	2.38	0.0641 ns
	C:Grape variety	5.9497	1	5.9497	1.57	0.2157 ns
	RESIDUAL	185.3200	49	3.7820		
	TOTAL (CORRECTED)	343.3660	59			
Syringic acid	A:Administrated enzymes	0.1017	5	0.0203	4.02	**0.0039****
	B: Fermentation stage	0.0843	4	0.0211	4.17	**0.0055****
	C:Grape variety	1.6295	1	1.6295	322.31	0
	RESIDUAL	0.2477	49	0.0051		
	TOTAL (CORRECTED)	2.0633	59			
Trans-Resveratrol	A:Administrated enzymes	0.3471	5	0.0694	0.26	0.9321 ns
	B: Fermentation stage	21.6120	4	5.4030	20.31	**0.0000***
	C:Grape variety	1.3410	1	1.3410	5.04	**0.0293*****
	RESIDUAL	13.0327	49	0.2660		
	TOTAL (CORRECTED)	36.3328	59			
Cis-Resveratrol	A:Administrated enzymes	4.9931	5	0.9986	1.46	0.2211 ns
	B: Fermentation stage	58.3319	4	14.5830	21.27	**0.0000***
	C:Grape variety	49.8135	1	49.8135	72.67	**0.0000***
	RESIDUAL	33.5883	49	0.6855		
	TOTAL (CORRECTED)	146.7270	59			

The superscript symbols denote different statistical significances at the 95.0% confidence level (* $p < 0{,}001$, ** $p < 0.01$, *** $p < 0.5$, n.s. indicates that the factor does not have a statistically significant influence).

followed by 0.26 μg/mL in V2 variant (Endozym® β-Split, AEB), the lowest one being recorded in the control sample (0.10 μg/mL).

Resveratrol, the major active biological compound of the stilbene phytoalexins in wine, represents a polar compound that exists as *trans*- and *cis*-isomers (Vlase, Kiss, Leucuta, & Gocan, 2009). The content of *trans*-resveratrol in resulted wines ranged from 0.29 to 2.12 μg/mL in Feteasca regală samples and 2.20 to 2.50 μg/mL in Sauvignon blanc samples. The *cis*-resveratrol concentration varied from 2.42 to 3.91 μg/mL in Feteasca regală samples and 2.55 to 3.21 μg/mL in Sauvignon blanc variants. These results are in accordance with the hydrolysis ability of some enzymes such as pectinase, cellulase and β-glucosidase in grape pomace and wine reported by Kammerer, Claus, Schieber, and Carle (2005).

Trans-resveratrol has a significant function defining the organoleptic particularities of wine, conferring astringency and structure due to formation of protein-tannin complexes (tannin interacts with proteins mainly through hydrogen bond formation between the phenolic donor and the peptide acceptor) (Kammerer et al., 2005).

Dependent variables in this experiment are represented by the identified phenolic compounds while three factors influence their concentration (administrated enzymes, fermentation stage and grape variety). The contribution of each factor was statistically interpreted independent to the effects of all other factors.

p-coumaric and gentisic acid showed a statistical significance ($p < 0.05$) of each of the factors, while chlorogenic, ferulic, gallic acids, *trans*- and *cis*- resveratrol contents were significantly influenced only by 2 factors (fermentation stage and grape variety). Martins, Roberto, Blumberg, Chen, and Macedo (2016) also registered a significant increase of gallic and caffeic acid and their content was influenced by both grape variety and pectinolytic/cellulolytic enzyme treatment ($p \leq 0.0001$). Caftaric and protocatechuic acids were significantly affected by the type of administrated enzyme, while caffeic acid showed a statistical significance with the fermentation stage.

Since the activity of enzymes is usually dependent on sugars existing during the fermentation stage, the ethanol of wines, or the administration of sulfur dioxide, some enzymatic activities can be inhibited by high levels of these parameters. That can explain why some enzymes showed a minor impact on the analysed substances.

Since this article aimed to evaluate the influence of some commercial enzymes on the evolution of phenolic compounds in white wines, the data confirms that different enzymes act on wine's phenolic composition in varying degrees. The effects of enzymatic treatments on the chemical composition of wines have been widely studied; several works studying similar products (Bartowski, Costello, Villa, & Henschke, 2004; Bautista-Ortín et al., 2012; Fernández-González, Úbeda, Cordero-Otero, Thanvanthri Gururajan, & Briones, 2005; Masino, Montevecchi, Arfelli, & Antonelli, 2008). Generalić Mekinić et al. (2019) have reported significant increases in wine phenolic composition and only a minor influence on physicochemical parameters when enzymes are used. The obtained results confirm that enzymatic preparations significantly contribute to the enrichment of the phenolic profile of wines.

4. Conclusions

The evolution of phenolic compounds content during the fermentation process was influenced by the varietal factor, administrated enzyme and fermentation stage. Enzymatic treatments did not significantly affect the physicochemical composition of the obtained wines. The type of administrated enzymes showed a statistical significance on *p*-coumaric, syringic, gentisic, caftaric and protocatechuic acids final concentrations. *p*-coumaric, gentisic and protocatechuic acid showed the highest increase under the influence of applied treatments. Enzymes generate enhancement of the concentrations of the phenolic compounds in wines, with minimum techniques and energy consumption.

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CRediT authorship contribution statement

Elena-Cristina Scutarasu: Data curation, Writing - original draft, Formal analysis. **Camelia Elena Luchian:** Methodology. **Laurian Vlase:** Resources, Investigation, Validation. **Lucia Cintia Colibaba:** . **Ana Maria Gheldiu:** Investigation, Visualization. **Valeriu V. Cotea:** Resources, Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127910.

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