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“GLOBAL CHALLENGES THROUGH THE PRISM OF
RURAL DEVELOPMENT IN THE SECTOR OF
AGRICULTURE AND TOURISM”



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TABLE OF CONTENTS

AGRICULTURE AND FOOD	9
ORGANIC ANIMAL NUTRITION	
Tugay Ayasan, Esra Gursoy, Milena Milojević.....	10
SYSTEM FOR REARING BEE COLONY AND OBTAINING BEE PRODUCTS INDOORS AT HOME	
Slobodan Dolašević	20
EVALUATION OF THE BIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF THE FRUITS OF EARLY STRAWBERRY VARIETIES	
Stefan Marković, Ljiljana Tanasić, Nemanja Stošić.....	32
INTRODUCTION OF A NATIONAL SYSTEM OF FOOD QUALITY AND SAFETY CONTROL IN UKRAINE	
Kameniev Anatolii	41
THE EFFECT OF THE APPLICATION OF DIFFERENT QUANTITIES OF NITROGEN FERTILIZER ON THE GRAIN YIELD OF DIFFERENT CORN GENOTYPES	
Vladimir Stepić, Vesna Stepić	48
CLASSIFICATION OF NOISE EFFECTS ON WILD ANIMALS	
Milan Glišić, Suzana Knežević, Željko Ignjatović	56
YIELD COMPONENTS AND GENETIC POTENTIAL OF WINTER WHEAT ON SMONICA SOIL OF CENTRAL SERBIA	
Vera Rajčić, Dragan Terzić, Violeta Babić	63
TREATMENT OF BIOREMEDIATION AND PHYTOREMEDIATION OF LAND IN THE PROCESS OF RETURNING LAND TO AGRICULTURAL PURPOSES	
Marija Bajagić, Nemanja Stošić, Vera Rašković.....	71
WINE COMPOSITION AND QUALITY	
Violeta Ivanova-Petropulos	78
AGRICULTURAL TOOLBOX FOR EXPLOITING SENTINEL DATA TO IMPROVE DROUGHT MITIGATION (AGROSEND) – the project proposal	
Gordan Mimić.....	87
THE ROLE OF DIGITAL TECHNOLOGY IN MODERN AGRICULTURE	
Borislav Kolarić	92
APPLICATION OF VEGETABLES IN THE PREPARATION OF EXPRESS RESTAURANT DISHES	
Gordana Jovanović, Ana Vasić, Aleksandra Krsmanović	98
DAIRY COWS PROTECTION FROM ENVIRONMENTAL HEAT STRESS AND SUSTAINABILITY OF MILK PRODUCTION	
Maja Došenović Marinković, Biljana Delić Vujanović, Mira Majkić.....	104
INFLUENCE OF DIFFERENT VARIETIES OF TRITICALE ON PRODUCTION CHARACTERISTICS AND CARCASS YIELD OF BROILER CHICKENS	
Vera Rajčić, Dragan Terzić, Aleksandar Miletić.....	110

ENVIRONMENTAL PROTECTION AND SUSTAINABLE DEVELOPMENT	117
RESEARCHING THE DEGRADATION OF ROADSIDE AREA PLANT COMMUNITIES	
Ganna Zhelnovach	118
CONCEPTUAL APPROACH TO CIRCULAR ECONOMY IMPLEMENTATION: CASE STUDY OF COMPANY ELIXIR ZORKA - MINERAL FERTILIZERS	
Nemanja Tošković, Alija Salkunić, Jelena Ignjatović	126
SUSTAINABLE TECHNOLOGIES AND THE ZERO EMISSIONS IN THE FOOD INDUSTRY	
Žaklina Andjelković, Nemanja Gligorijević, Slobodan Glišić	134
APPROACHES TO THE IMPLEMENTATION OF THE EFFICIENT FUNCTIONING OF THE MECHANISM OF ECOSYSTEM SERVICES TO REDUCE RISKS IN THE AGRO SECTOR	
Natalia Vnukova	143
GEOTHERMAL ENERGY SOURCES AND THEIR SIGNIFICANCE FOR THE SUSTAINABLE DEVELOPMENT OF MAČVAN DISTRICT	
Suzana Knežević, Ljiljana Tanasić, Milena Milojević	150
THE ROLE OF INTERNATIONAL ORGANIZATIONS IN ENVIRONMENTAL PROTECTION	
Zoran Filipovski.....	157
RELATIONSHIPS BETWEEN ECOSYSTEM INVASIBILITY AND SPECIES DIVERSITY	
Milan Glišić, Bojan Damnjanović	163
PROMOTION AND PRESERVATION OF HUMAN HEALTH	172
THE LEGAL FRAMEWORK OF A HEALTHY ENVIRONMENT– CHALLENGES OF MODERN SOCIETY	
Emina Karo	173
AGRICULTURE AND THE PROFESSIONAL REHABILITATION OF PERSONS WITH MENTAL DISABILITIES	
Miroљjub Nikolić, Snežana Lozanović, Andrijana Nikolić	178
ASSESSMENT OF HEALTH-RELATED QUALITY OF LIFE IN BURN PATIENTS	
Sunčica Ivanović, Milena Cvetković Jovanović, Sanja Trgovčević.....	187
KNOWLEDGE AND ATTITUDES TOWARD BREASTFEEDING AMONG POSTNATAL MOTHERS AND PREGNANT WOMEN USING PREGNANCY SCHOOL IN ŠABAC, SERBIA	
Marijana Srećković, Nikola Beljić, Aleksandra Krsmanović	197
IMPROVEMENT AND PRESERVATION OF HUMAN HEALTH	
Suzana Milutinović, Ljubica Krivokapić, Ivana Vukosavljević.....	208
RISKS FOR PATIENT SAFETY IN EMERGENCY SERVICES	
Milena Cvetković Jovanović, Sunčica Ivanović, Sanja Trgovčević.....	216
HEALTH STATUS OF WOMEN	
Ivana Vukosavljević, Suzana Milutinović, Ljubica Krivokapić	223

AGRICULTURAL PRODUCTION AS A RISK FACTOR FOR HUMAN HEALTH IN MACVA DISTRICT Igor Dragičević	228
IMPROVING THE HEALTH OF CHILDREN AND YOUNG PEOPLE Ljubica Krivokapić, Aleksandar Anđelković, Stefan Jovanović	235
QUALITY OF LIFE OF COLORECTAL CARCINOMA SUFFERERS Stefan Jovanović, Gordana Repić, Aleksandar Anđelković	241
ECONOMICS AND MANAGEMENT.....	251
REENGINEERING OF MARKETING AND BUSINESS PROCESSES IN THE COMPANY Nikola Vojvodić, Mladen Ivić, Veljko Vuković.....	252
THE ROLE OF KNOWLEDGE MANAGEMENT AS A STRATEGIC TOOL IN MODERN AGRIBUSINESS Borislav Kolarić, Saša Spasojević	259
ECONOMIC INSTRUMENTS AND SUSTAINABLE DEVELOPMENT IN THE FOOD INDUSTRY Žaklina Andjelković, Nemanja Gligorijević, Slobodan Glišić	268
THE IMPORTANCE OF THE ACCOUNTING TREATMENT OF PROVISIONS FOR THE REALITY OF FINANCIAL REPORTING Ivana Vladimirović, Dejan Grujić, Suzana Grujić.....	280
THE REPRESENTATION OF WOMEN IN TOP MANAGEMENT POSITIONS IN THE SPORT INDUSTRY Vesna Jovanova-Simeva, Azemina Mashovic	290
HUMAN CAPITAL AS A DISPOSITION OF SOCIAL WELFARE Zoran Jokić, Jelena Jevtić	298
THE INFLUENCE OF EXCHANGE RATE POLICY ON THE DEVELOPMENT AND COMPETITIVENESS OF COMPANIES IN CERTAIN COUNTRIES OF SOUTHEAST EUROPE (SEE) Dejan Grujić, Suzana Grujić, Ivana Vladimirović	305
TOURISM - RURAL DEVELOPMENT.....	312
ANALYSIS OF HOW POWISLANSKI UNIVERSITY STUDENT ARE INVOLVED IN LOCAL DEVELOPMENT, INCULUDING RURAL AREA DEVELOPMENT Katarzyna Strzała-Osuch, Beata Pawłowska, Julia Osuch	313
SUSTAINABLE DEVELOPMENT OF RURAL TOURISM: CASE STUDY OF THE REGION OF WESTERN SERBIA Jelena Ignjatović, Aleksandra Đorđević	321
TOURIST DESTINATION OF UPPER SANA AND PLIVA RIVERS AS A FACTOR OF RURAL DEVELOPMENT Nikola Dragović, Željko Grublješić.....	328
CHILDREN'S EDUCATIONAL TOURISM AS A COMPONENT OF LIFELONG ENVIRONMENTAL EDUCATION FOR SUSTAINABLE DEVELOPMENT Sarkisian Kateryna	336

EDUCATION AND KNOWLEDGE FOR THE 21ST CENTURY-INCLUSION.....	346
ENVIRONMENTAL METAPHORS AND NATURAL WORLD IDIOMS WITH IN ENGLISH AND SERBIAN: CONTRASTIVE ANALYSIS AND IMPLICATIONS FOR ESP TEACHING	
Nada Buzadžić Nikolajević.....	347
INFORMATION TEHNOLOGIES	353
THE CONNECTION BETWEEN APPLIED STATISTICS AND INFORMATION TECHNOLOGIES	
Tatjana Bajić	354

WINE COMPOSITION AND QUALITY

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ABSTRACT

Wine is a very complex matrix consisting of variety of compounds, such as polyphenols, aroma compounds, organic acids, proteins, carbohydrates, biogenic amines, minerals etc. The wine quality depends mainly on grape variety, but also on temperature, soil, climate conditions and ripening stage. Also, wine-making practices such as intensity of pressing, fermentation temperature, maceration time, yeast strain, enzymes, SO₂ doses, storage temperature affect the wine composition and quality. Various analytical techniques have been used for determination of wine composition. Reversed phase high-performance liquid chromatography (RP-HPLC) is commonly employed for analysis of polyphenols, biogenic amines, organic acids, carbohydrates and other nonvolatile compounds, using mostly C18 column, and a binary solvent system with an polar acidified solvent, such as aqueous phosphoric acid or formic acid solution (solvent A) and organic modifier such as methanol or acetonitrile, possibly acidified (solvent B). Various detection systems have been coupled to HPLC, such as UV-Vis, DAD, MS and Q-TOF-MS detectors. Gas chromatography is the techniques for analysis of volatile compounds in wine, while multielement analysis is performed with atomic absorption spectroscopy (AAS), electrothermal atomic absorption spectroscopy (ETAAS), inductively coupled plasma - optical emission spectrometry (ICP-OES) and inductively coupled plasma - mass spectrometry (ICP-MS). This paper is focused on the chemical composition of wine and most important advanced instrumental techniques applied for wine analysis, including spectroscopic and chromatographic methods.

Keywords: wine quality, composition, analytical techniques.

INTRODUCTION

Wine is one of the most complex and heterogeneous beverage, consisting of organic and inorganic compounds that have a great influence on its quality. Knowledge about wine composition at various stages of winemaking is very important in order to control the technological process and obtain quality wine. Wine composition is influenced by the grape variety, but also by the climate conditions, vine cultivation and protection and ripening stages. Moreover, winemaking practices, such as grape pressing, fermentation temperature, maceration duration, addition of yeasts, fining chemical, as well as stabilization and aging conditions have influence on wine composition and quality (Ivanova et al., 2009b; Ivanova et al., 2012; Ivanova-Petropulos et al., 2016). Therefore, controlling the changes in wine composition during all stages of production is necessary in order to produce high quality wine, or wine quality according to the costumer's requirements.

Chemical composition of wine

Wine is a complex and heterogeneous mixture that contains a large number of compounds. Generally, wine is composed of water (~ 80 %), ethanol (~12 %) and other compounds (~ 8%), including carbohydrates, organic acids, polyphenols, aromatic compounds, proteins, minerals, biogenic amines etc. (Ivanova et al. 2011a; Fogarasi et al. 2018; Jakobová et al. 2021). *Water* is the dominant component in wine in which a large number of chemical reactions that take place during the alcoholic fermentation, stabilization and aging.

Alcohol is a product of alcoholic fermentation, and it determines the quality of wine. The alcohol content ranges from 10 to 15%, while the higher concentrations may be derived from additionally added sugar during the fermentation, or additionally added ethanol. At higher contents, alcohol causes a tingling, burning sensation, especially noticeable in dry wines. Ethanol increases the intensity of bitterness, but reduces the astringency of tannins. The main factors affecting the formation of alcohol are the sugar content, the fermentation temperature and the yeast.

Organic acids are important compounds influencing the stability, flavor, aroma and color of grapes and wine and contributing to the pH, and to the chemical and microbiological stability of the wines (Tašev et al., 2016; Ivanova-Petropulos et al., 2020).

The main organic acids in wine are tartaric, malic, lactic, citric succinic, and acetic acids. Their content in wines ranges from 5.5 to 8.5 g/L. Since tartaric acid is the dominant component from the group of organic acids, the total acidity of wines is usually expressed as g/L tartaric acid equivalents. Tartaric acid, together with malic acid (Figure 1), constitute more than 90 % of the total acidity of wines. The content of tartaric acid decreases during the aging of wines, as a result of precipitation in form of tartrate crystals. During malolactic fermentation, the content of malic acid decreases because lactic acid is formed (which content increases) under the action of malolactic bacteria. Citric acid is also present in grapes and wine, which can also affect overall acidity.

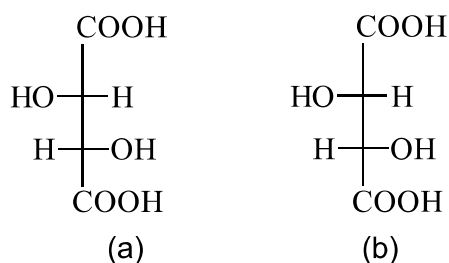


Figure 1. Structure of tartaric acid (a) and malic acid (b)

Carbohydrates are divided into three groups: monosaccharides (glucose, fructose), disaccharides (sucrose) and polysaccharides (starch, cellulose, pectins, glucans, dextrans). The basic monosaccharides present in grapes are glucose and fructose (Figure 2). They are present in almost equal amounts in grapes, except in overripe grapes where the amount of fructose is often higher. Sucrose is very rarely present in grapes of the *Vitis vinifera* variety, while in non-*Vitis vinifera* varieties, it can constitute up to 10% of the total amount of carbohydrates in the grape. Sucrose accumulates in vine leaves during photosynthesis, but upon transfer to the bunches, it hydrolyzes and forms the essential sugars, glucose and fructose.

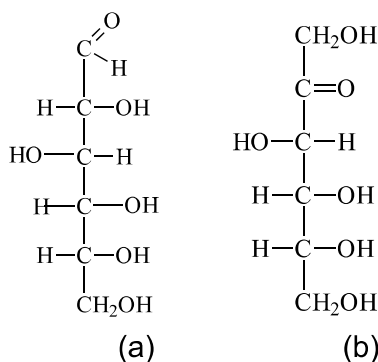


Figure 2. Structure of glucose (a) and fructose (b)

During the fermentation, yeasts convert sugars to ethanol and carbon dioxide. The glucose/fructose ratio decreases from 0.95 at the beginning to 0.25 towards the end of fermentation.

Polyphenols are a large and complex group of compounds that are responsible for the characteristics, color and quality of wines, especially red wines. They are a heterogeneous family composed of two main groups: flavonoids and non-flavonoids. Flavonoids are a group of compounds composed of anthocyanins, flavonols, flavan-3-ols (tannins or proanthocyanidins), while non-flavonoids include phenolic acids and their derivatives and stilbenes (Ivanova et al., 2011a, Ivanova et al., 2011b, Raičević et al., 2020). Polyphenols significantly influence the sensory characteristics of both grapes and wine, as they are responsible for some organoleptic properties, such as aroma, color, taste, bitterness and astringency. Thus, anthocyanins are responsible for the color of red wines, while proanthocyanidins, which are also called condensed tannins, are responsible for the bitterness and astringency of wines. The content of polyphenols in white varieties is lower compared to red varieties because anthocyanins are not synthesized in white grapes.

Anthocyanins are the red components responsible for the color of red grapes and wine. These compounds are located in the vacuoles of grape skin cells. Anthocyanins are based on five anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin. The most abundant anthocyanins are 3-monoglucosides, and among them malvidin-3-glucoside is the main component. Anthocyanins are also present in the form of 3-acetylglucosides, 3-*p*-coumaroylglucosides and 3-caffeoylglucosides (Figure 3).

Flavan-3-ols are a large family of polyphenols that are responsible for the astringency, bitterness and structure of wine. These compounds can be monomers, oligomers and polymers. The main flavan-3-ol monomers in grapes and wine are (+)-catechin and (-)-epicatechin, while (+)-gallocatechin, (-)-epigallocatechin and (-)-epicatechin-3-*O*-gallate are present in lower concentrations (Figure 3). Flavan-3-ol oligomers and polymers are known as condensed tannins or proanthocyanidins. Their properties depend on their structure. Thus, low molecular weight flavan-3-ols are responsible for the bitterness of wines, while polymeric flavan-3-ols largely influence astringency.

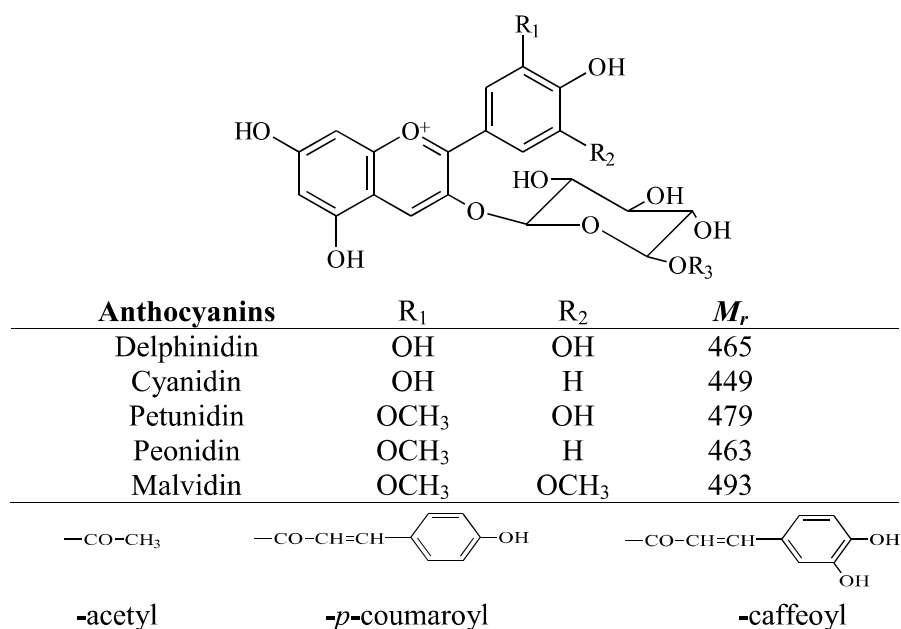
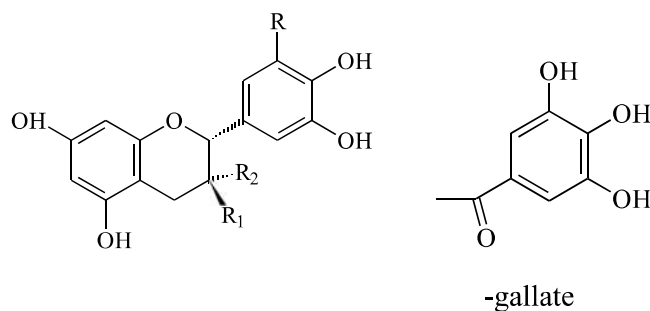


Figure 2. Structures of anthocyanins



Flavan-3-ols	R	R ₁	R ₂	M _r
(+)-Catechin	H	OH	H	290
(-)-Epicatechin	H	H	OH	290
(+)-Gallocatechin	OH	OH	H	306
(-)-Epigallocatechin	OH	H	OH	306
(-)-Epicatechin-3-gallate	H	H	OGallate	442

Figure 4. Structures of flavan-3-ol monomers

Flavonols are flavonoids that absorb the UV radiation and protect the internal tissues of grapes from the negative effects of sunlight (Ivanova et al., 2011b). Flavonols are mainly present in the form of 3-glucosides of myricetin, quercetin, kaempferol and isorhamnetin laricitrin and syringetin.

Phenolic acids are non-flavonoids, divided into two groups: hydroxybenzoic and hydroxycinnamic acids. The hydroxybenzoic group includes gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid and vanillic acid. Gallic acid is the dominant hydroxybenzoic acid in both, grapes and wine. The main hydroxycinnamic acids in wine are caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid. In free form, these components are present in small concentrations. In grapes and wine, they are mainly present as esters of tartaric acid and among the derivatives of hydroxycinnamic acids, the most common are caffeoyltartaric (caftaric) acid, which is present with more than 50% of the total content of hydroxycinnamic acids, *p*-coumaroyltartaric (*p*-coutaric) acid and feruloyltartaric (fertaric) acid (Ivanova et al. 2011b).

Stilbenes are non-flavonoids that can be synthesized in the grape vine as a defense system against fungal infection, mostly *Botrytis cinerea*, and from UV radiation. During the vinification, stilbenes are transferred from the grapes into the wine in very low concentrations (Mattuvi et al., 1995; Raičević et al., 2020). Stilbenes in the form of glucosides are often called piceides. The most significant and commonly studied stilbene is resveratrol, which exists in two isomeric forms (*cis*- and *trans*-form).

Wine and grapes are rich in *mineral* components. The content of minerals is an important factor affecting the quality, stabilization and nutritional value of wine. The analysis of certain elements in the wine is very important from a nutritional point of view, because it contains essential elements that the human body needs, such as Ca, Co, Fe, K, Mg, Cu, Se, Zn, Ni, Cr, and Mn. On the other hand, wine contains potential toxic elements such as Pb, Cd and As and therefore, it is very important to monitor their concentration in wines (Ivanova-Petropulos et al. 2013). Most common elements in wine are: Ca, K, Na and Mg, in concentrations of 2-2000 mg/L, minor elements are: Al, Fe, Cu, Mn, Rb, Sr and Zn and they are present in concentrations from 0.1 to 10 mg/L, while: Ba, Cd, Co, Cr, Li, Ni, Pb and V are trace elements present in concentrations of 0.001 to 0.5 mg/L.

Iron (Fe) and copper (Cu) are undesirable metals if they are present in concentrations above the maximal allowed because they catalyze oxidation reactions, change the taste or lead to the appearance of cloudiness in the wine.

Vitamins are chemical substances that participate in the regulation of cellular functions. They are present in small amounts in grape cells and in wine. The most important vitamins in wine are ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), *p*-aminobenzoic acid, biotin, niacin. During the fermentation and maturation of the wine, the vitamin content decreases. For example, ascorbic acid (vitamin C) oxidizes very easily; thiamine is reduced as it is degraded by reaction with SO₂ or exposure to heat, as well as by absorption of bentonite used for wine treatment. Riboflavin is oxidized after the wine is exposed to light. The only vitamin whose content increases during fermentation is *p*-aminobenzoic acid.

Various groups of *aromatic compounds*, such as alcohols, esters, aldehydes, lactones, terpenes and volatile phenols, have been identified in wine. These compounds affect wine aroma even at low concentrations. Alcohols and esters are the main components that are present in highest concentrations in wine. Esters have fruity odors and significantly affect the wine aroma (Ortega-Heras et al., 2002; Zhang et al., 2021).

Advanced analytical techniques for wine analysis

Reverse phase liquid chromatography (HPLC) coupled with UV-Vis detection is the standard method for the analysis of organic acids, biogenic amines, vitamins and various classes of polyphenolic compounds (anthocyanins, flavan-3-ols, phenolic acids, flavonols), using C18 column, a binary solvent system with an polar acidified solvent, such as aqueous acetic acid, formic acid, phosphoric acid or perchloric acid solution (solvent A) and organic modifier such as methanol or acetonitrile, possibly acidified (solvent B). Organic acids show an absorbance maximum in the UV/Vis region at 210 nm. Before HPLC analysis, sample pretreatment should be performed, including: (a) simple pretreatment, such as dilution and filtration, or more complex treatment, such as solid-phase extraction (SPE) (Ivanova et al., 2011a; Ricci et al., 2019). A typical chromatogram of organic acids obtained for a wine sample (Vranec), recorded at 210 nm is presented in Fig. 1.

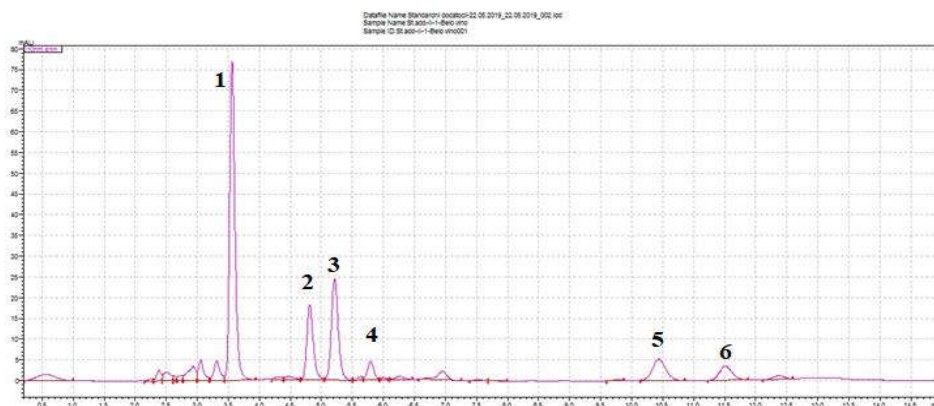


Figure 1. UV-Vis chromatogram of organic acids in red wine

Order of analytes: (1) tartaric acid; (2) malic acid; (3) shikimic acid; (4) lactic acid; (5) citric acid; and (6) succinic acid. Separation conditions: Shimadzu Shim-pack GIST C18 column, room temperature, isocratic elution with aqueous solution of H₃PO₄, concentration 5·10⁻³ mol/L, pH 2.1, flow rate 1 mL/min, injection volume 10 µL, monitoring at 210 nm wavelength, and dilution of the samples (1:5 for white wine; 1:10 for red wine).

Phenolic compounds show characteristic absorption in the UV–Vis region enabling the distinction of the various classes: anthocyanins have an absorbance maximum around 520 nm, as well as in the UV range around 280 nm, flavonols at around 360 nm and hydroxycinnamic acids can be detected at their absorption maximum at 320 nm. Flavan-3-ols exhibit maximum around 280 nm and these substances possess fluorescence properties that the other wine polyphenols do not have that enable their more specific detection and determination. A typical chromatogram for anthocyanins separation obtained for a wine sample (Vranec), recorded at 520 nm is presented in Fig. 2.

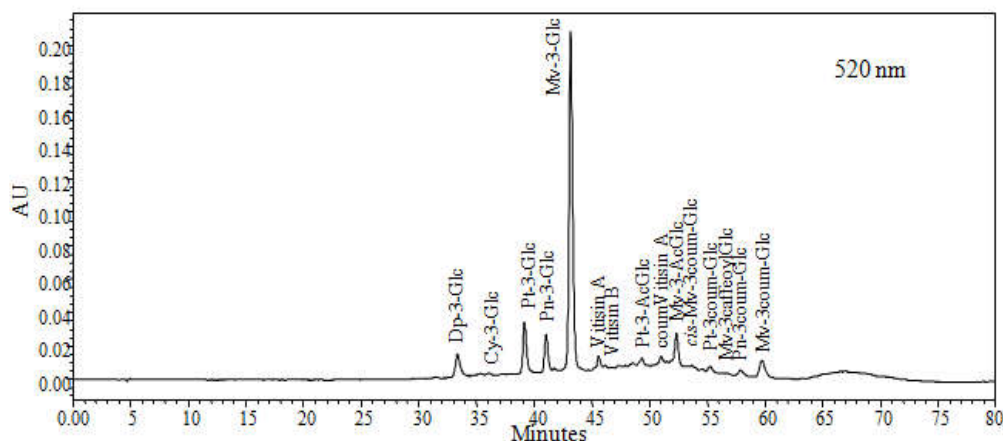


Fig. 2. Chromatogram for separation of anthocyanins in red wine monitored at 520 nm.

Chromatographic conditions: C18 column (250 mm x 2.1 mm i.d., 5 μ m packing, Waters, Milford, MA) protected by a guard column of the same material (20 x 2.1 mm i.d.; Waters, Milford, MA); mobile phase consisting of water/formic acid (95:5; solvent A), and acetonitrile/water/formic acid (80:15:5; solvent B) at a flow rate of 0.25 mL/min at 38 °C. Gradient program of solvent B: isocratic for 2 min with 0 %; 2-5 min, 0-2 %; 5-12 min, isocratic with 2 %; 12-15 min, 2-3 %; 15-25 min, 3-8 %; 25-40 min, 8-20 %; 40-45 min, 20-25 %; 45-55 min, isocratic with 25 %, 55-70 min, 25-65 % and 70-75 min, 65-0 %.

Source: Data from Violeta Ivanova, 2009a. PhD Diss., Ss. Cyril and Methodius Univ., Skopje.

Liquid chromatography coupled to mass spectrometry (LC/MS) is applicable to a wide range of compounds, focused on separation, detection and structural characterization of novel compounds in wine. This technique is very effective for glycoside compounds, allowing characterization of the aglycone and sugar moiety (Ivanova et al. 2011a; Causon et al., 2019). LC/MS is sensitive, selective, fast, and effective providing capability to analyze phenolic compounds either in the positive or in the negative ion mode, generating cations ($[M+H]^+$, $M+Na^+$) or anions ($[M-H]^-$, $[M-Cl]^-$). Figure 3 presents extracted ion chromatograms relative to analysis of wine (Vranec) flavan-3-ols in negative-ion mode.

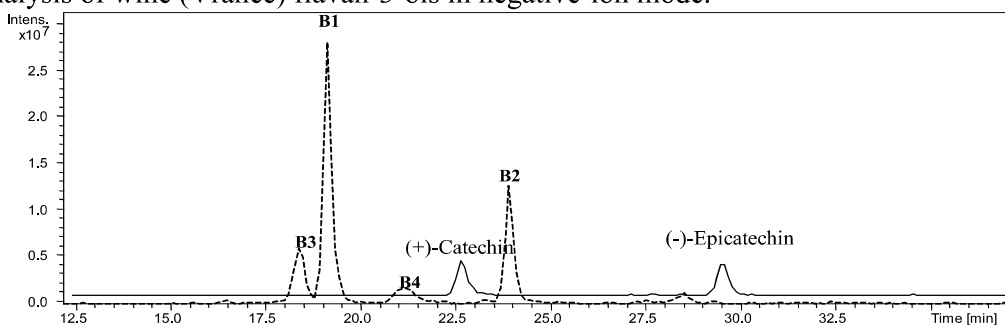


Fig. 4. Negative ion-mode extracted ESI-MS chromatogram of flavan-3-ols obtained from wine analysis in the m/z range 200-1200

Extracted m/z values correspond to the ions of (+)-catechin (m/z 289), (-)-epicatechin (m/z 289) and procyanidins: B1 (m/z 577), B2 (m/z 577), B3 (m/z 577) and B4 (m/z 577)

Source: Data from Violeta Ivanova, 2009a. PhD Diss., Ss. Cyril and Methodius Univ., Skopje.

GC-MS is the most sensitive and suitable technique for analysis of volatile components present in low concentrations, and therefore, it is used for qualitative and quantitative characterizations of aroma compounds in wine. This technique is highly efficient separation technique for volatiles' analysis and for characterization of the wine bouquet, applying polar column for separation. In addition, before GC-MS analysis, aroma compounds are usually extracted from the wine applying various extraction methods. Liquid-liquid extraction method using different organic solvents is a very suitable technique for extraction of a wide range of volatile components (Ortega-Heras et al. 2002), solid-phase extraction (Hernanza et al. 2008), solid-phase microextraction (Ivanova-Petropulos et al., 2014) or stir bar sorptive extraction (Coelho et al. 2009; Andujar-Ortiz et al., 2009).

Atomic absorption spectroscopy (AAS) is a suitable technique for direct determination of trace elements in wine (Stafilov and Karadjova 2009). In addition, electrothermal atomic absorption spectroscopy (ETAAS) technique offers high sensitivity and selectivity for determination of low levels of metals, and therefore, it is suitable and widely used for determination of toxic and heavy metals Pb and Cd, present at low concentration (Ivanova-Petropulos et al., 2015). ETAAS is not suitable for fast multielement analysis, this technique allows direct determination of Pb and Cd in wine samples, which is advantageous for routine analyses. Inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) are the most versatile techniques for wine multielement analysis. Both techniques provide high detection power, high selectivity, and high sensitivity. Since wine is a complex matrix, containing high ethanol content and other organic compounds, sample pretreatments are necessary for analysis of elements. The most commonly used pretreatments are dilution, heating the samples (digestion) with mineral acids (HNO_3 , HClO_4 , H_2SO_4) and microwave heating for sample digestion in high pressure digestion vessels (Gonzalvez et al., 2008; Seeger et al., 2015).

CONCLUSIONS

Red wine is an alcoholic beverage associated with various compounds which determine its quality, such as organic acids, polyphenols, aroma compounds, proteins, carbohydrates, minerals, vitamins etc. Varietal composition of wines depends on geographical origin of the grapes, cultivation techniques, as well as vinification process applied for production. Therefore, determination of wine composition is important in relation to quality control and consumer information. Various analytical technique are used for chemical characterization of wine, such as spectroscopic and chromatographic methods.

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