THE DETERMINATION OF TITRATABLE ACIDITY AND TOTAL TANNINS IN RED WINE

Miloš B. Rajković and Ivana D. Sredović [1]

Abstract: Titration acidity and content of total tannins in mass-market red wines are analysed in this paper.

The content of total acids in wine, expressed through wine acid, was analysed by potentiometric titration on 7.00 pH value. According to titratable acidity in analysed wines, all wines (only) with analysed parameters according to Regulations about wine quality.

The analysis of differential potentiometric curves shows that these curves can give the answer to the question if non organic substances, amino groups and phenols are present in wine in large quantity, as they are always present in wine. However it doesn't give the clear answer which substances are present in analysed samples. The answer to this question can be received only by the method of ionic chromatography.

The content of total tannins shows short time of storage in wine cellars so wine wasn't stored long enough, i.e. they are commercial wines made for mass market production and consumption.

Although they have less content of total tannin materials of given values, wine taste gives the correlation with other polyphenol materials that only give odour, aroma and "bouquet" to wine.

The relative error of mean value is very low which indicates the reproduction of results and reliability of the method of determination of total tannins in red wine.

Kew words: red wine, potentiometry, titrable acidity, tannins, phenol, polyphenols, deacidification.

Introduction

[1] Miloš B. Rajković, rajmi@agrif.bg.ac.rs, Ivana D. Sredovć, Institute of Food Technology and Biochemistry, Faculty of Agriculture, P.O.Box 14, 11081 Belgrade-Zemun, Nemanjina 6, Serbia

The European Union (EU) directive 1493 (OIV, 1999) defines wine as a product obtained exclusively from the total or partial alcoholic fermentation of crushed or intact fresh grapes or grape musts. During the process of vinifacation, grapes undergo two types of transformation:

1. The alcoholic fermentation whereby fermentable sugars (sugars and fructose) are converted into ethanol by the anaerobic action of yeast:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

This reaction also produces 103,34 kJ/mol (25,4 kcal/mol) of energy that heats the mass in fermentation. The must contains between 150 and 250 g/dm^3 of sugar, which in the case of dry wines drops to less than 2 g/dm^3 when fermentation is completed after 4-10 days. If the fermentation is deliberately stopped, for example, by the addition of ethanol or sulfur dioxide, the resulting sweet wine will contain residual sugars of up to 60 g/dm^3 and an alcohol content slightly higher than that of a dry wine (15-17 vol. %).

2. The malolactic fermentation (MLF), where as a result of bacterial action malic acid is converted into lactic acid. This process is not, in reality, a "fermentation" process, rather a biochemical process involving the removal of one of the carboxylic acids groups of malic acid by the action of bacteria of the *Pedicoccus*, *Leucomastoc* and *Lactobacillus* genera. Since the MLF reduces the acidity of a wine, it is a process that is encouraged in red wines and frequently prevented (by the addition of sulfur dioxide) in white and rosé wines in order to preserve the perception of freshness on the palate (Kelly and Blaise, 2005).

Wine is divided to: common wine (*In stricto sensu*), special wine, aromatized wine, distillation wine.

Common wine is wine received from fresh grapes by the allowed processes of treating grapes, crushed grapes or must. Wine is divided *according to quality* and put on market as: table wine, table wine with geographic origin, quality wine with geographic origin and high quality wine with geographic origin.

Table wine is wine made of grapes from one or more sorts of vine without specific geographic origin. Table wine with geographic origin is wine made of grapes from one or more sorts of vine which origin from one or at most two neighboring regions but the amount of the grapes of neighboring region is up to 15%. Quality wine with geographic origin is wine made of grapes from one or more sorts of vine with specific quality characteristics for sort or sorts that originate from one or at most two neighboring sub region but the amount of the grapes of neighboring sub region is up to 15%. High quality wine with geographic origin is wine made of grapes from one or more sorts of vine with specific quality characteristics for sort or sorts that originate from one or at most two neighboring

vineyards areas, but the amount of the grapes of neighboring vineyards is up to 15 %. Table wine and table wine with geographic origin which are sold on mass markets could be filled out of region with obligatory manufacturer's declaration and authorization from a specific Ministry (Encyclopedia Britannica. Encyclopedia Britannica Online; Harper, 2001).

According to its color the wine is divided into: white wine made from grapes of white sorts of vine; rosé wine made mostly of grapes of red or black sorts of vine and red vine which is made by allowed process of vinification from grapes of black sorts of vine (Official Gazette of SRY, 1999 and 2002; Official Gazette of SRY, 2003).

Acids and Acidity

Wine is one of the most acidic alcoholic beverages with pH values ranging from 2.8 to 3.8 ; white and rosé wines are, in general, more acidic than red wines due to the natural desacidification as result of the malolactic fermentation (MLF). It is also possible to adjust the pH of a wine, a procedure that may be required for technological reasons or to protect against bacterial growth. Tartaric acid (the most abundant acid in grapes and wines) is normally used for acidification, whereas desacidification – a more unusual and delicate procedure – involves the addition of either potassium nitrate, or calcium carbonate or potassium hydrogen carbonate.

The acidity of a wine encompasses a number of parameters
1. pH value,
2. Individual wine acids,
3. Total acidity, and
4. Volatile acidity.

The total of a wine is defined as the fraction of non ionized acids in the wine; officially, it is determined by the volume of sodium hydroxide required to bring the pH of a specified volume of wine to 7.00.

Individual wine acids. Acids are very important components of wine that in larger degree determine its quality. Basic acids that exist in wine are tartaric acids and its salts, and malic acid and its salts. Among these acids, in the wine can be found also acetic, succinic, citric, lactic acid and their salts. Dissociation constant (acidity) of acids which are present in wine is shown in Table 1 (Usseglio-Tomasset and Bosia, 1978; Segal, 1976; Dawson et al., 1969; Stranks et al., 1965; Weast, 1977).

Tab. 1. -   The aqueous dissociation constants for some wine constituent

Acid	pK_{a1}	pK_{a2}	pK_{a3}
Tartaric	3.07 [1]	4.39 [1]	
Malic	3.48 [1]	5.10 [1]	
Lactic	3.89 [1]		
Succinic	4.21 [1]	5.64 [1]	
Sulfurous	1.80 [2]	7.20 [2]	
Citric	3.06 [3]	4.74 [3]	5.40 [3]
Oxalic	1.19 [3]	4.21 [3]	
Glutaric	2.47 [4]	4.68 [4]	
Galacturonic	3.50 [1]		
Gluconic	3.81 [1]		
Glucoronic	3.26 [1]		
Pyruvic	2.71 [1]		
Ascorbic	4.10 [3]	11.79 [3]	
Arginine	2.17 [3]	9.04 [3]	
Proline	1.99 [3]	10.60 [3]	
Aspartic	2.09 [3]	3.86 [3]	9.82 [3]
Glutamic	2.19 [3]	4.25 [3]	9.67 [3]
Ammonia	9.20 [2]		
Carbonic	6.40 [2]	10.30 [2]	
Acetic	4.78 [1]		
Sorbic	4.76 [3]		
Phosphoric	2.1 0[2]	7.20 [2]	12.40 [2]
Sulfuric	-9.00 [5]	2.00 [2]	
Nitric	-1.40 [2]		
Gallic	4.41[3]		
Hydrosulfuric	7.0 [2]	12.9 [2]	

Volatile Acidity

The volatile acidity is a test of the state of health of a wine. This is because it is represented principially by acetic acid that may be formed by the oxidation of ethanol by *Mycoderma acetil*, the degradation of tartaric acid by *Lactobacillus plantarum* (reaction I) or by the degradation of citric acid by *Leuconostoc oenos* (reaction II).

Acetic acid then combines with ethanol to form ethyl ethanoate (ethyl acetate), which contributes to the pungency perceived wines with high volatile acidity.

Although limits are imposed for the amount of this acid in a wine (20 meq/dm^3) (OIV, 1999), it is a significant increase in the concentration of acetic acid (rather than its absolute value) during the course of vinification or aging that signals a problem with the wine, In order to correctly determine the concentration in volatile acidity, it is necessary to eliminate, CO_2 and subtract the concentration of various antiseptics or preservatives that may have been added, i.e. sulfur

dioxide, sorbic acid, salicylic acid, and sodium salicylate. Volatile acidity is expressed in terms of meq/dm^3, or in terms of g/dm^3 sulfuric acids, or more frequently acetic acid. The method involves steam distillation since 100 % recovery of acetic would not be possible with direct distillation.

$$\begin{array}{cccc}
COOH & CH_3 & CH_3 & \\
| & | & | & \\
CHOH \rightarrow & CO & \rightarrow & CO \\
| & | & | & \\
CHOH & CH_2 & COOH \rightarrow [Ox] \rightarrow CH_3COOH + CO_2 \\
| & | & \\
COOH & COOH &
\end{array}$$

Tartaric acid

$$[I]$$

$$CH_2COOH-CHCOOH-CH_2COOH \rightarrow HOOC-CO-CH_2-COOH \rightarrow CH_3-CO-COOH + CO_2$$
$$\text{1 mol} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow \qquad [II]$$
$$CH_3COOH \ (1.2–1.5 \ mol)$$

Titratable Acidity

Titratable acids represent the sum of all acids in wine, except carbonic acids. *Titratable acidity* (*acidity*) is determined by wine titration (after removal CO_2) till the end point of titration by strong basis and is expressed by proton number received as equivalent concentration of selected acid. In the United States the end point chosen is pH = 8.20 and the acid for reference is tartaric acid. In some other countries, France for example, the end point is pH = 7.00 and the reference acid is sulphuric. Titration acidity is commonly in the range 6.0-12.0 g/dm^3 expressed in tartaric acid (Peynaud and Maurie, 1956; Boulton, 1980a; Boulton,1980b).

The term titration acids is often wrongly equalized with the term total acids. Titration acidity (acidity) consists of acids that can be titrated by the strong basis solution and these are their free carboxylic functions (–COOH). However, part of wine acids has carboxylic functions totally or partially neutralised by cations (potassium, sodium and/or calcium). The term total acids becomes correct if wine is first put through colon with ion-exchange resin and all carboxylic functions are released, and then it is started to their neutralization with strong basis (Vine and Harkness, 1997).

The amount of total acids in must vary in very wide limits and mostly depend on sorts of grapes and climate conditions in the period of ripening. Most sorts of common – table wines have less total acids than sorts of quality and high quality wines, under the same conditions of ripening. Also, at the same sort of wine, the content of acids could vary depending on particular years (American Heart Association).

The amount of titration acid in must is in the largest number of cases in the range 5.0-8.0 g/dm^3. Wines, as the rule, contain less acids than the regulations

require and according to Regulations (Official Gazette of SRY, 1999 and 2002), titratable acidity is in the range 4.0-8.0 g/dm^3 expressed in tartaric acid, because the part of tartaric acid is deposited in the form of salts (*tartar* or *argol*) during alcohol fermentation.

In the process of fermentation a certain amount of *succinic acid* (ordinary about 1 g/dm^3) and a small amount of evaporated acids are created, but, as a rule, their amount doesn't compensate the loss of tartaric acid.

For wines that contain less than 4 g/dm^3 of titratable acids there arise suspicion about their origin, that is, that during the preparation some illegal acts had been done (Daničić, 1984).

Tannins (*acidum tannicum*) in red vine

The phenolic compounds are among the most important grape and wine constituents. They are responsible for all the differences in color and taste between red wines. Phenol compounds are the substances widely spread in plants, which, in their aromatic ring, contain one or more hydroxyl groups (Krstić, Sukdolak and Solujić, 1998). Several hundreds of structures of natural phenol compounds are known at the present. Flavonoids are the most common group among them, although monocyclic phenols, phenyl propanoid and phenol quinone are also present in great number. Other groups of polymer products such as: lignin, melanin, tannin, than polyphenolics and phenols bounded to proteins, alkaloids, steroids and terpenoids can also be found in plants. The functions of some of these groups of phenols compounds are well known, while for the rest we can say they are not researched enough.

Free phenols and phenylic acids in plants are mixed with other compounds (mixtures). They manly appear as methyl-ethers with lignin and easily free by acid hydrolysis and extraction with ether. Sometimes, during the extraction, they can be found in alcohol fractions bonded as glycoside. Simple phenol compounds such as oxy-benzene (pyrocatechin, resorcinol, hydroquinone and phloroglucin) and their methyl-ethers in plants are rare. Vicine trioxybensons (pyrogallol) in esters form are placed in highly polymerised structures of lignin. Sometimes, as gallic acid, they represent the important part of hydrolyzed solutions of tannin substances (gallotanin) (Meyer, 1960). Gallic acid appears directly from shikimic acid in biosynthetic processes (Pryce, 1972).

Salicyl and oxy-salicyl acids appear in similar way. Trimethyl-gallic acid that is a part of alkaloid *reserpin* is very interesting. Lower phenlyc acids C$_7$-oxy and methylated oxy-aromatic acids such as p-hydroxy-benzoic, vanillic, protocatechin and sinapine acids can often be find in plants. Other methylated phenolyc acids (anise, veratric) are rare in plants.

Phenol compounds are very significant for physiology of live organisms because they have pharmacological influence (Harbone, 1982). Phenol components exist as biologically active substances in many diet products. Like in

tea, citruses, apple, pear, plum, forest fruit, granular products, hop, coffee, cacao, grapes etc. Fruit and vegetables couldn't have specific taste without phenol compounds. Tannins are components of most of fruit, juices, beer and red wines.

In the analysis of all tasty aspects of all numerated fruit it is observed that their taste depends on the content of several phenol compounds. These fruit contain the class of flavonoids, flavonoide glycoside, aglucons and methoxylated flavonone. Simple phenol compounds are equally represented in these fruit as aglucons. These compounds exist in skin and eatable part of fruit in great concentration.

Phenol compounds have sharp taste, but it doesn't mean that they must express sharp features. Sharpness is attributed to glycoside structure of flavons. The content of disaccharins greatly influences on it, especially the bond between ramnose and glucose. Sharpness degree of the group of glycosides doesn't depend on the kind of aglucon. On the other hand, the position of carbonyl group of flavonoids (Horowitz, 1961) has special significance on tasty features of these compounds. It is known that biological activity of flavonoids depends on the substituent position on the phenol part of a flavonoid molecule. This activity can be enlarged by introduction of new substituent (Krstić, Sukdolak and Solujić, 1996).

The influence of phenol compounds in live organisms and human beings has not still been fully searched. It is known that they represent an important factor in the process of nourishment and metabolism. It is established that phenol compounds (Majid, Khanduja and Gandhi, 1995) help or take part in all biochemical processes in organism, and the analysis showed activity in above all coronary, vasodilating, anti-cancer, bacterial, anti-inflammatory and other activities.

Wine phenols are commonly referred to as "polyphenols", due as may be seen in Figure 1, to the presence of multiple phenolic groups in their structures, which confer on these compounds various properties linked to health benefits, specificially the antioxidant properties attributed to the consumption of moderate amounts of red wine.

The phenolic acids of wine (Figure 1A) consist mainly of derivatives of benzoic and cinnamic acids. The flavonoids (Figure 1B) are yellow-colored pigments that give the pale yellow color to white wine. They are also present in red wines but their color is masked by the anthocyanins (Figure 1C), the coloring substances of red wine (Figure 2). The tannins naturally occurring in wine are known as condensed tannins, polymeric structures resulting from the polymerization of elementary phenol compounds, polyhydroxyflavan-3-ols known as the catechins (Figure 1D).

(A) Phenolic acids
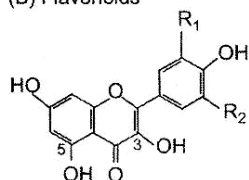(i) Derivatives of *o* - and *p*-hydroxybenzoic acid
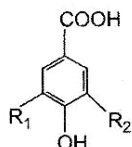
R = H	R′ = OH	Catechic acid
R = OH	R′ = OH	Gallic acid
R = OCH₃	R′ = OH	Vanillic acid

(ii) Derivatives of cinnamic acid
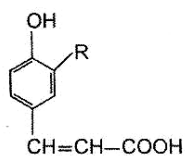
R = H	Coumaric acid
R = OH	Cafeic acid
R = OCH₃	Ferulic acid

(B) Flavonoids

R₁	R₂	Aglycone
H	H	Kaempherol
OH	H	Quercitin
OH	OH	Myrcitin

(C) Anthocyanins
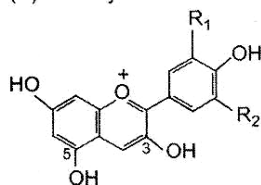
R₁	R₂	Aglycone
OH	H	Cyanidin
OCH₃	H	Paeonidin
OH	OH	Delphinidin
OH	OCH₃	Petunidin
OCH₃	OCH₃	Malvidine

(D) Tannins
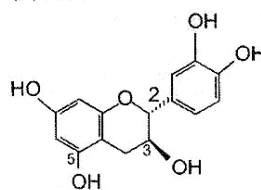
(+) Catechin 2*R*, 3*S*

(−) Catechin 2*S*, 3*R*
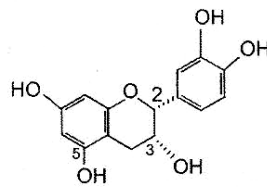
(+) Epicatechin 2*S*, 3*S*

(−) Epicatechin 2*R*, 3*R*

Fig. 1. - Structure of phenolic compounds. (A) Phenolic acids: (i) derivatives of *o*- i *p*-hydroxybezoic acid, (ii) derivatives of cinnamic acid; (B) Flavonoids; (C) Anthociyanins; and (D) Tannins
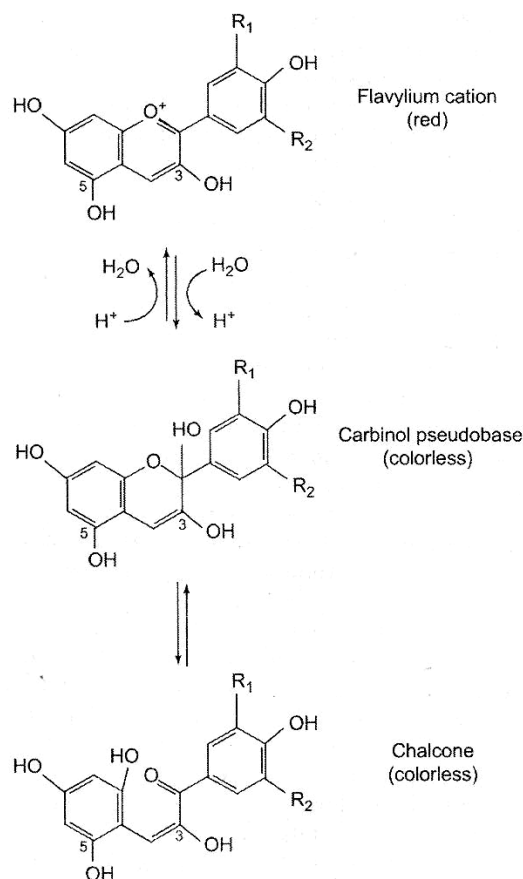
Fig. 2. - Different forms of anthocyanins

Determination of total tannins and permanganate index (I Mn)

Tannins (*Acidum tannicum*) are very wide spread substances in plants, they easily dissolve in water, taste sharp, sediment proteins. Glucose, *m*-digallic acid and small quantity of gallic acid are received by the hydrolysis of tannin.

General formula of tannins could be presented in the following way:

(D-rest of gallic or *m*-galloilgallic acid or H)
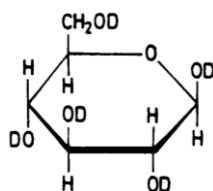
Tannin is white, amorphous dust, of sour and sharp taste. It dissolves in alcohol, water and glycerine, but not in c*hloroform* and ester oils. It distinguishes with very sharp taste and features with contractions or causing astringency.

The increased content of tannins in green fruit causes unwilling sharpness and soreness.

Tannin acids oxidize and change into products from dark to red color under the influence of oxidized ferments. They can be divided into two basic groups: hydrolyzable (soluble) tannins and condensate (non-soluble) tannins.

Hydrolyzable (soluble) tannins represent esters of aromatic oxicarbonic acids which, under the influence of specific tenaza enzymes, hydrolyze on their basic components. Besides enzyme, hydrolysis can emerge under the influence of acids or basis. They are derivates of gallic, protocatechin, elaginic and other acids. This group of tannins mostly represents esters of these acids with sugars, mostly glucose.

Condensed (non-soluble) tannins represent dimer or polymer derivates of compounds of flavane, which are present in plants. They are made from compounds of catechin and their derivates. Catechin is, according to their attributes, similar to antocians and their products: flavonols and flavans.

For the technology of alcohol drinks, tannins are significant in the "aging process", especially during aging of wine distillates in oak barrels for reaching strong spirits like cognac. The taste of cognac and other spirits is significantly improved by their oxidation. Many reactions between primary components of wine distillate and secondary components of oak's rainbow appear during "aging process" of wine distillates, and oxidation-reduction reactions are the most common and can be divided into three phases:

a) separation of air oxygen in ethanol,

b) creation of unstable compounds of peroxide, their disintegration and
   parting of active oxygen (–O–O–),

c) creation of many oxidized products.

During the "aging process" of alcohol drink appears oxidation-reduction type which intensity mainly depends on:

a) content of composition of wine's distillate,

b) content of wood (oak) of building barrel,

c) temperature,

d) presence of air oxygen,

e) catalyst and activator.

Mechanism of transformation of ethanol in the process of "aging" can be presented in the following way:

a) ethanol + $O_2 \rightarrow$ acetaldehyde

b) acetaldehyde + $O_2 \rightarrow$ acetic (ethanoic) acid

c) wooden barrel $\rightarrow$ acetic (ethanoic) acid

d) ethanol + acetic (ethanoic) acid $\rightarrow$ ethyl acetate + H_2O

During the aging process of wine distillates in oak barrels its component, lignin, has the important role. It separates under the influence of ethanol (hydrolyzed) which is popularly called ethanolysis of lignin. Two aromatic

aldehides, vanillin and siringin appear as final products and have great influence on creation of final "bouquet".

Oxidized tannins and extracts of strong distillate of drinks (type of brandy) give viscosity, richness, softness and harmony among "bouquet", aroma and taste. Glycides which give full taste to drinks appear from transformation of other oak component, chemicelulosis. Too large amount of tannin materials in distillate gives unpleasant, sour and sharp taste.

Potentiometry

Potentiometry represents the method of chemical analysis (or technique of volumetric (titrimetric) analysis) based on measurement of potentials of electrode put into the solution which contains the analyzed ionic kind. Usability of the method is in concentration up to 10^{-5} mol/dm^3.

Potential of electrode is conditioned by creating a double electric layer that emerges as the result of balancing electrochemical reaction between metal/solution. Such received potential on electrode, which can be measured, is called equilibrium potential and represents the function of activity of the components of corresponding oxidation-reduction system. As each reaction on electrode is the oxidoreduction reaction, the potential of electrode can be determined by oxidation-reduction system which all components are in the solution:

$$Oks + ne^- \rightleftarrows Red$$
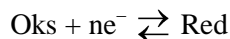
or one of components is electrode itself:

$$Me^{n+} + ne^- \rightleftarrows Me$$
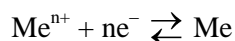
In the first case, inert metals (platinum, gold etc) are used as electrodes. They are used as transmitters of electrons from one component of system to the other, but they themselves don't participate in the reaction. Potential of such electrode depends on the relationship of activity of reduced and oxidized form of substance according to Nernst equation:

$$E = E^0_{Oks/Red} - \frac{RT}{nF} \cdot \ln \frac{a_{Red}}{a_{Oks}}$$

where: $E^0_{Oks/Red}$ - is the standard potential of electrochemical reaction, in V; RT/F - is the constant, which, during changing from natural logarithms to decade ones and on temperature of 298.15K, has value 0.059 V; n - is the number of electrons which participate in reaction; a_{Red} and a_{Oks} – are activities of analyzed substance in reduced and oxidized form.

In case when activities are less than 0.1, the ratio of activities can be changed with the ratio of concentrations:

$$E = E^0_{Oks/\mathrm{Re}\,d} - \frac{0,059}{n} \cdot \log \frac{c_{\mathrm{Re}\,d}}{c_{Oks}}$$

If H^+ ions participate in oxidoreduction reaction, the potential of electrode depends, also, from concentration of these ions:

$$E = E^0_{Oks/\mathrm{Re}\,d} - \frac{0,059}{n} \cdot \log \frac{c_{\mathrm{Re}\,d}}{c_{Oks}} \cdot c_{H^+}$$

In the other case, the electrode itself represents one of the components of system which determines the potential, and as pure metal concentration considers to be equal to one, for these systems Nernst equation is:

$$E = E^0_{Oks/\mathrm{Re}\,d} - \frac{0,059}{n} \cdot \log \frac{1}{c_{Me^{n+}}}$$

In this way, the potential of electrode represents the linear function of logarithm of ions concentration in solution. The electrodes, which potential depends only on concentration of cations in solution, are called first row electrodes. Examples of this are: silver, mercury, hydrogen (hydrogenous) electrode etc. Second row electrode shows the dependence from corresponding anion potential. The potential of such electrode is determined by equation:

$$E = E^0_{Oks/\mathrm{Re}\,d} - \frac{0,059}{n} \cdot \log c_{A^{n-}}$$

The examples of this are calomel and silver chloride electrode, which are mostly used. Beside these electrodes that influence (sensitive) on changing of concentration of ions correspondent (which corresponding) to those from electrode content, there are also third row electrodes which potential shows Nernst dependence from concentration of cations or anions that are not correspondent to those from electrode content. The examples of this are ion-selective electrodes with hard and liquid membrane, which in recent years have wide usage.

Direct measurement of absolute value of electrode potential is not possible. Because of that, measurement of electrode potential is most often done by the measurement of electromotor force (EMS) of couple that consists of the electrode which potential is measured (indicator electrode) and the auxiliary electrode which potential is known (reference electrode), both put into the analyzed solution.

Electromotor force of couple is determined by the difference of the potentials of both indicator and referent electrode: $E_s = E_{ind} - E_{ref}$. The potential of electrode is calculated according to the measured values of electromotor force, and from here it is calculated the concentration of one of the components of the analyzed solution or ratio of the concentration of the system components.

Value of electrode potential depends not only on the ions which circumstantially determine the potential, but also on the other ions present in solution. Such measured values of the potential are called serial (formal) potential.

Potentiometry represents wide field and according to the way of the analysis it can be divided into two groups: direct potentiometric and potentiometric titration (R a j k o v i ć, 2007).

The aim of this work was to determine the titration acidity in different sorts of red wine and to, according to the received data, identify their origin, and by determination of the content of total tannine materials to set the quality of red wine that can be bought as mass market product in our supermarkets.

Material and Methods

Acids are very important components of wine that in larger degree determine its quality. Basic acids that exist in wine are tartaric acids and its salts, and malic acid and its salts. Among these acids in wine there can be found also acetic, succinic, citric, lactic acid and their salts. There are some adequate chemical methods for determination of some acids existing in wine. However, determination of every acid by itself is a long and complicated process, so in practice titration wine acidity is determined by the method of neutralization.

### Determination of titratable acidity

In this paper titratable acidity in red wine was determined by potentiometric titration.

Examined red wines for determination of titratable acidity were: sample 1. Crnogorski vranac (manufacturer: Agrokombinat „13.juli" Plantaže, Podgorica, Montenegro, dry red wine, content of alcohol – 12.50 vol.%); sample 2. Vranac (manufacturer: Vino Župa, Aleksandrovac, table dry red wine, content of alcohol – 11.00 vol.%); sample 3**.** Crni Međaš (manufacturer: Vino Župa, Aleksandrovac, table dry red wine, content of alcohol – 11.50 vol.%); sample 4. Ždrepčeva krv (manufacturer: Vinarija Čoka, Subotica, table sweet wine, content of alcohol – 11.30 vol.%); sample 5. Vranac (manufacturer: Vinarija Ohrid, Macedonia, dry red wine, content of alcohol – 13.00 vol.%).

Reagents: Sodium carbonate as primary standard solution and hydrochloric acid and potassium hydroxide as standard solution. The approximate concentration of solution is 0.25 mol/dm^3.

Sodium carbonate solution was prepared in order to standardize of hydrochloric acid solution. For standardization of hydrochloric acid solution, 10.00 cm^3 Na$_2$CO$_3$ solution was taken by pipette, two drops of methyl orange

indicator and small quantities of distilled water were added. Such prepared solution was titrated by hydrochloric acid solution till changing the indicator color from yellow to first orange. Standardization of hydrochloric acid solution was developing according to the following reaction (R a j k o v i ć and N o v a k o v i ć, 2005):

$$2HCl_{(aq)} + Na_2CO_{3\,(aq)} \rightarrow 2NaCl_{(aq)} + CO_{2\,(g)} + H_2O_{(l)}$$
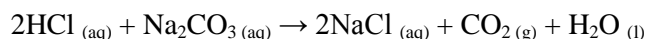
Concentration of hydrochloric acid solution is calculated in the following way:

$$c(\text{HCl}) = 2 \cdot \frac{c(\text{Na}_2\text{CO}_3) \cdot V(\text{Na}_2\text{CO}_3)}{V_s(\text{HCl})}$$

After HCl solution standardization, KOH solution standardization was done. In 250 cm^3 erlenmeyer 20.00 cm^3 of hydrochloric acid solution was taken by pipette and two drops of indicator phenolphthalein and small quantities of distilled water were added. Such prepared solution was titrated by KOH solution till changing the indicator color from colorless to first pink. Standardization of KOH solution by HCl solution was developing according to the following chemical equation:

$$KOH_{(aq)} + HCl_{(aq)} \rightarrow KCl_{(aq)} + H_2O_{(l)}$$
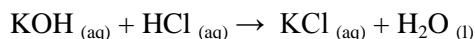
Concentration of base solution is calculated in the following way:

$$c(\text{KOH}) = \frac{c(\text{HCl}) \cdot V(\text{HCl})}{V_s(\text{KOH})}$$

Apparatus for potentiometric titration consisted of pH-meter (Eutech, Netherland) and magnetic stirrer. In a 100 cm^3 glass 20.00 cm^3 of sample was taken by pipette, magnetic nucleus was put in and a combined pH electrode for the analysis was placed in the solution (R a j k o v i ć and N o v a k o v i ć, 2005). Titration was done by the solution of potassium hydroxide with stirring by magnetic agitator where titration solution was added in 0.2 cm^3 aliquots. After every adding of the KOH solution, pH value of the solution was observed.

Determination of content of total tannins

Analyzed red wines for determination of total tannins: sample 1. Vranac, (manufacturer: Agrokombinat „13.juli" Plantaže, Podgorica, Montenegro, qualitative red wine of Montenegro, content of alcohol – 12 vol. %); sample 2. Merlot (proizvođač: Agrokombinat „13.juli" Plantaže, Podgorica, Montenegro, the top red wine of Montenegro, content of alcohol – 12.5 vol. %); sample 3. *Game* (manufacturer: Navip – Vinarstvo d.o.o., Vranje, qualitative dry red wine of Vranje, content of alcohol – 10-11 vol. %); sample 4. Cabernet, (manufacturer:

„Podrum Palić", Subotica, qualitative dry red wine, content of alcohol – 11.7 vol.%); sample 5. Ždrepčeva krv (manufacturer: Vinarija Čoka, Subotica, table sweet wine, content of alcohol – 11.30 vol. %).

The content of total tannins was determined by Ribereau-Gayon-Maurié method (D a n i č i ć , 1973; R a d o v a n o v i ć , 1986).

For determination according to this method the following solutions were prepared:

- Indigo-carmine solution – 3 g of indigo-carmine was put into a mortar and 100 cm^3 of diluted H_2SO_4 was added (1 : 4) (250 cm^3 H_2SO_4 and 750 cm^3 H_2O). Mass was well mixed and put into a 1000 cm^3 measurement dish and left for 24 hours. Then the measurement dish was filled up by distilled water till the measurement line and solution was intensively mixed. In the end the solution was filtrated and left in a dark bottle. Working solution: Just before the determination 50 cm^3 of basic solution is made, put into a 1000 cm^3 dish and 45 cm^3 of diluted H_2SO_4 (1 : 4) is added and filled up by distilled water till the measurement line.
- Tartaric acid – 0.5000 g of tartaric acid was dissolved in 50 cm^3 of distilled water, put into a 100 cm^3 measurement dish, 9.50 cm^3 of NaOH solution of 0.1 mol/dm^3 and 100 cm^3 concentration was added, then 3.50 cm^3 of NaOH solution of 0.1 mol/dm^3 concentration and 10 cm^3 of 96 % alcohol (absolute) were added and filled up with distilled water till the measurement mark.

Work process: In 100 cm^3 Erlenmeyer is put 50 cm^3 of indigo-carmine working solution which has already been acidify by sulphuric acid during preparation. Then 2.00 cm^3 of wine acid solution which contains basic wine ingredients is added. Titration is done by gradual adding of potassium permanganate solution of 0.002 mol/dm^3 concentration from burette till total loss of blue color, that is, till the appearance of gold yellow color.

The loss of potassium-permanganate for oxidation of indigo-carmine is notified. Now the other titration is done in the same way, but instead of wine acid solution, 2.00 cm^3 of analyzed wine is added. If the wine is of dark color the smaller amount should be taken (1.00 cm^3), and if it is of light color (rosé) 3-5.00 cm^3 can be taken. During this titration, the oxidation of indigo-carmine and polyphenole materials in added wine is done, so the loss of $KMnO_4$ is higher. The $KMnO_4$ solution is added till the complete loss of azure blue color and emerging of green-yellow shade.

Based on the differences in loss between these two titrations the so called permanganate index (I Mn) is calculated according to equation: (Dančić, 1973)

$$I Mn = (n - n_1) \times 10 \times F KMnO_4$$

where:

n – is loss of $KMnO_4$ solution of 0.01 mol/dm^3 concentration for oxidation of indigo-carmine and wine in cm^3;

n_1 – is loss of $KMnO_4$ solution for oxidation of indigo-carmine in cm^3;

10 – is the factor for calculation of $KMnO_4$ to normal solution and to1 dm^3 of wine. If for analysis used 1 cm^3 this factor is 10 if used 10 cm^3 this factor is 1.

The permanganate index presents of potassium permanganate solution of 1 mol/dm^3 concentration to be needed to oxidation all varies of polyphenols in 2 dm^3 of wine.
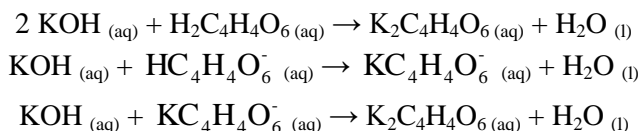
Nonetheless, it is possible to classify wines according to their permanganate index (milliequivalents of permanganate used in the titration) as follows.

Red press wine   100-150
Tannic red wine   75
Soft red wine   60
Rosé wine   27-35
White wine   1-15

Results and Discussion

Determination of titratable acidity of wine is based on neutralization of all acids and their acid salts, by potassium hydroxide solution. Potassium hydroxide is used for neutralization of all substances in wine that have acid character, so the amount of total acids in wine is expressed as the mass of tartaric acid, as for the wine quality this acid is one of the most important (Durliat and Comtat, 2005; Prenesti, *et al*., 2004).

Presuming that neutralization of tartaric acid and its acid salts has been done by the following reactions:

$$2 \, KOH_{(aq)} + H_2C_4H_4O_{6\,(aq)} \rightarrow K_2C_4H_4O_{6\,(aq)} + H_2O_{(l)}$$

$$KOH_{(aq)} + HC_4H_4O_6^-{}_{(aq)} \rightarrow KC_4H_4O_6^-{}_{(aq)} + H_2O_{(l)}$$

$$KOH_{(aq)} + KC_4H_4O_6^-{}_{(aq)} \rightarrow K_2C_4H_4O_{6\,(aq)} + H_2O_{(l)}$$

mass of tartaric acid is calculated in the following way:

$$m(H_2C_4H_4O_6) = \frac{1}{2} \cdot c(KOH) \cdot V(KOH) \cdot M(H_2C_4H_4O_6)$$

As the content of tartaric acid is expressed in g/dm^3 the above equation should be multiplied by factor 50:

$$m(H_2C_4H_4O_6) = \frac{1}{2} \cdot c(KOH) \cdot V(KOH) \cdot M(H_2C_4H_4O_6) \cdot 50$$

The results obtained by potentiometric titration were processed with software Origin 6.1 for maths data processing.

Obtained potentiometric titration curves are shown in Figure 3.
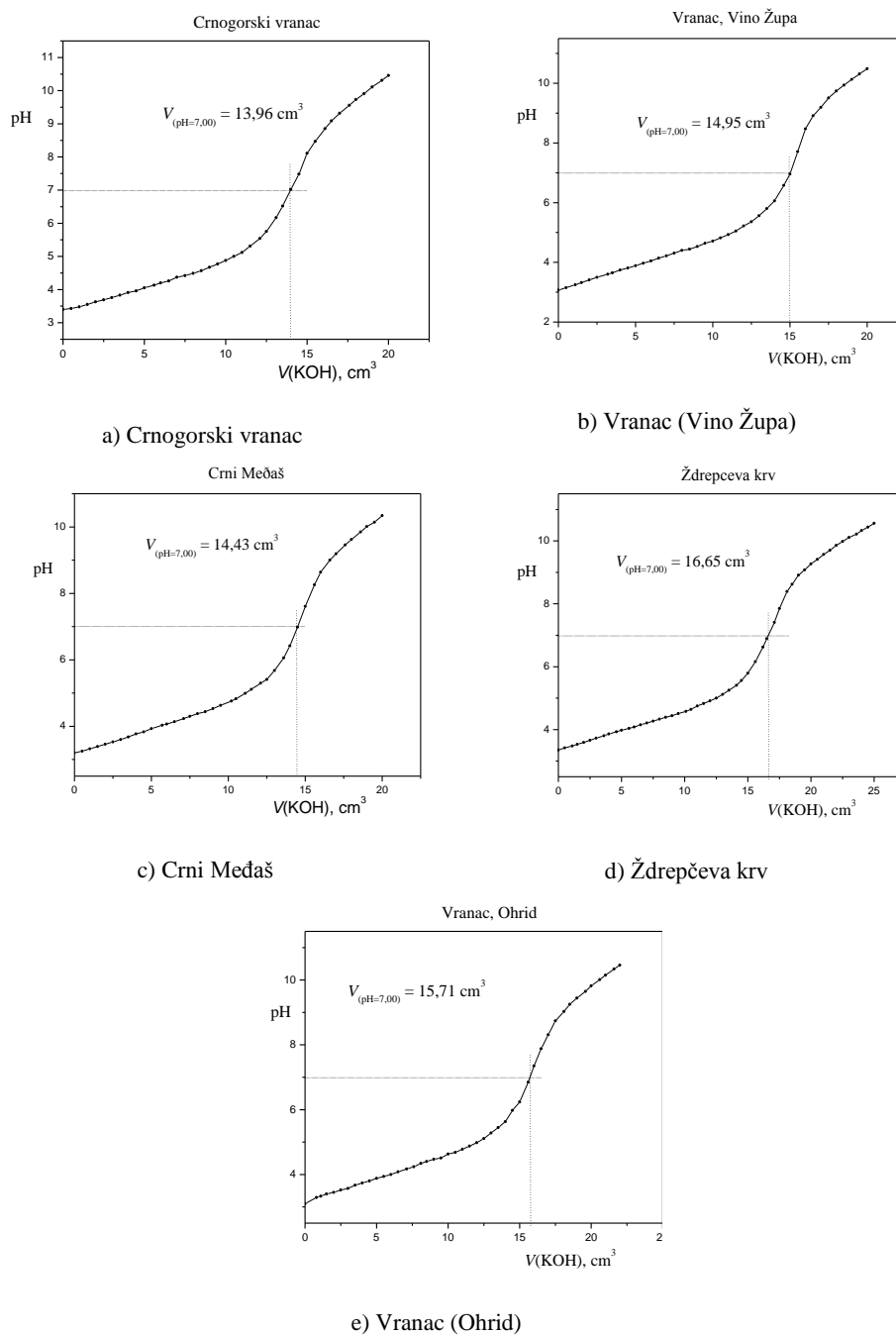In Figure 4 differential potentiometric curves are shown.

a) Crnogorski vranac

b) Vranac (Vino Župa)

c) Crni Međaš

d) Ždrepčeva krv

e) Vranac (Ohrid)

Fig. 3. - Potentiometric titration curves obtained of examined red wine

a) Crnogorski vranac

b) Vranac (Vino Župa)

c) Crni međaš

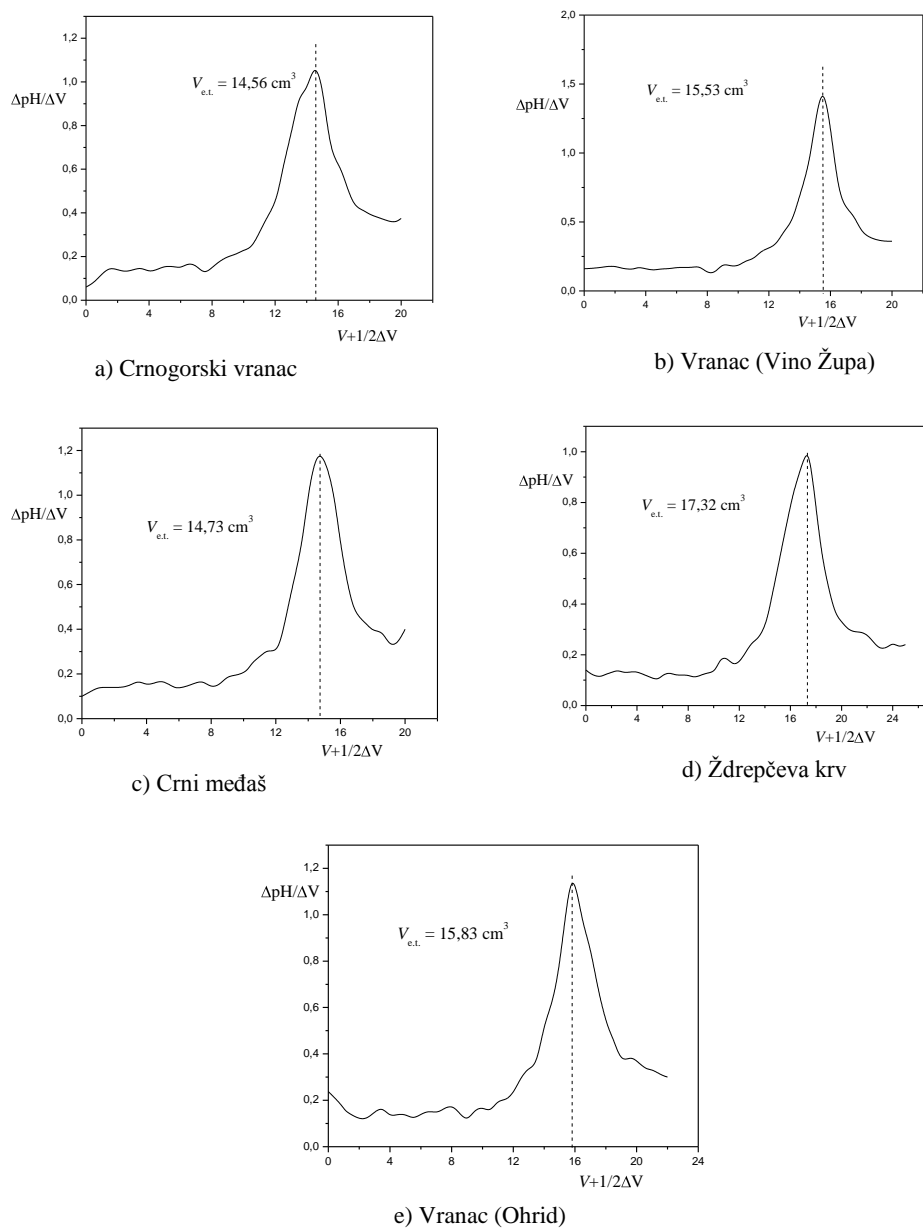d) Ždrepčeva krv

e) Vranac (Ohrid)

Fig. 4. - Differential potentiometric titration curve obtained of examined red wine

In Table 2 the results of measurements of pH values of vine samples that mark concentration of free H$^+$ ions in vine are shown. Values of real pH depend from the amount of total acids and intensity of their dissociation. Of carboxylic acids that often exist in wine, tartaric acid is most strongly dissociated, malic acid less than it, and other carboxylic acids are even weaker. So, pH values of wine samples mostly depend of the amount of tartaric acid in vine.

Tab. 2. - pH value of samples and titratable acidity of analyzed wines expressed by the mass of tartaric acid in1 dm^3 of wine

Sample	pH value	$m(H_2C_4H_4O_6)$, g/dm^3	
		at pH = 7.00	extract
Crnogorski vranac	3.40	5.81	6.06
Vranac (Vino Župa)	3.07	6.22	6.50
Crni Međaš	3.20	6.00	6.13
Ždrepčeva krv	3.35	6.93	7.23
Vranac (Ohrid)	3.10	6.54	6.59

Concentration of H$^+$ ions, that is pH value, is not directly proportional to the amount of total acids in wine. With increasing of titratable acidity, concentration of H$^+$ ions, that is pH value of wine, is not always increasing proportionally. According to the results shown in Table 2 it can be seen that Crnogorski vranac has the highest pH value, while Ždrepčeva krv has the highest content of total acids.

Detailed analysis of differential potenciometric curves doesn't indicate the existence of smaller jumps before and after the highest jump on the curve, as it was case with determination of white wines by potentiometric (Prlina and Mažibrada, 2006; Rajković, Novaković and Petrović, 2007). From common theoretical observation it is realised that every rise on differential curve corresponds to one end point of titration (Prenesti, et al., 2004).

In Figure 4 it can be seen that in the titration of analysed red wines, jumps which precede to the highest jump on the curve are not noticed, as it was observed with the titration of white wines. These jumps emerged in the pH values scope from 5.40 to 6.70. They probably correspond to the titration of nonorganic substances which are present in wine, among all the titration of SO$_2$ (Prlina and Mažibrada, 2006; Rajković, Novaković and Petrović, 2007).

Although these rises can't be seen in titration of examined red wine, it can't be said that non organic substances are not present in wine, but that their concentration is very low.

Rises on differential potentiometric curves that occur after the highest rise correspond to pH range from 10.20 to 11.56. These results show the content of substances in wine such as biogenic amines, amino acids (amino groups) and phenol compounds, and that have less constants of acidity than carboxylic acids present in wine. These compounds do not significantly influence to total wine

acidity, and on the other hand determination of the end point of titration for these compounds is always discussible (Prenesti *et al.*, 2004). Namely, oxido-reduction and/or processes of sedimentation (as the oxidation of phenol and so) have advantage in relation to acid-base reactions, so they make it impossible that this part of titration curve be registered precisely and reproductively (De Lange, 2007; Corder *et a*l., 2006). From Figure 4 it can be seen that the reproducible presence of these compounds on titration curve cannot be registered for all examined wines.

The highest rise on differential titration curve for all wine samples occurs in pH range from 7.25 to 8.70. As only carboxylic acids are significant for wine quality, it is obvious why in practice potenciometric determination of total acidity stops in a moment when pH values of titrated solution is 7.00.

Determination of total tannins using method of Ribereau-Gayon-Maurié

The Ribereau-Gayon-Maurié method is used for determination and expression of total   polyphenol materials in the form of so called permanganate index. It is considered that it is more practical and simple to express total colored and tannin materials in the amount of $KMnO_4$ spent for their oxidation, so this is called index of wine oxidability. For secure reasons, working solution of indigo-carmine indicator is prepared with such amount of H_2SO_4 to avoid its additional adding during the analysis.

Loss of $KMnO_4$ for "blank test" with wine acid and for different sorts of wine and permanganate index (index of wine oxidability) are shown in Table 3. Statistic analysis of received results of the analysis of total tannin materials in different sorts of red wine is shown in Table 4.

Statistical processing obtained results determination of total tannins in different sort of red wine is shown in Table 4.

Tab. 3. - The determination of permanganate index (I Mn)–oxidative index in different sort of red wine

Sort of wine	n-consumption of $KMnO_4$ for wine (in cm^3)	n_1-consumption of $KMnO_4$ for "blank test" (in cm^3)	I Mn
Vranac	7.70	4.90	28.00
Marlot	7.35	4.95	24.00
Game	7.50	4.95	25.50
Cabernet	8.48	4.95	35.25
Ždrepčeva krv	7.12	4.95	21.75

At wine type rosé the permanganate index is between 25 and 35, with red wines it reaches value up to 60, and with very sharp it can be over 70.

According to the received results it can be observed that, according to content of total tannin materials, all wines belong to rosé type (or red) of wine. The lowest value of tannin has Ždrepčeva krv only 21.75, which, according to manufacturer's declaration is red half sweet table wine. The most tannin materials has Cabernet, declared as dry wine.

Tab. 4. - Statistical processing determination of tannins in different sort of red wine

Sort of wine	Number of measurements	Deviation (v)	Standard deviation (σ)	Coefficient of deviation (σ %)	Mean deviation (σ_m)	Relative mean deviation (in %)
Vranac	5	0.0100	0,200	2.63	0.09	3.29
Merlot	5	0.0025	0.050	0.68	0,02	0.84
Game	5	0.0125	0.112	1.49	0.05	1.85
Cabernet	5	0.0031	0.056	0.66	0.03	0.82
Ždrepčeva krv	5	0.0081	0.090	1.26	0.04	1.56

Obtained results indicate the less content of total tannins that means that wine wasn't stored long enough, i.e. they are commercial wines made for mass market production and consumption.

The difference in content of total tannins, beside Ždrepčeva krv, is not drastic.

Conclusion

Titration acidity and content of tannins in mass-market red wines are analyzed in this paper.

According to the obtained results it can be seen that wine titration with indicator gives sufficient reliable values of titration acidity in wine (OIV, 2005). However, as potentiometric titration for pH value 7.00 is more reliable and objective method, values for content of total amount of acids in wine, expressed through tartaric acid, have been given according to these results. According to titratable acidity in analyzed wines, all wines (only) with analyzed parameters according to Regulations about wine quality.

The analysis of differential potentiometric curves shows that these curves can give us the answer to the question if non organic substances, amino groups and phenols are present in wine in a larger amount, as they are always present in wine. However, neither of analyzed methods gives strict answer to the question which substances are present in analyzed samples. The answer to this question can be given only by the method of ionic chromatography.

The content of total tannins shows that means that wine wasn't stored long enough, i.e. they are commercial wines made for mass market production and consumption.

Although they have less content of total tannin materials of literature values, wine taste gives the correlation with other polyphenol materials that give odour, aroma and "bouquet" to wine.

The relative mistake of mean value is very low which indicates the reproduction of results and reliability of the method of determination of total tannin materials in wine.

REFERENCES

1. American Heart Association: Alcohol, Wine and Cardiovascular Disease. http://www.americanheart.org/presenter.jhtml?identifier=4422.
2. Boulton, R. (1980a): The General Relationship Between Potassium, Sodium and pH in Grape Juices and Wines. Am.J.Enol.Vitic., 10, 105-109.
3. Boulton, R. (1980b): A Hypothesis for the Presence, Activity and Role of Potassium/ Hydrogen Adenosine Triphosphates in Grapevines. Am.J.Enol.Vitic., 31, 283-287.
4. Corder, R., Mullen, W., Khan, N. Q., Marks, S.C., Wood, E.G., Carrier, M. J. and Crozier, A. (2006): Oenology: Red Wine Procyanidins and Vascular Health. Nature,444(7119),566.
   http://www.nature.com/nature/journal/v444/n7119/abs/444566a.html.
5. Daničić, M. (1973): Praktikum iz tehnologije vina. Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd-Zemun (in Serbian).
6. Daničić, M. (1984): Tehnologija vina - praktikum. Poljoprivredni fakultet, Beograd-Zemun (in Serbian).
7. Dawson, R. M. C., Elliott, C., Elliott, W. H. and Jones, K. M. (1969): Data for Biochemical Research, Oxford, UK: Clarendon Press.
8. De Lange, D. W. (2007): From Red Wine To Polyphenols and Back: A Journey Through The History of The French Paradox. Thrombosis Research, 119(4), 403-406. http://www.sciencedirect.com/science/article/B6T1C-4KCXJ95 2/2/78efd7cd7ed0ce19403d64d649518c2a.
9. Durliat, H. and Comtat, M. (2005): Critical Evaluation of Potentiometric Redox Titrations In Enology. Analytica Chimica Acta, 545, 173-181.
10. Encyclopedia Britannica. Encyclopedia Britannica Online: Wine
11. http://www.britannica.com/eb/article-9106005/wine.
12. ETS Laboratories (2002): Sulfides in Wine.
13. http://www.etslabs.com/scripts/ets/pagetemplate/blank.asp?pageid=350.
14. Harbone, J. B. (1982): Biochemistry of Phenolic Compounds. Acad.Press, London, 1964; Darbarwar, M. and Sundaramurthy, V., Synthesis, 5, 337-338. Reviews
15. Harper, D. (2001): Wine. Online Etymology Dictionary.
16. http://www.etymonline.com/index.php?term=wine
17. Horowitz, R. M. (1961): The Orange its Biochemistry and Physiology, W.B., Sinclair, Berthley.
18. Ignjatović, B. i Đorđević, I (2002): Određivanje ukupnih taninskih materija (*acidum tannicum*) u različitim sortama crnog vina metodom Ribereau-Gayon-Maurié. Regionalni centar za talente Beograd 1 – Zemun (in Serbian).
19. "Introduction to Wine". 2basnob.com.http://www.2basnob.com/wine-introduction.html.
20. Kelly, M. T. and Blaise, A. (2005): Wine. in "Food and Nutritional Analysis".
21. Elseiver Ltd. pp. 292-302.
22. Krstić, Lj., Sukdolak, S. i Solujić, S. (1996): Nosioci biološke aktivnosti kod flavonoida. Hemijski pregled, 37(1-2), 32 (in Serbian).

23. Krstić, Lj., Sukdolak, S. i Solujić, S. (1998): Značaj i uloga fenolnih jedinjenja. Hemijski pregled, 39, 81-87 (in Serbian).
24. Majid, S., Khanduja, K. L., Gandhi, R. K., Kapur, S., Sharma, R. R. (1991): Influence of Ellagic Acid on Antioxidant Defense System and Lipid Peroxidation in Mice. Biochemical Pharmacology, 42(7), 1441-1445.
25. Meyer, L. H. (1960): Food Chemistry. Reinchold Publishing Corporation, New York, Chapman&Hall, Ltd., London.
26. OIV (1999): Compendin of International Methods of Analysis of Wines and Musts.
27. Paris: OIV Publications.
28. Prenesti, E., Toso, S., Daniele, P. G., Zelano, V. and Ginepro, M. (2004): Acid-base Chemistry of Red Wine: Analytical Multi-Technique Characterisation and Equilibrium - Based Chemical Modelling. Analytica Chimica Acta, 507, 263-273.
29. Prlina, I. i Mažibrada, I. (2006): Određivanje ukupnih kiselina u belom vinu. Regionalni centar za talente Beograd 1 – Zemun (in Serbian).
30. Pryce, R. J. (1972): Gallic Acid As a Natural Inhibitor of Flowering in Kalanchoe Blossfeldiana. Phytochemistry (Elsevier), 11(6), 1911-1918.
31. Radovanović, V. (1986): Tehnologija vina. Drugo prerađeno i dopunjeno izdanje, Građevinska knjiga, Beograd (in Serbian).
32. Rajković, M. B. i Novaković, I. D. (2005): Praktikum iz analitičke hemije - klasične metode. Poljoprivredni fakultet, Zemun (in Serbian).
33. Rajković, M. B. (2007): Uvod u analitičku hemiju klasične osnove. Drugo, prerađeno i dopunjeno izdanje. Pergament, Beograd (in Serbian).
34. Rajković, M. B., Novaković, I. D. and Petrović, A. (2007): Determination of Titratable Acidity in White Wine. Journal of Agricultural Sciences, 52(2), 169-184.
35. Segal, L. (1976): Biochemical Calculations, 2nd ed., New York, John Wiley&Son.
36. Službeni list SRJ: Pravilnik o kvalitetu i drugim zahtevima za vino, br. 54/99 i 38/2002 (in Serbian).
37. Službeni list SCG: Pravilnik o kvalitetu i drugim zahtevima za vino, br. 56/2003 i dr. pravilnici (in Serbian).
38. Stranks, D. R., Heffernan, M. L., Lee Dow, K. V., McTigue, P. T. and Withers, G. R. A. (1965): Chemistry – A Structural View. London: Melbourne University Press.
39. Usseglio-Tomasset, L. and Bosia, P. D. (1978): Determinazione delle constanti di dissociazione dei principali acidi del vino in soluzioni idroalcoliche di interesse enologico. Rivista di Viticoltura e di Enologia, 31(9), 380-403 (in Italian).
40. Vine, R. and Harkness, E. (1997): Winemaking. Chapman&Hall, New York.
41. Weast, R. C. (1977): CRC Handbook of Chemistry and Physics. 58th ed. Cleveland, OH: CRC Press.

ODREĐIVANJE TITRACIONE KISELOSTI I UKUPNIH TANINA U CRVENOM VINU

Miloš B. Rajković i Ivana D. Sredović[1]

R e z i m e

U radu su ispitivana titraciona kiselost i sadržaj ukupnih tanina u komercijalnim crvenim vinima.

Sadržaj ukupnih kiselina u vinu, izraženih preko vinske kiseline, ispitivan je potenciometrijskom titracijom pri pH vrednosti 7,00. Na osnovu titracione kiselosti u analiziranim vinima, sva vina (samo) po analiziranim parametrima odgovaraju Pravilniku o kvalitetu vina.

Analiza diferencijalnih potenciometrijskih krivih, ukazuje da ove krive mogu pružiti odgovor na pitanje da li su u vinu prisutne neorganske supstance, amino grupe i fenoli u većoj količini, pošto su one uvek prisutne u vinu, međutim, ne daje jasan odgovor koje supstance su prisutne u analiziranim uzorcima. Odgovor na ovo pitanje može pružiti jedino metoda jonske hromatografije.

Iako imaju niži sadržaj ukupnih taninskih materija od literaturnih vrednosti, ukus vina daje i korelacija sa drugim polifenolnim materijama, što tek vinu daje miris, aromu i buke.

Relativna greška srednje vrednosti ima jako nisku vrednost što ukazuje na reproduktivnost rezultata i pouzdanost metode određivanja ukupnih tanina u vinu.

[1] Miloš B. Rajković, rajmi@agrif.bg.ac.rs, Ivana D. Sredović, Institut za prehrambenu tehnologiju i biohemiju, Poljoprivredni fakultet, P.O. Box 14, 11081 Beograd-Zemun, Nemanjina 6, Srbija