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A STUDY OF THE PHYSICOCHEMICAL PROPERTIES OF SOME OXIDIZED ENOTANNINS

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New samples of Enoxil (Enoxil I, Enoxil II and Enoxil III) were obtained by oxidation of enotannins. The antioxidant properties of enotannins and Enoxils were determined by means of the chemiluminescence method. It was demonstrated that the antioxidant activity of novel chemical compounds is by 29-32% more pronounced than that of enotannins used as raw material.

By applying the potentiometric method of "Boehm titrations" the amount of acidic functional groups (carboxyl and phenolic) in enotannins and the mentioned Enoxil preparations was determined. The obtained results reveal that new substances contain about 3-4 times more acidic functional groups than Enotannins.

Keywords: *chemiluminescence, antioxidant, polyphenols, enotannins.*

STUDIUL PROPRIETĂȚILOR FIZICO-CHIMICE ALE ENOTANINELOR OXIDATE

Prin oxidarea enotaninelor au fost obținuți compuși noi chimici de Enoxil (Enoxil I, Enoxil II și Enoxil III). Proprietățile antioxidante ale enotaninelor și ale enoxilurilor au fost determinate prin metoda chemiluminescenței. S-a demonstrat că activitatea antioxidantă a noilor compuși chimici este cu 29-32% mai pronunțată decât cea a enotaninelor utilizate ca materie primă.

Prin aplicarea metodei potențiometrice a „titrărilor Boehm" a fost determinată cantitatea de grupări funcționale acide (carboxil și fenol) în enotanine și în preparatele Enoxil menționate.

Rezultatele obținute arată că substanțele noi conțin aproape de 3-4 ori mai multe grupări funcționale acide decât enotaninele.

Cuvinte-cheie: *chemiluminescență, antioxidant, polifenoli, enotanine.*

Introduction

Tannins are vegetal compounds with a complex chemical structure. They are actually the result of structural polymerization elementary possessing phenolic fractions. Tannins reactivity depends on their structure [1].

Tannins can be found in green and blanck tea, grapes [2-4], vegetables [5-7], fruits [8-11]. Natural tannins form two large groups: hydrolisable and condensed or catehinic tannins. Tannins from the first group decompose under the action of mineral acids to give a monosacharide, usually D-glucose, and gallic acid or its derivative. Condensed tannins do not contain a sugar component and can be cleaved in simplier fragments only by alkaline melting. The hydrolisable tannins are transformed under dry heating into pyrogalol and the condensed tannins into pyrocatehine [12].

Among the various vegetal sources of antioxidants, grape seeds are an important source of enotannins-polyphenolic tannins of condensed structure, exhibiting enhanced antioxidant properties due to high content of flavonoids [13,14]. The antioxidant activity of polyphenols, the most studied plant constituents, is controlled by the presence of hydroxyl groups at 3' and 4' positions in the B ring and, to a lesser extent, by the presence of hydroxyl group at 4' position in the C ring (Figure 1) [15,16]. The degree of polymerization strongly influences the inhibitory characteristics of the polyphenols. Polyphenolic fractions with different degrees of polymerization that were extracted from grapes have different antioxidant / antiradical effects [15]. Flavonoids contain phenolic hydroxyl groups attached to the ring structure, which confers them reducing properties.

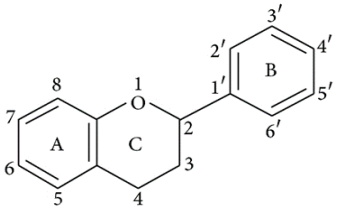

Fig.1. General chemical structure of flavones.

One of the priority direction of the moldavian wine industry is the valorition of by products. Due to the availability of industrial quantities of grape seeds, that are one of the wine production wastes, a series of research programs was initiated, aimed at developing efficient procedures for extracting tannins from grape seeds. As a result, a series of extracts from grape seeds, representing reliable sources of tannins, appeared on the market of wine secondary products. But medical use of grape seed extracts endowed with the amplified antioxidant, antibacterial and antifungal properties is limited by their low water solubility. In order to improve the therapeutic efficecy and physico-chemical properties of tannins, new methods of solubilisation (in water) and modification have been applied [17].

The aim of this work was to obtain and modify enotannies from grape seeds (of local origin). The physic-chemical properties (antioxidant activity and content of acidic groups) of initial and modifiend samples were evaluated.

Experimental

Materials

Catechin hydrate (±)-Cat, Luminol (3-Aminophthalhydrazide), Tris-HCl buffer solution (pH 8.6) BioUltra. Hydrogen Peroxide solution (35% H_2O_2), NaOH 1 M solution and 33% HCl solution were purchased from a local chemical supplier. Enotanin products extracted from Red grapes (Enotanin II and Enotanin III) and white grapes (Enotanin I) were purchased from a local wine merchant.

Obtaining of enotannin

Enotannins have been extracted from the grape seeds originated from different grapes varieties: Enotannin I form white grapes (Chardonnay), and Enotannin II, III form red grapes (Cabernet). Extraction of enotannins was carried out by standard method (weight ratio of product/solvent =1:5), using as solvent the ethyl alcohol 70% [12].

Oxidation of enotannin

It is known that tannin products are insoluble in water, which minimizes their use as medicinal products. In this research new hydrophilic compounds Enoxil (Enoxil I, Enoxil II and Enoxil III) were obtained. In order to increase the water solubility of tannins, the procedure of depolymerization reactionis is applied [17-19].

Enoxil samples were prepared as follows: 0.5 g of Enotannin 1 (the same amount for Enotannin 2 and Enotannin 3) was subjected to oxidation of the polymer chain. All samples were left to dry in oven at 60-80^0, and the final product was obtained with the yield of 66% (Enoxil I), 76% (Enoxil II) and 64% (Enoxil III).

Determination of total (carboxylic and phenolic) groups content

For the analysis of total (carboxylic and phenolic) groups (T_{CF}), the Boehm titration method has been used with some slight parameter changes [20]. Abaut 1 g of 0.1% sample solution was transferred into a 150 mL flask, and then bidistilled water (25 mL) was added and stirred on a magnetic stirrer. Further 25 mL of 0.05 mol / L NaOH solution was added to the sample solution and the excess of NaOH was titrated with 0.05 mol / L of HCl solution. Exactly at pH value of 4.5 the volume of consumed HCl solution was taken for further calculation of T_{CF}, expressed as mg-eq/g of sample solution and calculated according to the relation:

$$T_{CF} = \frac{C_{NaOH} \cdot V_1 - C_{HCl} \cdot V_2}{m} \quad (1)$$

where: V_1 – volume of NaOH solution, in mL;
C_{NaOH} – concentretion of NaOH solution;
V_2 – volume of HCl titrant solution, in mL;
C_{HCl} – concentretion of HCl solution;
m – mass of the samples solutions, in g.

Measurements were carried out at Titrator "Titroline 6000" instrument (SI Analytics, Germany).

The antioxidant properties

For the evaluation of the antioxidant properties of the obtained compounds, the chemiluminescence method was used. The chemiluminescence (CL) assay is fast, reliable and comparaively often used for the analysis and determination of the percentage of inhibition of free radical by antioxidants [21].

The oxidative cleavage reaction of Luminol compound by free radicals was used to measure the antioxidant activity of polyphenols. In this assay hydrogen peroxid solution (10^{-5}M) was used as a source of free radicals, which produces hydroxyl, singlet oxygen and superoxide radicals in a slightly alkaline pH solution [22]. Thus, inhibition percentage of free radicals produced in the Luminol/Tris-HCl CL generation system by different concentrations of antioxidants, between 0.01% - 0.25% (w/w), have been determined. Finally, the emission of light was triggered by adding 50 µL of aqueous hydrogen peroxide (C(H_2O_2) 10^{-5}M) to 200 µL of Luminol solution (C(Lum) 10^{-5}M), 650 µL Tris-HCl solution (pH 8.6) and 100 µL aqueous or alcohol solution of flavonoids.

The antioxidant activity was calculated according to the relation:

$$AA(\%) = \frac{(I_0 - I)}{I_0} \cdot 100 \quad (2)$$

where: I_0 and I are relative light emissions of blank and sample solutions after 10 seconds, respectively.

Measurements were carried out at chemiluminometer "Turner Desing TD".

Results and discussion

The Enotannins were extracted from grape seeds with the yield of 10,06% (Enotannin I), 12,58% (Enotannin II) and 11,88% (Enotannin III).

The antioxidant behaviour of obtained of Enoxil samples is presented in Figure 2.

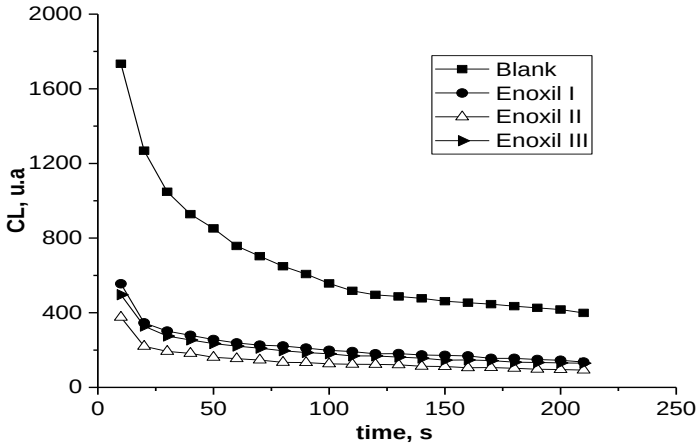

Fig.2. Intensities of the chemiluminiscence signals for Blank, Enoxil I, Enoxil II and Enoxil III.

As shown in Figure 2, the values of antioxidant activity of the new compounds are very close, but at the same time are quite high.

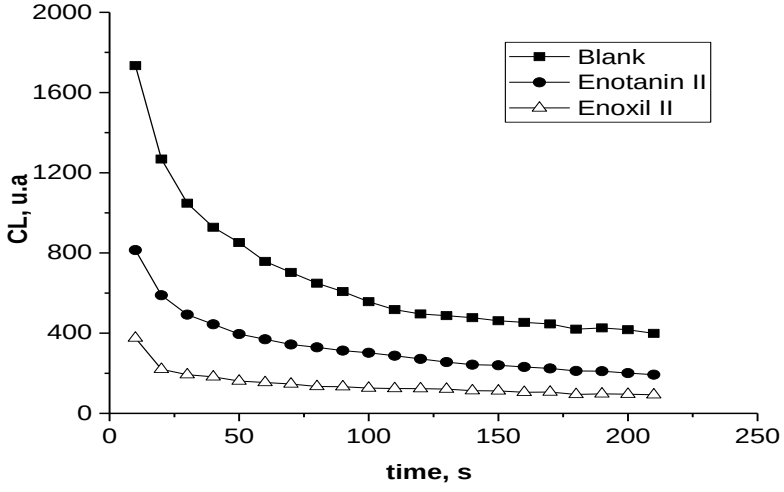

Fig.3. Time variation of the chemiluminescent signal for Blank, Enotannin II and Enoxil II.

Analysis of the data presented in Figure 3 reveal that the antioxidant activity of the novel compound Enoxil is significantly increased in comparison with blank and the initial - Enotannin. Analysis of the obtained data allows us to conclude that the antioxidant activity of enotannins constitutes 53.03% and of Enoxil – 85.72% (Table). Thus, the antioxidant activity of the novel compounds is by 32% more pronounced than of the initial enotannins. The increase of antioxidant activity for new compounds in comparison with that of enotannins is explained by the fact, that in the process of enotannin hidrosolubilization, in parallel with multiple oxidation processes, the process of depolymerization of the proanthocyanidin polymer chain occurs. As a result, from one enotannin molecule, a large number of monomers of catechin, epicatechin etc is formed [8].

We can conclude that these new compounds of natural origin possess amplified antioxidant properties and are attractive for future microbiological and pharmacological research.

Determination of the acidic functional groups is an important parameter required to characterize the physico-chemical properties of Enotannins. The oxidation of Enotannins leads to increasing the contenit of acidic functional groups by 3-4 times, in comparason with initial samples (Table).

Table

Parameters of antioxidant activity (AA) by chemiluminescence and T_{CF} content of polyphenolic compunds

Samples	CL, AA(%)	$T_{CF,}$ mg-eq/g
Enoxil I	77.10 ± 0.32	0.22±0.01
Enoxil II	85.72 ± 0.19	0.29±0.01
Enoxil III	78.58 ± 0.17	0.26±0.01
Enotanin I	46.91 ± 0.28	0.06±0.03
Enotanin II	53.03 ± 0.44	0.08±0.02
Enotanin III	51.18± 0.36	0.07±0.04

It should be noted that these polyphenolic compounds play an important role in inhibiting the development of pathogenic micro-organisms, by complex bacteriostatic action.

Conclusions

New samples of Enoxil (Enoxil I, Enoxil II and Enoxil III) were obtained by oxidation of enotannins. The antioxidant properties of enotannins and Enoxils was determined by chemiluminescence method. It was demonstrated that the antioxidant activity of novel chemical compounds is by 29-32% more pronounced than that of enotannins used as raw material.

By applying the potentiometric method of "Boehm titrations" the amount of acidic functional groups (carboxyl and phenolic) in enotannins and the mentioned Enoxil preparations was determined.

The obtained results reveal that new substances contain about 3-4 times more acidic functional groups than Enotannins.

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