

STUDIES ON THE WATER SOLUBILIZATION PROCESSES OF OENOTANNINS AND THEIR PHYSICO-CHEMICAL PROPERTIES

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Abstract: The results of investigation of oenotannins content in the seeds of different grape varieties are presented in the paper. It was demonstrated that the oenotannins content depends on the grape variety as well as on the geographical zone of grape cultivation. A new procedure for water solubilization of oenotannins by depolymerization has been elaborated. The mechanism of the oenotannins depolymerization has been revealed by mass-spectrometric studies. It has been established, that the depolymerization process brings about formation of carboxylic acids and organic peroxides. The presence of the carboxylic and peroxidic groups was determined on the basis of IR spectral analysis. It was demonstrated that the antioxidant properties of modified tannins are higher than those of initial unmodified ones.

Introduction

Tannins are wide spread in natural sources. They can be found in fruits, fruit shells, in leaves and seeds of different plants. Natural tannins form two large groups: hydrolysable and condensed or catechinic tannins. Tannins from the first group decompose under the action of mineral acids to give a monosaccharide, usually D-glucose, and gallic acid or its derivative. Condensed tannins do not contain a sugar component and can be cleaved in simpler fragments only by alkaline melting. The hydrolysable tannins are transformed under dry heating into pyrogallol and the condensed tannins into pyrocatechine [1,2].

The grape seeds represent in the Republic of Moldova the most convenient source of tannins. Depending on grape variety, they contain from 3 to 14% tannins. Tannins obtained from grape seeds are called oenotannins. Most of these are water insoluble. They dissolve only in ethanol, methanol, ethylacetate, acetone and other organic solvents.

Oenotannins represent a complex mixture of catechine and epicatechine oligomers. Their composition varies from monomers to decamers of catechine or epicatechine. The polymeric chain is formed on condensation of functional groups at C4 carbon atom in C-cycle and C8 carbon atom in A-cycle. Polymerization processes of catechine and epicatechine and its mechanism have been studied previously [3].

There have been reported in the literature on the fungicide and antibacterial action of oenotannins [4], but these properties are weak. It is the limited water solubility that makes difficult utilization of these compounds in medicine and agriculture.

We provide in the present paper a new procedure for oenotannins solubilization in water, the possible chemistry of this process, as well as some physico-chemical properties of the modified tannins.

Table 1.

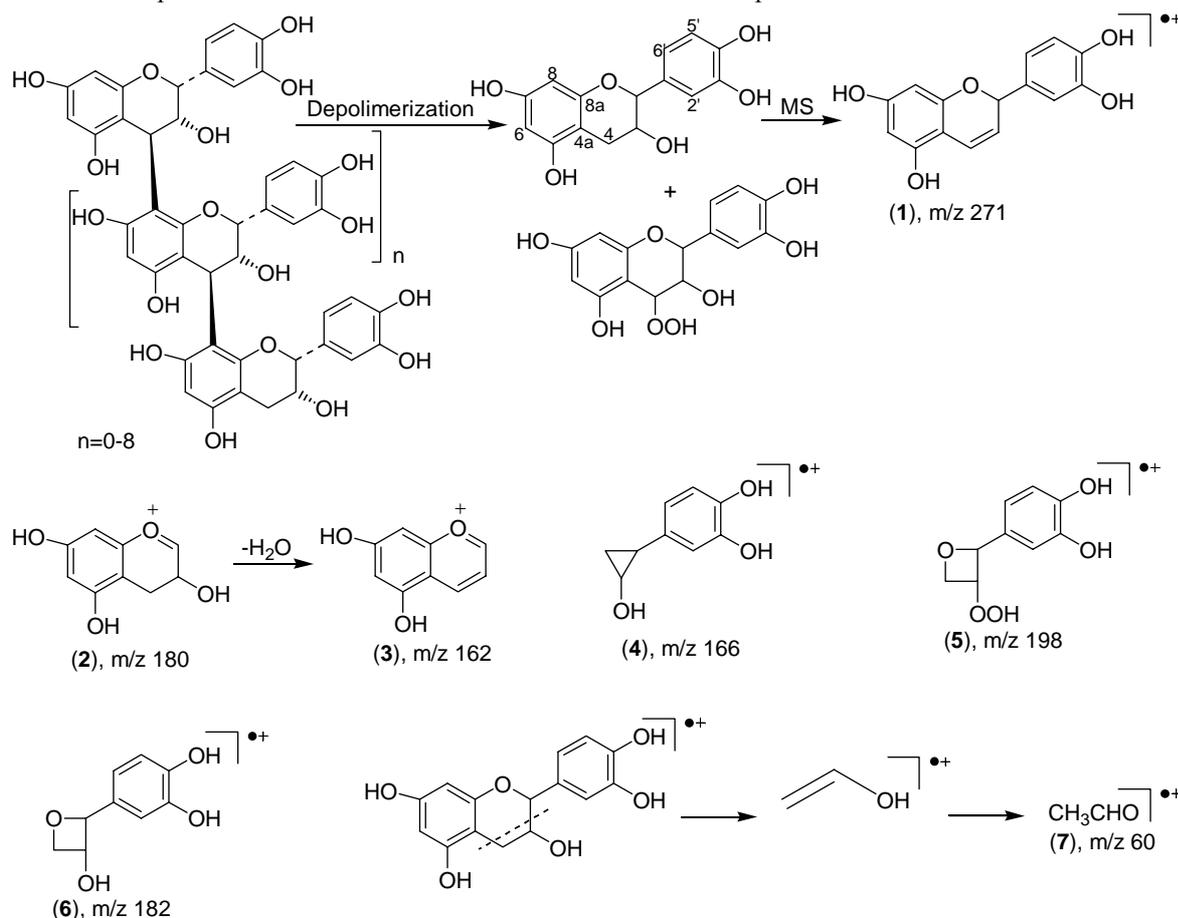
The oenotannins content in grape seeds

Grape variety	Geographical zone of cultivation	Tannins content, %
Rcaţeteli	Bulboaca, Anenii Noi	6,4
Sovignon	-,-,-	4,0
Feteasca	-,-,-	3,1
Cabernet	-,-,-	3,7
Rcaţeteli	Căuşeni	5,4
Sovignon	-,-,-	5,1
Merlot	-,-,-	5,3
Feteasca	-,-,-	4,0
Cabernet	-,-,-	10,7
Rcaţeteli	Bardar, Ialoveni	6,6
Aligote	-,-,-	6,4
Cabernet	-,-,-	9,6
Merlot	-,-,-	5,9
Fraga neagra	-,-,-	14,1

Results and discussions

The oenotannins content in different grape varieties is presented in table 1. An examining of the results presented in this table can lead us to the conclusion that the oenotannins content in the grape seeds depends on the grape variety as well as the geographical zone of grape cultivation. The content of oenotannins lies between 3,1 and 14,1%.

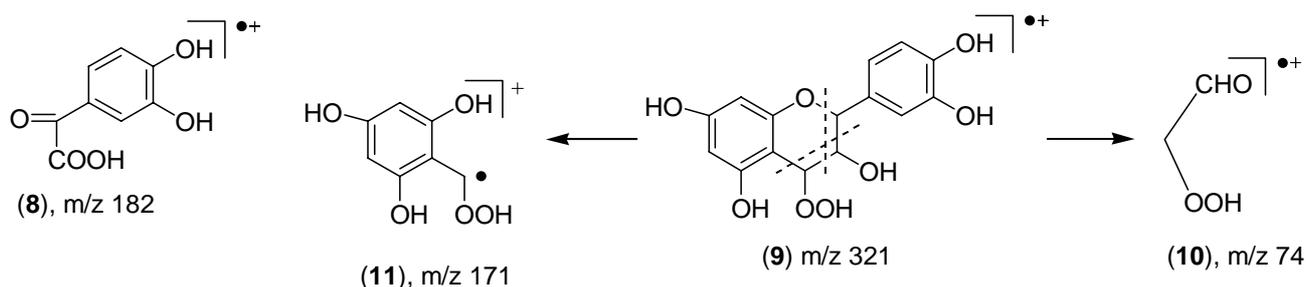
Mass-spectrometric data confirm the catechic nature of compounds from the studied mixture. According to



the peaks intensities, the monomeric structures predominate, but there are also dimeric (peaks in the region of m/z 620-670), trimeric (m/z 918) and tetrameric (m/z 1118-1158) structures. There are no peaks with higher m/z value in the spectra. Trimeric and tetrameric compounds are in smaller amounts. The ions with m/z 271 (1), 180 (2), 162 (3), 166 (4), 198 (5), 182 (6) and 60 (7) belong to the catechic structure.

A series of peaks show the presence in the studied mixture of compounds with hydroperoxidic residues in positions C-3 [ions with m/z 198 (5) and 182 (8)] and C-4 [peaks with m/z 74 or 75, formed by transformation (9)→(10) and m/z 171 (11)] of catechine.

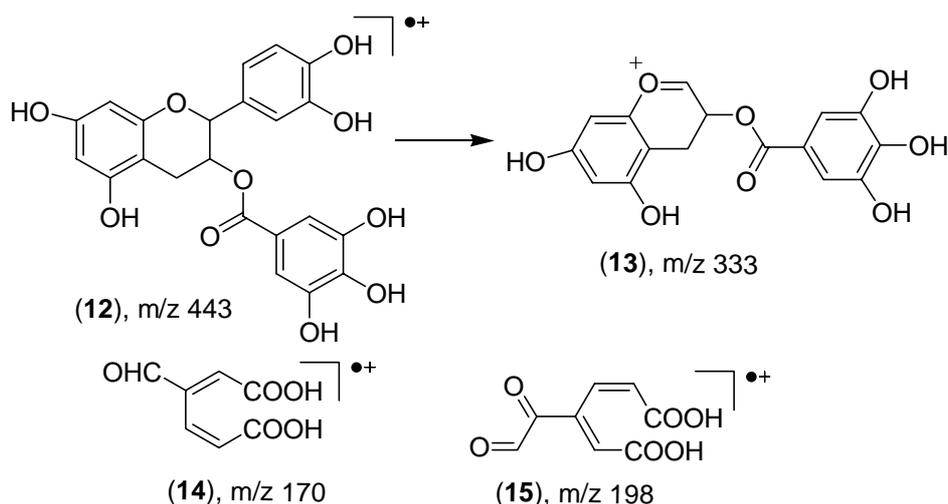
The presence of hydroperoxide groups is confirmed too by the presence of the peak with m/z 17 (OH- group) in one of the spectra.



The peaks of ions with m/z 443 (12) and m/z 333 (13) confirm the presence in the mixture of gallic acid esters, linked with an esteric group with catechine at C3 position.

Above mentioned data prove that chemical treatment of oenotannin with H₂O₂ brings about depolymerisation of catechine and epi-catechine oligomers to form monomeric derivatives both in the free form and esterified with gallic acid at C-3. Most probably, breaking the C4-C8 bond between the catechic residues leads to introduction of the hydroperoxidic residue at C4 position. There also a possibility of introduction of the hydroperoxidic group at C3 position by nucleophilic substitution.

The possibility of deep oxidative cleavage of cycles B and C of catechic skeleton is not excluded too. There is obviously possible that a certain MS peak corresponds to different ions with the same m/z but with different structures and this is characteristic for MS cleavage.



The IR spectra of the original and modified oenotannins are presented in figures 1 and 2.

Figure 1.

IR spectrum of unmodified oenotannins

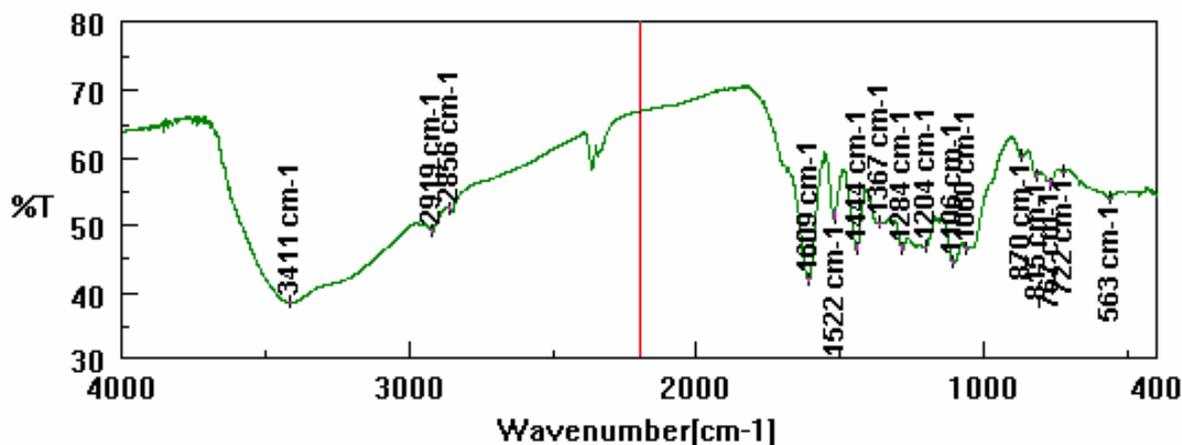
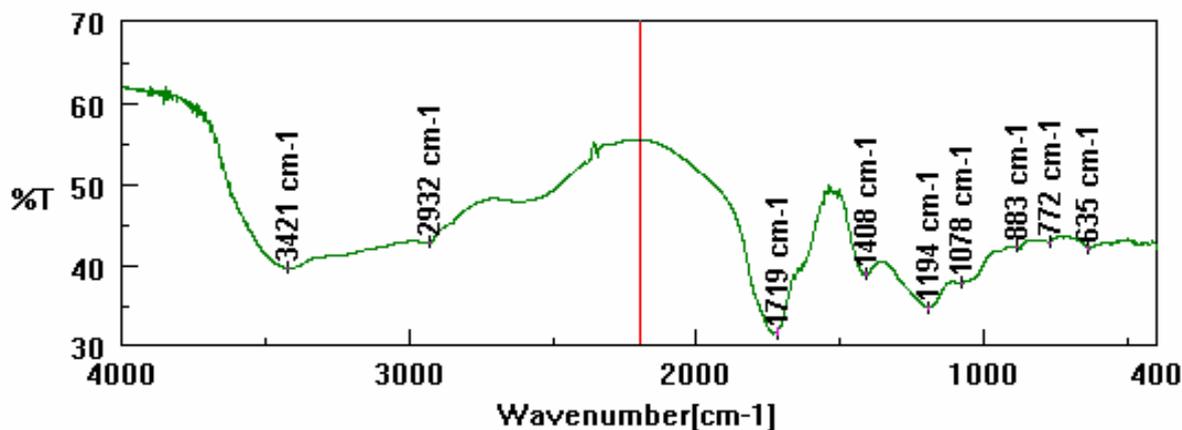


Figure 2.

IR spectrum of modified oenotannins



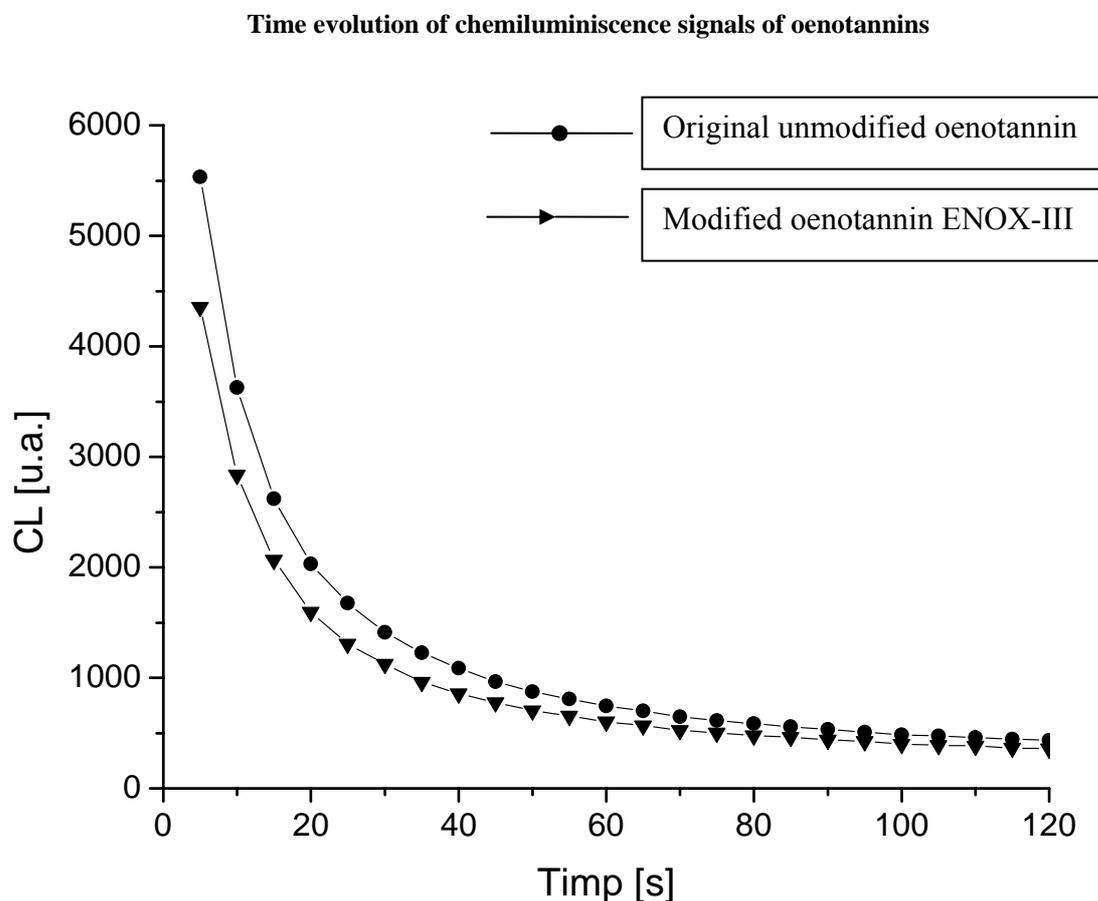
Spectral analysis reveals that in both cases the samples contain strong hydrogen bonds (broad bands at 3411 and 3421 cm^{-1} respectively). An essential difference between is seen in the region of the digital imprints of original and modified oenotannins spectra. The original unmodified sample shows bands characteristic to aromatic rings with different substitution pattern (bands at 762 cm^{-1} , 815 cm^{-1} , 1,2,3 substitution; 870 cm^{-1} 1,2,4 substitution; 1060 cm^{-1} 1,3 substitution; 1444 cm^{-1} and 1609 cm^{-1} benzene ring). The most intensive peak in the modified product is detected at 1719 cm^{-1} that is a characteristic signal of carboxylic acids (dimers). This is the essential difference between the two products, since this band is absent in the original unmodified oenotannin. The spectrum of the modified product shows also a strong band at 1194 cm^{-1} that is also absent in the original oenotannin. This band can be attributed to the hydroperoxidic group. Its presence was also confirmed by chemical means, making use of the oxido-reductive test with potassium iodide in acidic conditions.

The IR spectrum of modified tannins shows bands characteristic to aromatic compounds ($772, 883, 1078\text{ cm}^{-1}$) but their intensity is much smaller than those of the unmodified product and this can be accounted for a substantial degree of aromatic rings cleavage in the modified product.

The antioxidative activity was determined on both original and modified tannins using the luminol/hydrogen peroxide system (Fig. 3). The antioxidant activity of original oenotannins represents 38,1% and of the modified product – 51,3%.

The obtained results denote that the modified oenotannins have a more prominent antioxidative activity than the original ones.

Figure 3.



Additional investigations on biological activity of the oxidized oenotannins were performed in the “N. Testemiteanu” Moldova State Medical and Pharmacy University, Institute of Microbiology and Biotechnology and Institute of Genetics and Plant Physiology, Academy of Sciences of Moldova. These investigations showed that oxidized oenotannins are more water soluble and possess relevant antimicrobial activity.

Experimental part

Oenotannins have been extracted from the grape seeds originated from different grape varieties grown in different geographical zones of the Republic of Moldova. Extraction has been performed in a glass column with 30 mm i.d. and 500 mm length. The seeds have been crushed in an electrical mill to a fraction not larger than 1 mm in diameter. As extraction solvent ethanol has been used. The solvent was passed in a descending mode through the column with a 10 ml-min rate until oenotannins extraction was complete. The obtained extract was concentrated on a rotary evaporator under vacuum, until the density of the residue reached $1,12\text{ g/cm}^3$, then dried in an oven at 60°C to a constant mass [5].

Water solubilization of oenotannins was realized by their oxidation with hydrogen peroxide at $70\text{-}90^\circ\text{C}$. The obtained product was dried in an oven at $45\text{-}60^\circ\text{C}$ to a constant mass [6]. The mass spectrometric investigation was performed on a Bruker Autoflex II mass spectrometer using laser ionization. The IR spectra have been recorded on a Jasco FT-IR 620 spectrometer. Antioxidative activity of both modified and original oenotannins was measured in the luminol-peroxide system on a Turner Design TD 20/20 chemiluminometer.

Conclusions

1. The oenotannins content in the investigated grape seeds varies from 3,1 to 14,1%. The oenotannins content is higher in black grape varieties.

2. The hydrogen peroxide promote depolymerization of catechine and epicatechine oligomers in oenotannins to provide monomeric derivatives of free and esterified carboxylic acids, as well as peroxidic compounds.
3. The presence of carboxylic and peroxidic functional groups was demonstrated on the basis of IR spectra analysis.
4. The enhanced antioxidant properties of modified tannins was demonstrated by the chemiluminescence method.

Acknowledgements

This work was performed within the INTAS project 05-104-7505. Thanks are due to the staff of the "Politehnica" University of Bucharest for antioxidant activity testing and of the Institute of Surface Chemistry, National Academy of Sciences of Ukraine, for mass spectrometric investigations.

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