

EVALUATION OF RESIDUE COMPOSITION IN CATECHIN COMPOUNDS FROM WINE INDUSTRY THROUGH SPECTOMETRIC AND CROMATHOGRAPHIC METHODS

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Abstract: Flavan-3-ols are the result of secondary plant metabolism, being a subclass of flavonoids, also distributed in grapes. The most important property of these compounds is the antioxidant activity, due to their –OH groups and double bonds. Flavan-3-ols have the generic name “catechins”.

There are known eight types of catechin structures: catechin (C), catechin gallate (Cg), gallocatechin (GC), gallocatechin gallate (GCg), epicatechin (EC), epigallocatechin (EGC), epigallocatechingalate (EGCg).

The aim of this study is the identification and isolation of some flavan-3-ols from different varieties of romanian grapes (*Vitis vinifera*) through HPLC High Performance Liquid Chromatography (HPLC), and UV-VIS analysis. The identification of catechins was made from different varieties of Romanian grapes. The total content of catechins is determined by UV-VIS analyses.

INTRODUCTION

In agricultural areas, crops such as grapes generate huge amounts of byproducts. In Europe alone 112 million tons of grapes were processed by the wine industry in 1998.

An estimated 13% (14.5 million tons) of this amount corresponded to the byproduct after pressing, consisting mainly of skins and seeds. Grape skins and seeds are rich sources of health-promoting polyphenols, including flavan-3-ols of different degrees of polymerization known as proanthocyanidins. Oligomeri proanthocyanidins, as well as other polyphenols, are potent free radical scavengers useful as preventative agents against cancer, cardiovascular diseases, and premature aging. [1]

Residues such as peels and seeds that result from fruit juice production may contain substantial amounts of valuable natural antioxidants

After the grape is crushed, the juice is used to make wine and the solid grape residues, or pomace, are typically waste. Valuable grape sugar and other grape components such as phenolic antioxidants and red pigments remain in the pomace and can be recovered.

This is very important because grape skins and seeds are rich sources of health-promoting polyphenols, including flavan-3-ols. Grape seeds contain lipid, protein, carbohydrates, and 5-8% polyphenols depending on the variety. Polyphenols in grape seeds

are mainly flavonoids, including gallic acid, the monomeric flavan-3-ols catechin, epicatechin, gallo catechin, epigallocatechin, and epicatechin 3-*O*-gallate, and procyanidin dimers, trimers, and more highly polymerized procyanidins. The most abundant phenolic compounds isolated from grape seed are catechins, epicatechin, procyanidin, and some dimers and trimers. [2]

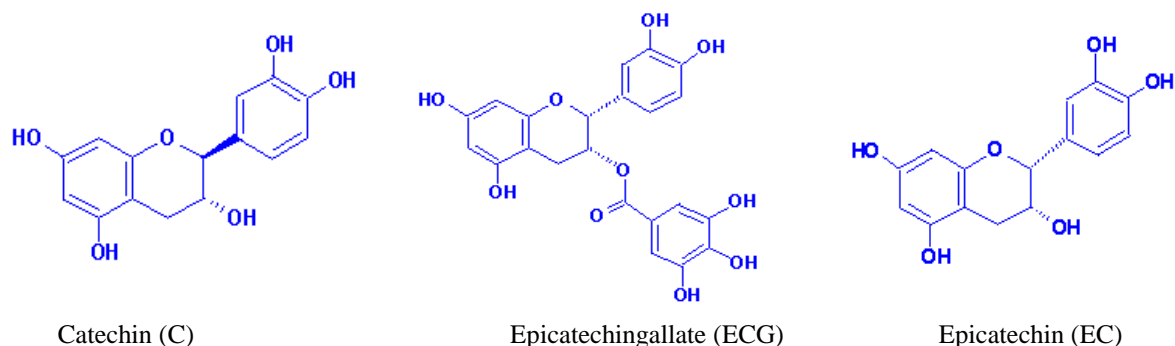


Fig 1. Chemical structure of some catechins from grape seed

The catechins isomers (**fig.1**) can vary among different varieties of grapes, depending on the species, the climate, the cultural practices and in the case of grape extract, on the conditions and technology used for the extraction and storage. [3]

Catechins may reduce oxidative stress through one of several mechanisms that relate to their structural chemistry. For example, catechins directly scavenge free radical species through hydrogen/electron donation. The activity of an antioxidant is determined also by: its reactivity with other antioxidants and the transition metal-chelating potential.[4]

The structure–activity relationship of these compounds suggests that the presence of a catechol group and the hydroxyl group at position 3 on the B-ring are absolutely essential to their ability to scavenge free radicals. [5]

MATERIAL AND METHOD

The chemical used were from the following sources: standard of catechin from Sigma Chemical Co., phosphoric acid, acetonitrile, methanol, etil acetat formic acid, BSA were from Nordic, silica plates G-25 from Merck. Doubled distilled water was prepared in our laboratory.

The biological material seeds and skins from different grape varieties: Merlot Recas, Burgund Recas, Cabernet Sauvignon Recas, Merlot Dealu Mare, Muscat Hamburg were dried at 65 °C and grounded. The extraction was made in 50% methanol with 1% HCl using 0.5 g of grape seeds and grape skins probe is then sonicated two hours and filtrated.

From final filtrate 1ml is introduced in a flask with 2 ml of BSA (bovine seric albumin) for precipitation of tannins, which is centrifugated 15 min. At room temperature 0.9 ml from supernatant with 0.1 ml FeCl₃ (10mM solution in HCl 0.01N) was considered our sample. The mator sample contain 0.9 ml BSA and 0.1 ml FeCl₃.

The UV-VIS spectra were recorded on a JASCO V-500 spectrophotometer. UV spectra were measurement at wavelengths 510 nm.

HPLC analysis was carried out on a PERKIN –ELMER LC–55 B system coupled to a diode array detector. HPLC of the catechins was carried out on a nucleosil C-18 column (250x 4 mm, 5µm particle size, Macherey & Nagel) with a solvent system of 0.1% H₃PO₄ /acetonitrile and a flow rate of 1ml/min. The absorbance at 279 nm were recorded for catechin

separation. The catechin standard in concentration 30 mg/ml was dilute in methanol 50%. Identification of catechin was made from co-cromathograms.

RESULTS AND DISCUSSIONS

UV-VIS spectra show the total content of catechins from grape seed extract. Values obtained for total catechins content 5 different varieties, exprimed in mg/kg or kg/l are represented in table 1.

Table 1. Total content of catechins from grape extract

Sample name	Total catechins from grape seeds mg/kg	Total catechins from grape bunches mg/kg	Total content of catechins from grape skins mg/kg	Total content of catechins from grape wine mg/l
Muscat Hamburg	11.526	3.480	The concetrations obtained are under 100 mg/kg	
<i>Merlot Recas</i>	8.180	3.880		
<i>Burgund Recas</i>	10.553	3.093		
<i>Merlot Dealu Mare</i>	9.933	3.906		
Cabernet Sauvignon	8.460	3.973		

In **fig 2.** are representative varieties for total content of catechins: Muscat Hamburg and Burgund Recas

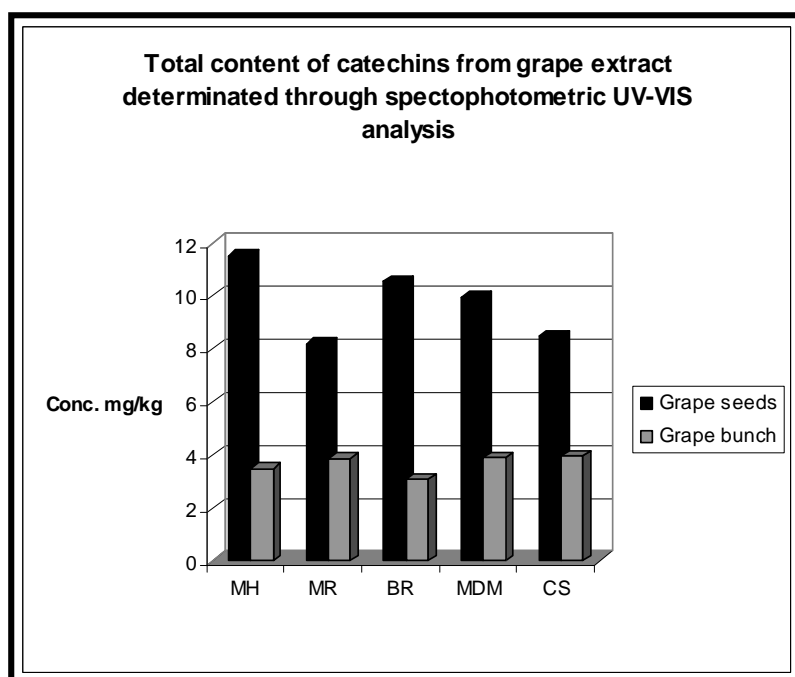


Fig 2. Total content of catechins from grape seed extracts determinated through spectrophotometric analysis. MR-Merlot Recas; BR-Burgund Recas; CS-Cabernet sauvignon Recas, MDM-Merlot Dealu Mare; MH-Muscat Hamburg

The PERKIN–ELMER LC–55 B apparatus has identified and separated catechin from 5 different varieties of grape. The HPLC analysis confirm that the Merlot Dealu Mare variety is the richest in catechin. The catechin identification was made using catechin standard.

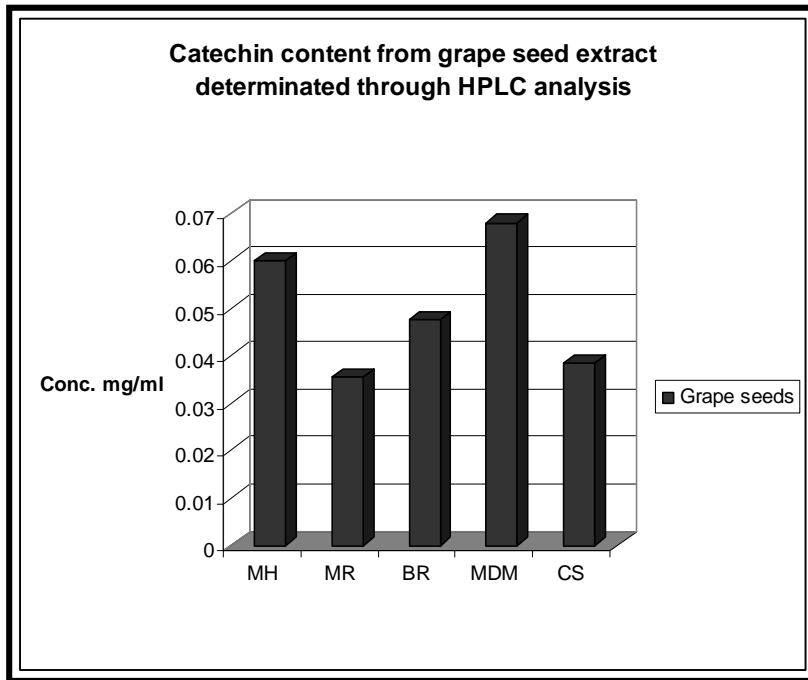


Fig 3. Catechin concentrations of grape seed extracts determined through HPLC analysis. MR-Merlot Recas; BR-Burgund Recas; CS-Cabernet sauvignon Recas, MDM-Merlot Dealu Mare; MH-Muscat Hamburg

In the **fig.4B** is the cromathoram of the sample Merlot Dealu Mare. The co-cromathograme from **fig 4A** show that the catechin peak appear at min. 7.57.

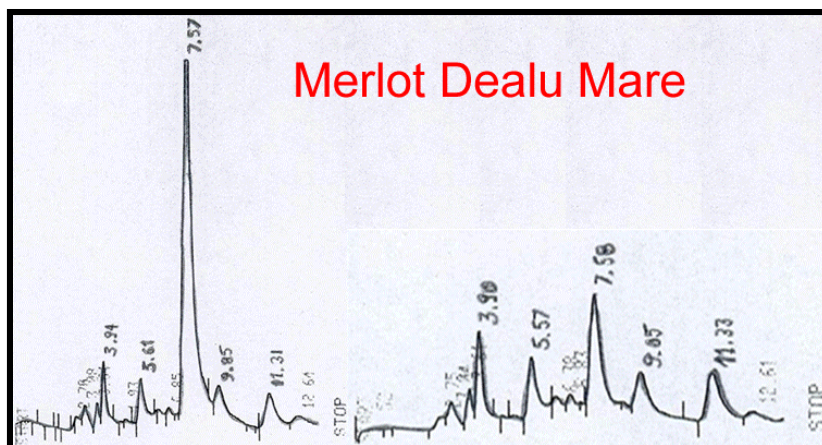


Fig 4. Identification and separation of catechin: A: co-cromathograme, using catechin standard (7.57), B: cromathogram of the probe.

CONCLUSIONS

- Using UV-VIS analysis, grape seeds show the greater content of total catechins, most important samples being Muscat Hamburg and Burgund Recas
- Values obtained for grape bunches through UV-VIS analysis are 3 times smaller than values for grape seeds
- Samples obtained from skins and wine show that the total content of catechins has concentrations 30 times smaller than the others from seeds, so present no interest for catechins.
- HPLC analysis shows that the Merlot Dealu Mare grape variety show a greater content of catechin than the other varieties tested
- HPLC represents the most accurate method tested by us for the catechin research

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