ORIGINAL PAPER

INFLUENCE OF EXTRACTION METHOD ON CHEMICAL COMPOSITION FROM RED GRAPES SKIN EXTRACT

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Abstract. *Chemical compounds from plant extracts offer unlimited opportunities as cosmetic additives due to their availability and chemical diversity. Increasing demand for chemical compounds from different classes made the plant extracts, edible plant extracts in particular, useful for cosmetic products. The profile of bioactive compounds contained by the so-called natural cosmetic products may be different according to the characteristics of the plant extracts used in their fabrication processes. The study proposes a comparative analysis of several characteristics of grape extracts obtained by solid-liquid extraction, using water as solvent, with and without ultrasonation. Extracts obtained from four red grape varieties harvested at maturity from two types of vineyards, ecological and traditional, were analyzed. Total flavonoid contents, pH and conductivity were measured; correlations of these properties with extraction method, grape variety and vineyard type were envisaged as well. It was concluded that slight differences may occur in extracts aciditiy and conductivity of studied red grape extracts, as well as in the total flavonoids contents, and that the use of the ultrasound power during the applied extraction procedures does not have a significant influence. The vineyards type however showed some influence on the studied extracts properties.*

Keywords: *red grapes, skin extract, total flavonoids content, acidity, conductivity, FTIR, UV-VIS.*

1. INTRODUCTION

Grapevine is one of most important perennial crops being cultivated mainly in tropical and temperate regions of Earth. The development of the human civilization is closely related to the development of the viticulture. It is very well known that the production of grapes is principally intended for wine, fresh fruit, juice and raisins. Therefore the Europe is the world's most important wine producing continent, with an area planted with vine of about 3.3 milion ha. Vine growing always had an important role in Romanian agriculture, as wine plays a major role in national economy. The increase of the requirements on the world market for

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products with a high quality and productivity grapes, require the application of newer and safer methods for characterization of vine genetic resources and for the improvement of amelioration process of it.

Due to the constant preoccupation of the consuming market for ecological products, several ecological vineyards were developed in Romania in the last years, and their products seem to become popular at international level. On the other hand, relying on their experience and specialized cultivation processes, traditional vineyards apply crops maintenance treatments to maintain good grape quality. Another point of view on the importance of vinegrape analysis is that recent data [1-4] suggest that beverages can significantly contribute to the total dietary intake of some trace elements with the possibility of influencing their levels in tissues and body fluids. Main differences between ecological and traditional grapes cultivation consist in the type of applied periodical bio-chemical treatments. In organic vineyards, the applied treatments must comply with certain standards imposed by the specific regulation organizations for the farm to obtain the “green label” [1]. The way that plants are treated can influence how they grow, but also the chemical composition [2-4].

Phytochemical characterization of grapes vegetative parts demonstrated their valuable content in various compounds with therapeutic actions for human health. Thus, the grape berries were found to contain chemical species with antioxidant, anti-inflammatory or other health protection roles. Some of these species, upon specific efficient extraction may be considered as potential bio-resources for industries like pharmaceuticals and cosmetics, as well as in other biomedical and biotechnological applications [5, 6]. In order to determine the optimum conditions for extraction depending on the target bioactive chemical compounds and the chemical matrix of the final product, different extraction methods were tested [7].

Flavonoids are an important class of bioactive chemicals that are generally found in grapes vegetative parts. They are natural polyphenols derived from plant metabolites, having a $C_6 - C_3 - C_6$ ring as basic structure (Fig.1), with varying substitues, thus leading to sub-classes as flavones, flavonols, isoflavones, anthocyanins [8, 9].

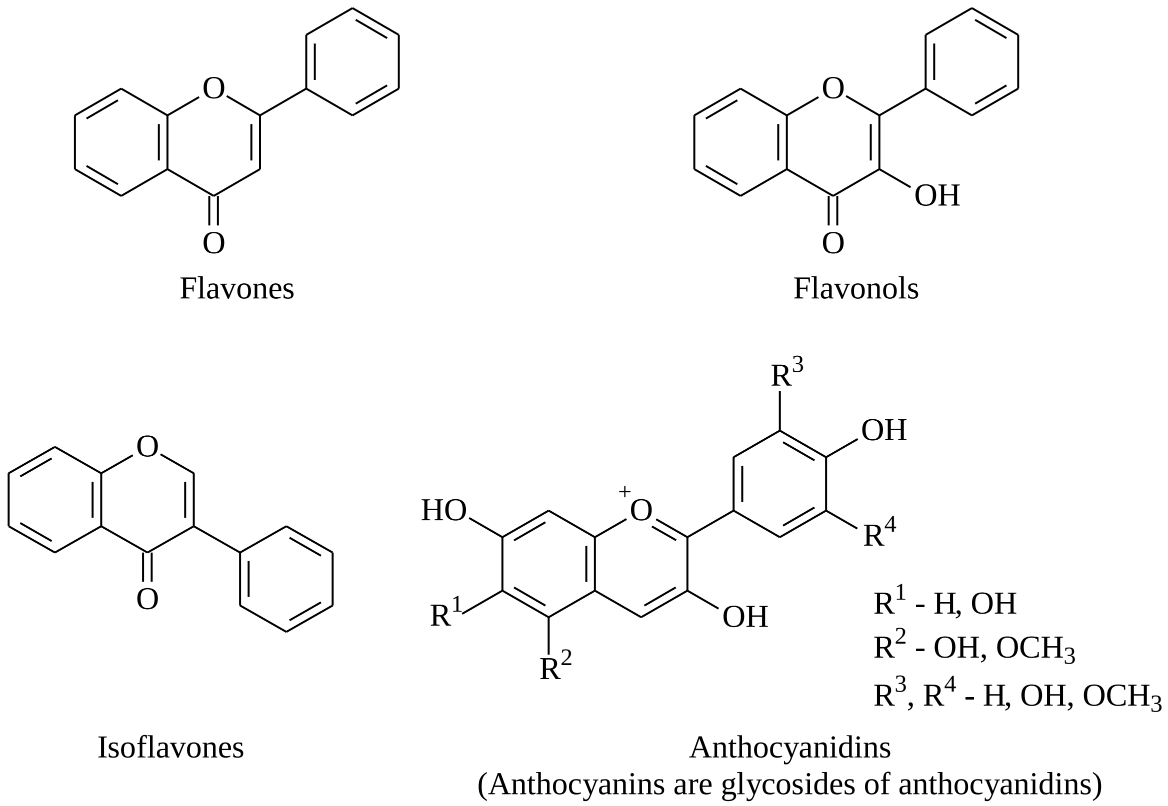

Figure 1. Chemical structures of flavonoids.

This study aimed to identify possible correlations between the vineyard type (i.e. ecological and traditional), grape varieties (i.e. Merlot, Feteasca Neagra, Pinoir Noir and Muscat Hamburg) and extraction methods (i.e. maceration and ultrasound assisted methods) upon some phytochemical characteristics of water extracts obtained from grape skins. In this respect, total flavonoids content (TFC), acidity and conductivity of aqueous grape skins extracts were monitored during experiments. These parameters were monitored from the perspective of achievement of new natural cosmetical products by using these aqueous extracts.

2. MATERIALS AND METHODS

2.1. MATERIALS

Chemical reagents used for all experimental determinations in this study were of analytical grade, and redistilled water (conductivity at 25°C bellow 0.5 $\mu S\cdot cm^{-1}$) was used as solvent and wash solution. Thus sodium nitrite (99% purity, manganometric assay), aluminiun chloride (99% purity, complexonometric assay), sodium hydroxide (purity > 97%, acidimetric assay, sodium carbonate < 1%) were purchased from Merck Millipore; ethanol of analytical grade was purchased from Chimopar SA. Quercetin hydrate with 95% purity (HPLC assay) was purchased from Sigma-Aldrich.

2.2. METHODS

Sampling

Grapes of four varieties (i.e. Merlot, Feteasca Neagra, Pinoir Noir and Muscat Hamburg) were collected in autumn of the year 2017, from two different vineyards from Romania: ecological culture, respectively traditional culture, with various pesticides treatments applied during growing period. Raw products of same batch were used during tests. Skins, pulp and seeds were manually separated from each sampled grapes variety and vineyard.

Extraction procedures

Water extracts of studied grapes varieties were obtained from skins that were first dried at 40 ºC for 48 hours, and then stored for further experiments at room temperature in closed labelled paper envelopes.

Two extraction methods were applied to obtain the grapes water extracts:

a) First procedure was the *classical maceration at room temperature*. Here, a weighted amount of approx. 2 grams of dried skin was placed in a covered laboratory flask, 100 mL redistilled water at room temperature was added, then kept under magnetic stirring for 3 hours, and centrifuged after 21 hours, thus reaching a total contact time of 24 hours. Same steps were followed for all studied grape varieties.

b) Second procedure was the *ultrasound assisted method at room temperature*, where same weighted quantity of grape skin samples (2 g) were contacted with same volume (100 mL) of same solvent (redistilled water) for 24 hours, out of which the first 30 minutes represented maintaining the covered flasks in the ultrasound bath. Higher times for the ultrasound treatment were tested (data not included), but were considered unappropriated, as grape-water mixtures temperature increased, exceeding the room temperature.

For both extraction methods, a centrifugation stage has followed; performed at a speed of 1000 rpm for 10 minutes, and then the resulted aqueous suspensions were filtered using Whatmann no. 4 paper. In order to be used in further tests, the liquid portions (supernatants) were stored at 4°C, while the solid ones were dried at 40 ºC for 48 hours.

Centrifugation was performed during extract preparation using a Rotofix 46 Hettich Lab Technology centrifuge, at a speed of 1000 rpm.

Electrochemical measurements

Electrochemical measurements of grapes water extracts, pH and conductivity, were performed at room temperature, using Inolab Multi 9430 meter (WTW), with automatic temperature compensation. Accuracy of the pH sensor model IDS SenTix 980 was $4 \cdot 10^{-3}$, and cell constant of the conductivity sensor TetraCon 925 was 0.475 cm^{-1} ± 1.5 %.

Fourier Transform Infrared Spectroscopy

The infrared spectroscopy (FTIR) is often used to identify chemical functional groups of organic compounds existing in various plant extracts samples, correlations with some of their phytochemical characteristics being envisaged [11-13]. For present study, dried grapes skin samples were scanned in the wavenumbers range of 4000 cm^{-1} to 400 cm^{-1} using Attenuated Total Reflectance (ATR) technique. The infrared spectra were collected with a Bruker Vertex 80 infrared spectrometer equipped with ATR device with diamond crystal.

Ultraviolet-visibile spectroscopy

Total flavonoid contents (TFC) were determined in freshly aqueous extracts using Thermo-Evolution-260bio spectrophotometer equipped with glass cuvettes of 1 cm.

Total flavonoid content was measured by the aluminum chloride colorimetric assay described in the literature [10], slightly modified. Method's principle is based on Al^{3+} ions that formed a coordinative compound by involving both carbonyl (i.e. C-4 carbon) and hydroxyl (e.g. C-3 or C-5 carbons) groups from flavonoids structure. Further, aluminium can bond the orthodihydroxyl groups from A- and B- nucleus of flavonoids. The effect of the formation of these bonds can be observed by coloration of the working solution in yellow due to resulted complex combinations.

Extract samples absorbances were measured at 510 nm, against redistilled water. The concentrations were calculated using the calibration curve drawn before each tests set, in the concentration range of 0.1 – 1.0 mg/mL of quercetin, used as reference flavonoid. Appropriate dilutions were prepared as needed from aqueous grape extracts, therefore that samples absorbances fit the calibration curves ranges. TFC were expressed as mg quercetin equivalents per mL of aqueous grapes extract. Analytical data were collected on triplicate samples, mean values together with standard deviations are reported in Table 2.

3. RESULTS AND DISCUSSION

3.1. RESULTS

Infrared spectral assay

FTIR data for all extract samples have shown similarities concerning spectra in the wavenumber range of 400-4000 cm^{-1} (Fig. 2 as example for Pinot Noir grape skin sample), regardless of the used extraction method. The observed peaks were assigned to different bonds according with their chemical structures. All FTIR spectra showed strong peaks at 3275 cm^{-1}, assigned to O-H stretching vibration, and at 1026 cm^{-1}, assigned to C-O stretching. Both 2918 cm^{-1} and 2850 cm^{-1} peaks were assigned to asymmetric and symmetric stretching vibrations of -CH_2-. The signal of 1722 cm^{-1} can be assigned to C=O stretching vibration. The weak peak size compared to absorption bands with maximum at 3275 cm^{-1} and 1026 cm^{-1} was considered to show that the number of carbonyl groups is lower than the number of hydroxyl groups. This experimental finding may be subsequently correlated with an indication of a higher content of anthocyanins in the total mixture of flavonoids from the grape skin.

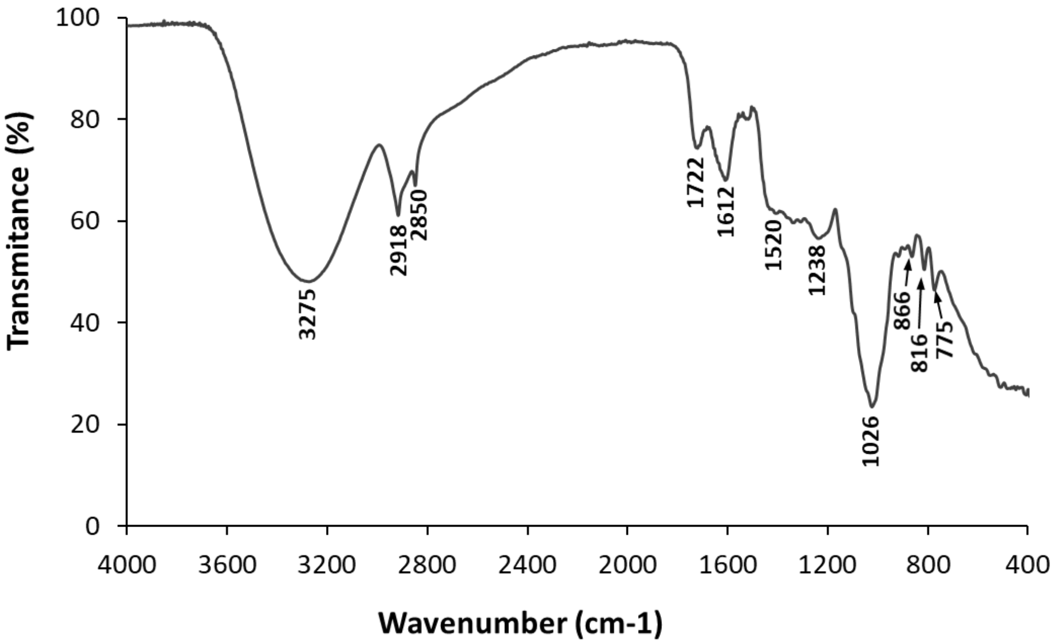

Figure 2. Infrared (IR) spectrum of grape skin sample.

The vibration at 1612 cm^{-1} is correlated with C=C stretching and the vibrations of 1520 cm^{-1} and 1238 cm^{-1} were assigned to correspond to C-H bending for methylene and methyl groups. C-H bending is assigned to absorption bands from 866 cm^{-1}, 816 cm^{-1} and 775 cm^{-1}.

Conductivity and pH measurements

The aqueous extracts obtained by the two methods, maceration and ultrasonication, from the studied red grape skins, were measured for their pH and conductivity, and the values found are showed in Table 1. Similarities of these values may be observed, indicating that the extraction method does not significantly influence either pH or conductivity. However, differences occur when comparing pH and conductivity values for samples obtained from ecological and traditional vineyards. Thus, in the case of Merlot grape variety, lower pH and conductivity values were observed for grapes cultivated according to the traditional method compared to grapes coming from the organic vineyards. In the case of Muscat Hamburg grapes, the extracts obtained from ecological vineyard grapes have lower pH values than extracts from traditional culture grapes that were treated with commercial pesticides according to regular procedures. The other two varieties, Feteasca Neagra and Pinot Noir, showed similar pH values. On the other hand, the conductivity values for extracts from Feteasca Neagra grapes treated with pesticides have lower conductivity values than aqueous extracts obtained from organic grapes.

These variations show that in certain situations, applied treatments can influence pH or conductivity values of skin grapes aqueous extracts, further specific research investigations are considered in this direction, so that potential correlations may be thoroughly defined.

Table 1. Measured values for pH and conductivity of aqueous red grapes skin extracts.

Grape variety	**Vineyard type**	**pH**		**Conductivity** (μS/cm)	
		Extraction Method		*Extraction Method*	
		Maceration	*Ultrasounds*	*Maceration*	*Ultrasounds*
Merlot	Ecological	4.079 ± 0.287	4.034 ± 0.309	1932 ± 306	1948 ± 392
	Traditional	3.462 ± 0.285	3.384 ± 0.272	1524 ± 166	1455 ± 132
Feteasca Neagra	Ecological	3.341 ± 0.252	3.170 ± 0.217	1935 ± 393	1981 ± 297
	Traditional	3.498 ± 0.290	3.240 ± 0.126	1636 ± 20	1612 ± 29
Pinot Noir	Ecological	3.306 ± 0.245	3.175 ± 0.173	1657 ± 225	1821 ± 213
	Traditional	3.592 ± 0.309	3.587 ± 0.293	1891 ± 183	1843 ± 121
Muscat Hamburg	Ecological	3.038 ± 0.219	2.823 ± 0.196	1810 ± 216	1916 ± 98
	Traditional	3.382 ± 0.362	3.358 ± 0.364	1928 ± 300	1765 ± 216

Total flavonoids content

Table 2 shows the total flavonoid contents expressed in mg Quercetin/mL extract. Comparison of the total flavonoid content values for the two types of extraction processes, maceration and ultrasonication indicates no significant differences. In the case of Merlot, it was noticed that ultrasonication was the process that favor extraction of higher quantities of flavonoids in water. The extract obtained from Feteasca Neagra grapes produced in organic vineyards shows the same trend indicating ultrasonication as the more efficient extraction procedure, while in the extracts obtained with Feteasca Neagra grapes variety from traditional vineyards, there are no differences between the two extraction procedures. The Pinot Noir and

Muscat Hamburg varieties extracts showed small differences in TFC and, in this situation, the procedure used to obtain the aqueous extract is not important when a higher content of flavonoids is desired.

Table 2. Values determined for total flavonoids content (mg/mL Quercetin).

Grape variety	**Vineyard type**	**Total Flavonoids Content (mg/mL) of grape skin water extracts**	
		Extraction Method	
		Maceration	*Ultrasounds*
Merlot	Ecological	0.253 ± 0.082	0.344 ± 0.082
	Traditional	0.440 ± 0.036	0.507 ± 0.039
Feteasca Neagra	Ecological	0.382 ± 0.089	0.467 ± 0.087
	Traditional	0.315 ± 0.062	0.288 ± 0.027
Pinot Noir	Ecological	0.317 ± 0.035	0.297 ± 0.028
	Traditional	0.109 ± 0.034	0.139 ± 0.074
Muscat Hamburg	Ecological	0.588 ± 0.079	0.549 ± 0.091
	Traditional	0.571 ± 0.075	0.533 ± 0.092

When compared the total flavonoids content of water extracts judging by their origin, traditional *versus* ecological vineyards, it was found that the grape skin extracts of Merlot variety had a lower content of flavonoids extracted in water for ecological grapes. Feteasca Neagra, Pinot Noir and Muscat Hamburg varieties showed a higher flavonoid content in grape extracts from organic vineyards. It is noticeable that the Pinot Noir extracts had TFC almost triple in ecological grapes compared to extract from grape skins from vineyards treated with pesticides.

4. CONCLUSIONS

In this study, comparison of total flavonoids contents, acidities and conductivities of some aqueous extracts obtained from four varieties of red grapes cultivated in Romania, in ecological and traditional vineyards, has been performed. Informations on the chemical structure of red grape skins were provided; a higher content in anthocyanins type of flavonoids in the grape skin was suggested as well.

It was found that extraction procedures applied, with and without use of the ultrasonication, does not significantly influence characteristics of obtained aqueous extracts. With regards to the vineyard types, it was found that Merlot type skins led to aqueous extracts with higher acidities and conductivity values when extract were prepared from grapes sampled from ecological vineyard, than water extracts prepared from grapes sampled from traditional cultures. When Muscat Hamburg variety grapes were used to prepare water extracts, those obtained from ecological vineyard had lower pH values than extracts from traditional cultures, treated according to regular procedures with various chemicals including pesticides. Grape varieties of Feteasca Neagra and Pinot Noir, showed similar pH values, however conductivity values showed different variations relative to the vineyard type where sampling was conducted. Another experimental finding was that Pinot Noir aqueous extracts prepared from ecological grapes skin had total flavonoids content almost triple than same extracts prepared from grapes skins sampled from traditional vineyards, treated with chemicals.

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