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Research on the content of trans-resveratrol in Romanian wines

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Abstract

Scientific activity in the field of oenology became more intense over time, being necessary regarding the quality of grapes and hence of wine. Recent proofs show that red wine has health benefits, and one in charge could be resveratrol. Moreover, there is evidence that resveratrol has chemo preventive activity against cardiovascular diseases and a variety of cancers. Given these, in this paper we intended to study the variation of resveratrol content in 8 samples of wines from different vineyards from Romania (Dealu Mare, Murfatlar respectively Recaş) which were differently packed in different years. Trans-resveratrol was quantified using a HPLC method, and it ranged from 0.728 to 14.131 mg L^{-1}. Eventually, Merlot variety had the highest concentrations of resveratrol of all wine samples.

Keywords: Trans-resveratrol, wine, HPLC

1. Introduction

Man started to drink wine 9000 years ago and medicine used it as a cure in the last 5000 years, making it the oldest human remedy [1]. Besides this, wine is also the best preventative medicine as shown by the Copenhagen study, being the first longitudinal study in progress to compare the death rates of abstainers and consumers of beer, strong drinks or wine. Previous studies have only compared abstainers to drinkers of alcohol with different rates of alcohol consumption. Copenhagen study showed a 50% reduction in the death rate of wine drinkers compared to abstainers. [2].

One of the topical research papers that discuss various antioxidants in wine and the major factors that influence their content in wine comes from the Czech Republic. J. Lachman J., [3] showed that long-term intake of red wine increased antioxidant activity in the body by 15% to 20%, compared to a control group. Thus, recent evidence shows that red wine has health benefits [4,5], and resveratrol in red wine might be responsible [6]. Resveratrol, a component of many plants, was probably first mentioned in Ayurveda almost 5000 years ago (called '*draksha*' which is fermented grape juice) as a cardio tonic. In addition to grapes, we now know resveratrol is also present in nuts, cranberries, currants, blackberries, jackfruit and other fruits and vegetables. [7]

Resveratrol was first identified in 1940 in white lily *Veratrum grandiflorum* [8], although the richest known natural source is the medicine from Asia the plant *Polygonum cuspidatum* (in Japanese '*Ko-jo-kon*'). The appearance of resveratrol was popularized in 1992 when it was discovered as a constituent of red wine, and engaged in the '*French paradox'* [7]. Therefore resveratrol helps to reduce vascular disease [9,10], dementia [11,12], cancer [13,14], insulin

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resistance in diabetes [15,16], as well as muscle degeneration [17] and other. In addition, it shows antioxidant activity [18-20] and anti-inflammatory activity [21-23].

In grapes, resveratrol is found only in the skin. The amount of resveratrol in grape skins varies depending on grape variety, geographical origin, winemaking technology and exposure to fungal infections. In red wine, trans-isomer concentration varies between 0.1 and 15 mg L^{-1}. The level of resveratrol and of cis – and trans – resveratrol ratio in wine vary by climate region, type of grape and the length of time the skin is kept in contact with the grapes during the winemaking [24].

The pharmacological activity of resveratrol has also stimulated the development of many chemical analytical methods to measure it in different matrices, such as plant extracts, wine and other beverages, as well as in derivate food products and biological fluids and tissues [7]. Thus in the last decade several methods have been developed to detect the presence and measure the levels of resveratrol, most often being used high performance liquid chromatography (HPLC) [25-27] and gas chromatography (GC) [28,29] Much attention has been focused on developing methods because resveratrol study required analysis of complex mixtures containing very small amounts of the analyte, and complete and rapid extractions were necessary to minimize loss due to isomerization or distortion. In general, the HPLC methods using C18 reverse-phase column coupled with UV detection were often used to adequately distinguish the isomers of resveratrol on the basis of the various maximum absorbance.[7] The objective of this study was the determination of resveratrol in wines purchased from three vineyards from Romania.

2. Material and methods

Sample description.Given that red wine is known to have a higher amount of resveratrol due to specific obtaining technology there were analyzed two types of wine from each winery, from red grape varieties as follows: Merlot and Cabernet Saugvinion. Designation of origin Dealu Mare, Murfatlar, respectively Recaș was attributed to wines made from grapes produced in the areas defined for this name.

Wines were purchased from the same harvest year at each winery, but from different years regarding a winery to another, as is depicted in Table 1.

The studied wines come from the following vineyards: Dealu mare – Buzău, Murfatlar and Recaș. These were purchased from Recaş showrooms and Carrefour supermarket from Sibiu in the case of Dealu Great and Murfatlar.

Wines purchased in bulk were from the same harvest year (2013) within each winery, but from different years (2011, 2012 and 2013) in terms of bottled wine.

Table 1. Studied wine characteristics

Vineyard	Variety	Packaging	Closing year	Code
Dealu Mare - Buzău	Merlot	Bottled	2011	DMM
	Cabernet Saugvinion			DMCS
Murfatlar	Merlot		2012	MM
	Cabernet Saugvinion			MCS
Recaș	Merlot		2013	RM
	Cabernet Saugvinion			RCS
	Merlot	Bulk		RMV
	Cabernet Saugvinion			RCSV

Chemicals and standards. Methanol and ultra-pure water of HPLC grade were obtained from Sigma Aldrich. Trans-resveratrol standard was also obtained from Sigma-Aldrich, having a concentration of > 96%.

Stock solution (0.02 mg mL^{-1}) was prepared by dissolving 10, 0 mg of trans-resveratrol WRS in 20 mL of methanol and sonication for 5 min then diluted to 25, 0 mL with the same solvent. The solution was stored in dark bottles at 4^0 C.

*Chromatographic procedure***.** Identification and assay of trans-resveratrol was carried out using high performance liquid chromatography (HPLC). This was performed according to an adapted method of the HPLC method of resveratrol assessment from Pharmacopoeia [30].

Samples of wine, previously homogenized, were passed through a filter Chromafil (Macherey-NAGEL) of 0.45 μm thickness and then were transferred to vials and positioned according to the sequence in the auto sampler. A volume of 7.5 ml of each sample and standard (the latter with a concentration of 0.16, 0.32 and 0.64 mg mL^{-1} respectively) were injected directly into the column. Thus, the calibration curve was plotted with the three standards, and by interpolation resveratrol concentrations of the samples were determined.

All measurements were performed three times (n = 3), and subsequently the mean and standard deviation were calculated.

Chromatographic parameters. The HPLC system (series KNAUER Smart line, Germany) used for the assay of trans-resveratrol was combined with a quaternary pump (Smart line 1000 Knauer), a column thermostat (Smart line 4050 Knauer), a UV detector (Smart line 2550 Knauer) and an auto sampler (Smart line 3950 Knauer). Trans-resveratrol was detected using a MACHEREY-NAGEL C18, 250 mm x 4 mm, 5 µm column, at a temperature of 25°C with a mobile phase consisting of methanol and ultrapure water in the ratio of 40: 60 (v / v) at a flow rate of 1.5 mL min^{-1}. UV detection was made at a wavelength of 303 nm in a time range up to 15-20 minutes. (Figure 1). Trans-resveratrol was identified by comparing the retention times with commercial standards. Chromatograms were analyzed by ClarityChrom software (version 5.0).

Statistical analysis. The concentrations of trans-resveratrol were measured and analyzed using one-way ANOVA. Values (n = 3) were reported as mean ± standard deviation. Moreover, ANOVA test was used to determine significant differences ($p < 0.05$) between variations using IBM SPSS statistical package version 21.

3. Results and discussion

Figure 1 shows the variation of resveratrol concentration in the studied wines. In the case of vineyards it cannot be seen any significant differences ($p > 0.05$) regarding the concentrations of resveratrol among them. The most notable variations in the concentrations was recorded in the Recaş vineyard and lowest in the Murfatlar vineyard.

In the matter of varieties there cannot be detected any significant differences ($p > 0.05$). Larger variations of resveratrol content was recorded in the case of Merlot variety and therefore smaller in the case of Cabernet Saugvinion variety. Grape variety affects at certain extent the concentration of resveratrol but not significantly. In general, it can be seen that trans-resveratrol in red wines varies between 0.728 - 14.131 mg L^{-1}. The most important content of resveratrol has been identified in the Recaş vineyard of 14.13 mg L^{-1}, Merlot variety, bottled in 2013. The lowest value of 0.72 mg L^{-1} was found also in the Recaş vineyard but in the Cabernet Sauvignon variety, bulk packaging, and year of closure 2013. Resveratrol content determined in analyzed Romanian wines do not significantly differ from the limits described by other researchers. Thus the concentration of resveratrol in red wine was similar to that reported [31] of 0.2 to 7.7 mg L^{-1}, [32] of 0.5 to 8 mg L^{-1} and [24] of 1 to 15 mg L^{-1} . Lower results than those obtained in this work have been reported in the Italian red wines of 0.56 to 2.86 mg L^{-1} [33] and Goldberg, Karumanchiri [34] reported trans-resveratrol levels ranging from 0.30 to 4.68 mg L^{-1} in commercial wines. Also, Padilla, Ruiz [35] found concentrations of trans-resveratrol in Spanish wines between 0.06 and 4 mg L^{-1} and Gambuti, Strollo [36] reported levels of trans-resveratrol between 2.1 and 2.5 mg L^{-1}.

Differences in concentration due to winemaking methods as a result of prolonged contact with grape skins during fermentation in the production of red wines [37,38] However resveratrol difference in content of the bulk and bottled wines is because the latter have a higher quality with denomination of controlled origin in comparison with bulk wines. Also the fluctuation in the content of resveratrol in wine is due to the quality of raw materials and production technology which supports resveratrol extraction from skin and its passage into wine and release from its glyosidic precursors during technological operations. The process can be conducted in such way in order to increase the level of resveratrol, and antioxidants in wine in general.

In this sense, one can follow to obtain high quality raw grapes, to harvest when optimal time, to review time and conditions for the maceration of red wines and to have a better knowledge of chemical reactions during malolactic fermentation regarding the antioxidant compounds. Trans-resveratrol appears after degradation due to enzymatic activity of β-glycosidase [39], so the must of which the RM wine with the highest concentration of trans-resveratrol was obtained of, was subjected to long maceration which favored an intense extraction of phenolic compounds.Given oenological practices, winemaking conditions seem to exert a significant influence on trans-resveratrol content [40].

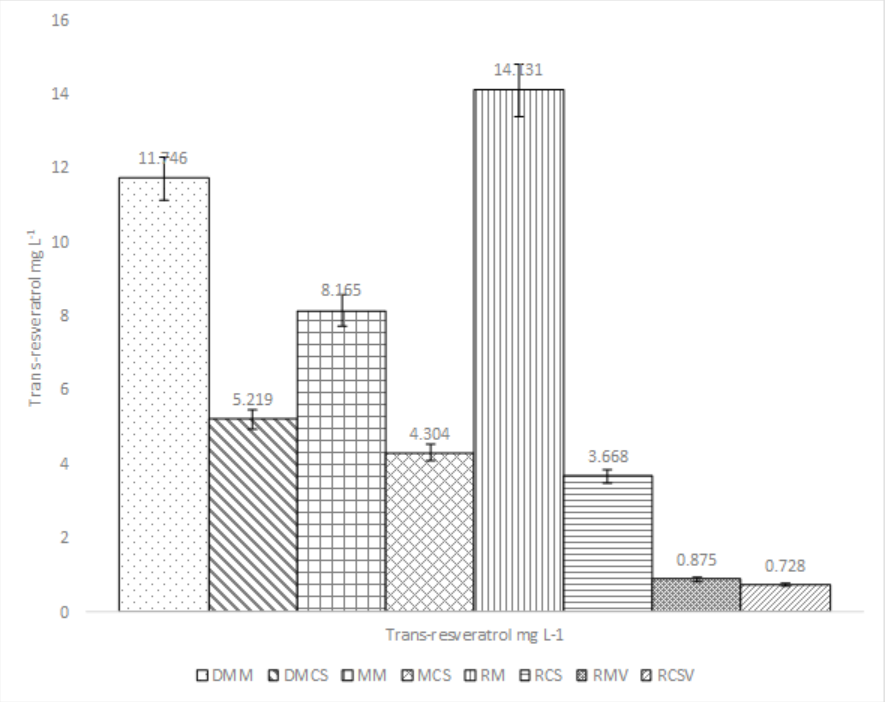

Figure 1. Variation of resveratrol concentration in studied wines, determined by HPLC

4.Conclusions

This research is intended to serve as a point of inspiration for further research and knowledge of resveratrol's development in time in different types of wine, for documenting oenologists and consumer information on the potential benefits of wine.

At this point, it is hard to say what dose is required for effective prevention of cancer in humans, certainly depends on many factors such as genetics and physical structure of each person, diet, lifestyle and environment. But it is safe to say that grapes and berries containing resveratrol have many health benefits.

Romania is among the European countries with the oldest viticulture having exceptional natural conditions favorable to achieve almost all types of wine, also being recognized abroad.

Globalization has influenced even the wine market, the number of states that contribute to the supply and demand in the wine market is growing so international trade provides opportunities for Romanian wine.

Romanian wine may only face international competition in quality. Therefore, the viticulture in Romania should benefit from a very competitive scientific and technical guidance.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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