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Determination of *Trans*-Resveratrol Levels in Different Fruits, Vegetables and Their Skin by HPLC

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The aim of this study is to identify and compare the amount of resveratrol in various fruits, vegetables and their skin or shell. Resveratrol levels in samples were measured using HPLC. Some of the samples did not show a presence of resveratrol while a significant amount was detected in the remainder of samples. The analysis results showed that the resveratrol levels were high in peanut (*Arachis hypogaea*) shell (39.1 µg/g), black mulberry fruit (*Morus nigra*) (32.5 µg/g), red grape (*Vitis vinifera*) seed (28.0 µg/g), cucumber (*Cucumis sativus*) skin (18.0 µg/g), and cherry laurel fruit (1.50 µg/g). Results indicate that some fruits and vegetables tested here could be consumed as a resveratrol source.

Key Words: *Trans*-resveratrol, Different fruits, Vegetables, Their skin, HPLC.

INTRODUCTION

Resveratrol is a phytoalexin produced naturally by several plants when they come under siege by bacteria or fungi. Phytoalexins are antibacterial and antifungal chemicals produced by different plants for protection against infection by pathogens. Resveratrol is found in the skin of red grapes and so is a constituent of red wine[1,2].

Due to its antifungal quality, resveratrol is considered an important substance for preventing mould infections in grapes. Fermented products contain a considerable amount of phenolic compounds. When grape is fermented, it is dissolved in alcohol and turns into wine. Thus, it is known that resveratrol is found in fruits, especially in the grape skin, but it is present only in low levels of concentration in the pulp, if ever present. This means that resistance in grapes against moulding takes place particularly in the skin[3-5]. There is only a small amount of resveratrol in grape juices that have not been fermented[6].

A number of beneficial health effects of resveratrol, such as anticancer, antiviral, neuroprotection, antiaging and antiinflammatory, have been reported, but all relevant studies have been carried out *in vitro* or in yeast, worms, fruit flies, fish, mice and rats. Because of its potentially strong antioxidant properties, the effects of this compound on human health have been studied more extensively. To this end, such properties as antithrombosis, heart-friendly, vascular relaxant, antiinflammatory and anticancer have already been studied[7-10]. Another quality of resveratrolis the protection of the kidney by modifying the NO metabolism in the kidney cells. Resveratrol slows down the development of neoplasm by reducing the prostaglandin synthesis[11].

It has previously been shown that the spasms caused by KCl in the rats' aorta with a healthy endothelium were relieved as a result of a targeted relief-effect produced by the grape extracts. The particular impact produced by resveratrol on the cardio-vascular system has been investigated. Similar research has been carried out on endothelium cells in the human umbilical cord and uterine arteries of the female guinea pigs[12]. Resveratrol also functions as a powerful agent in resolving thrombosis by suppressing the generation of tissue factors[13-15]. It inhibits the MAP-Kinaz in coronary arteries of pigs and plays an effective role in the heart and vascular diseases by reducing the amount of endothelium-1 present therein[6,16,17]. It was shown that resveratrol prevents cell reproduction by holding in check the mRNA and cyclin A gene that causes tumour in the oxen and rats. By increasing the level of fibrinolytic proteins in the umbilical and endothelium cells, resveratrol has proved to have beneficial and protective qualities against coronary hearth disease[18]. By injecting resveratrol before ischemia, the ischemia-reperfusion damage was averted following an increase in the adenosine release in a rat's heart[19].

Resveratrol also demonstrates a peroxide radical scavenging quality, *i.e.* an antioxidantal effect, by reducing the malondialdehyde levels, which tend to increase as a result

of ischemia[20,21]. It helps to reduce DNA refraction as a potent hydroxyl radical scavenger[22], and it is also effective in healing wounds by interacting with the hydrogen peroxide radicals and speeding up the development of capillary vessels[23].

Resveratrol is similarly effective in anticancer treatment as it inhibits tyrosine kinase reactions[24,25]. Resveratrol inhibits the cycloxigenite-1 (COX-1) enzyme and checks the spreading of tumour in the early stages of cancer development[2]. This has been demonstrated in experiments with mice and rabbits[26-30].

As a result of using resveratrol in glomerulonephritis, it was similarly observed that protein urine was reduced, serum albumin level was increased, and the hyperlipidemia was repressed[31]. Medicine containing *trans*-resveratrol is used for the treatment of osteoarthritis, as well as for cyclo-oxigenite-2 enzyme inhibition, for the care of blood vessels, for relieving the symptoms of menopause[32] and in the fight against cardio-vascular illnesses and cancer[33,34].

To date,the measurement of *trans*-resveratrol levels has been the main object of most studies. These studies were performed mainly in grapes and in a variety of wines produced from them, in some fruit juices and, to a lesser extent and in other foodstuff. Hence, in order to see whether this powerful antioxidant could be taken in sufficient amounts naturally and without fermentation, the resveratrol levels were determined by HPLC technique in a range of fruits, vegetables and their skin.

EXPERIMENTAL

Resveratrol levels were measured by HPLC in a variety of fruit and vegetable, especially in grape. Twenty five different types of fruit and vegetables were sampled, as well as grape molasses of the Zile vineyards.

We used a Perkin-Elmer HPLC, C_{18}. 100 New column (250 × 4.6 mm) LiChrosper® 100 RP-18 for colon and a UV detector. Because the colon and the detector were different than the ones used by Keller and his team[35], the programme duration was set to 15 min and as a result of the fact that the resveratrol peak was noticed within the first 5 min of our experiment. Finally, we set the wavelength to 330 nm for the measurement.

Resveratrol extraction: The modified method of Keller *et al.*[35] was used to measure the resveratrol levels by HPLC. 1 g of fruit, vegetable and the skin of some of these fruits and vegetables were weighed on a scale before the same amount was added into 10 mL sample of acetone of -20 ºC. The same sample was homogenized with a mechanic homogenizer and put to the mixer for 40 min. The mix was then centrifuged for 10 min at 3000 xg. The suspension on the tube was transferred into another tube and, in order to fully extract the resveratrol likely to remain in the skin fragments inside the tube, 5 mL methanol and 5 mL acetone was added to it and this was mixed for a further 40 min before it was centrifuged at 3000 xg for 10 min and the phase on top was poured into the earlier tube. The tube was now ready for a solvent removal exercise, which was achieved with an evaporator at 50 ºC. The evaporated extract was filtered through with an insulin injector by using a 0.45 µm filter and thus made ready for the HPLC analysis.

RESULTS AND DISCUSSION

The samples used in present research and the resveratrol values obtained from them are shown in Table-1.

TABLE-1
AMOUNT OF RESVERATROL IN DIFFERENT FRUITS AND VEGETABLES (MEASURED BY HPLC)

Samples	Measured the amount of resveratrol (µg/g)	Measured the amount of resveratrol in literature
Apple*Malus pumila*	0.00	*
Black mulberry *Morus nigra*	32.5	*
Blackberry *Rubus caesius*	0.00	
Blueberries *Vaccinum myrtillus*	0.00	1.07 nmol/g (Wang, 2002) (0.24 µg/g)
Cherry crust *Prunus laurocerasus*	0.25×10^{-4}	
Cherry seed *Prunus laurocerasus*	1.50	
Cucumber crust *Cucumis sativus*	18.0	
Inner crust peanuts *Arachis hypogaea*	0.00	2.17-5.31 µg/g (Ku, 2005)
Inner shell of pistachio, *Pistacia vera*	0.00	0.09-1.67 µg/g (Tokusoglu, 2004)
Leek *Allium porrum*	0.00	
Outer crust pistachio,*Pistacia vera*	4.00	0.09-1.67 µg/g (Tokusoglu, 2004)
Peanut shell *Arachis hypogaea*	91.0	0.02-1.79 µg/g (Potter, 2008)
Pear peel *Pyrus communis*	0.00	
Pomegranate crust *Punica granatum*	0.00	
Pomegranatemembrane *Punica granatum*	0.00	
Pomegranate seeds *Punica granatum*	0.00	
Potato crust *Solanum tuberosum*	0.25×10^{-4}	
Quince crust *Cydonia oblonga*	0.50	
Raspberry *Rubus idaeus*	0.35×10^{-6}	
Red grape seed *Vitis vinifera*	28.0	1.56-1042 nmol/g (Wang, 2002) (0.35-237.8 µg/g)
Red grape skin *Vitis vinifera*	0.50	
Strawberry *Fragaria vesca*	0.00	*
Tomato peel *Solanum lycopersicum*	0.00	*
White grape peel *Vitis vinifera*	0.00	1.56-1042 nmol/g (Wang, 2002) (0.35-237.8 µg/g)
White grape seed *Vitis vinifera*	0.00	
Zile molasses	0.25	

Scientists around the world have turned their attention to the study of coronary- and heart- disease as a consequence of the death rates rising relating to the illness[36].

A study conducted in Brazil in the year 1996 has concluded that the consumption of red wine in high-fat diets helped to prevent coronary heart diseases[37]. This finding has played an important role in directing the efforts of the researchers to the study of the wine contents. It is known that wine contains many substances, a high proportion those substances of which comes from the grape itself. A number of studies support the thesis that the substance, which is believed to play an effective role

in preventing the coronary heart disease belongs to the phenolic compounds with a flavonoid structure. It is therefore believed that the main impact on the heart defects and coronary malfunctions is derived from the phenolic compounds in wine.

Red wine's richness in phenolic compounds is due to the fact that the grape skin is added to the grape juice fermented throughout the wine production. Grape contains numerous flavonoid substances. The studies have shown that its protective quality is mostly derived from the resveratrol, a polyphenol with a "3,5,4'-trihydroxystilbene" structure. It is found especially in the *Vitis vinifera* genus of red grapes, resveratrol is a phytoalexin developed against mould infections in vineyards[1,2].

Scientists have reported different measurement values of *trans*-resveratrol in studying wines made from the red and white grapes alike. These differences in levels of *trans*-resveratrol could be the result of a number of factors including climate, the quality of grapes, conditions of growth, production and storage techniques, ageing and other factors related to vineyards[38-40].

The resveratrol concentration levels were compared in studies of the muscadet grape (*Vitis rotundifolia*), fruit juices, the apple puree, seeds and wine. More highly concentrated levels of resveratrol were discovered in the black muscadet grapes, apple puree and seeds than other muscadet grapes, apple puree and seeds. The resveratrol levels were also studied in 72 different plant categories including blueberries[2,41] but the findings were not pursued any further because of insignificant resveratrol levels detected in them. Wang *et al*.[42] measured the total amount of resveratrol in grape juice and blueberry juice by the MS method. They have found 1.56-1042 nmol/g in concord type of grape and 1.07 nmol/g in blueberry juice. The amount of resveratrol detected in peanut was higher than that of Wang *et al*. found in grapes when the same figures are taken as criteria.

We measured the resveratrol levels by HPLC in 26 different samples. We determined resveratrol levels of varying µg scale in quince skin, black mulberry, raspberry, potato skin, cucumber skin, red grape skin, pistachio shell, peanut shell, black fig pulp and seed and in Zile grape molasses. High amounts of resveratrol was detected in peanut shell (1.82 ppm), in cucumber skin (0.36 ppm) and in black mulberry (0.65 ppm).

Most studies conducted so far have used grape wine for the same research objectives and there is a shortage of research using other fruit-and-vegetable samples. Wang *et al.*[42] could observe only 1.56-1042 nmol/g resveratrol in grape in 2002. By contrast, high levels of the same substance (28.00 µg/g) has been noted for the red grape seed. We have not paid attention to the unfermented white grape skin due to the very low level resveratrol found in it. Whereas the resveratrol levels have increased with the fermentation of grape skins, no increase could be reported for the non-skin fermentations[43].

We also used pistachio shell in our experiment, which yielded 4.00 µg/g of resveratrol. The *trans*-resveratrol levels were also measured by HPLC method in a study of 15 different peanut and peanut oils grown in Korea. For the peanut, *trans*-resveratrol measurements varied between 0.09 and 0.26 µg/g and for the peanut oil, between 0.27 and 0.70 µg/g. The difference in resveratrol levels in peanuts could be explained by the fungal attacks as well as the biotic or a-biotic factors such as mechanical damages observed in low quality peanuts[44]. Ku *et al*.[45] have found 2.17-5.31 µg/g and 0.02-0.79 µg/g resveratrol in peanuts, respectively. The amount of the same substance that we have obtained a figure of 39.1 µg/g in our study.

In this study, high amounts of resveratrol have been detected not only in the outer shell of peanuts, but also in the black mulberry and cucumber skin. It contains high amounts of resveratrol, black mulberry can be consumed as a natural antioxidant. Grape molasses is another product prepared from grapes and it requires high temperatures for its preparation. In this case, the decrease in the level of concentration of resveratrol can be attributed to the temperature creating an impact on the structure of resveratrol.

Variations in the amount of resveratrol found in the same fruits and vegetables could also be the result of different methods used in the experiments. Different conclusions of the studies concerning the determination of *trans*-resveratrol levels are a consequence of a variety of factors that include the diversity of methods employed, the type of samples and whether these samples were subjected to different time frames during experimentation, differences in the environmental factors such as climatic conditions, the soil structure, the water pressure and availability, regional differences, conditions of growth and the diversity of production and storage techniques[38,39,46-49]. Scientists have so far studied resveratrol levels mostly in wine, and in the edible fruits to some extent. The interest in the inedible or discarded parts of fruits and vegetables has not been equally high. The main purpose of present study has therefore been to determine the amount of resveratrol in the unused parts of fruits and vegetables as well and to suggest ways in which people could have resveratrol naturally.

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