Research Paper

Total Resveratrol concentrations in some Syrian grape varieties

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ABSTRACT

Total resveratrol concentration in dried seeds, fresh seedless grape, and seedless raisins of some Syrian grape varieties was determined by high-performance liquid chromatography (HPLC) method, using DAD detector. Results showed that, resveratrol concentration in seedless raisins samples were higher than those found in fresh seedless grape samples. Resveratrol concentration in seedless raisins samples ranged between 1.23-8.2mg/100g. The highest concentration, (8.2mg/100g seedless raisin), was in Baladi variety and the lowest concentration, (1.23mg/100g seedless raisins), was in Roumi variety. In fresh seedless grape samples, the concentration of resveratrol ranged between 0.48-3.45mg/100g. The highest concentration, (3.45 mg/100g fresh seedless grape), was in Baladi variety and the lowest concentration, (0.48 mg/100g fresh seedless grape), was in Roumi variety. In dried seeds samples, resveratrol concentration range was 0.43-1.94mg/100g, where the highest level was in Baladi variety, and the lowest in Roumi variety. Significant differences in resveratrol concentration were found in dried and fresh fruits and in seeds among the different varieties as determined by LSD statistical test at *P* value 0.05.

Keywords: Resveratrol; Grape; HPLC.

INTRODUCTION

Grape (*Vitis vinifera*) Family-Vitaceae, is one of the world's largest fruit crop with an annual production of more than 60 million metric tons (Schieber, et al., 2001), 80% of this amount is used in wine production (FAOSTAT, 2007). The most studied group of grape phytochemicals are polyphenols, a group of secondary metabolites with different chemical structures functions, which are produced during physiological plant growth and/or as a response to various forms of environmental stress (Naczk and Shahidi, 2004).

Grape polyphenols are mainly flavonoids, stilbenses and phenolic acids, all well known for their strong biological actions (Monagas, et al., 2005). Among them, the non-coloured polyphenols which have attracted considerable research attention due to their ease of application by pharmaceutical, cosmetic and food industries. The most studied compound of non-coloured polyphenols is *trans*-resveratrol which is known for its strong biological effects *in vitro and in vivo* (Jang, et al., 1997; King, et al., 2006; Nakagawa, et al., 2001; Russo, et al., 2003).

Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is a natural phenolic compound, which belongs to phytoalexins synthesized in response to injury, ultraviolet irradiation, or fungal attack (Langcake and Pryce, 1976). This compound was detected in more than 70 plant species and was found in discrete amounts in wines and various human foods. Grapes and its products are considered as the most important dietary sources of resveratrol (Goldberg, 1995; Mattivi, et al., 1995).

Resveratrol is well known as anti-inflammatory (Chanvitayapongs, et al., 1997), antimutagenic agent (Langová, et al., 2005), and has a positive effect on the immune system (Fremont, 2000; Nakagawa, et al., 2001).

Many authors have described the effect of resveratrol in the prevention of cardiovascular diseases (so-called French paradox). Resveratrol modulates lipid metabolism and eicosanoid synthesis (Kimura, et al., 1983). It also blocks platelet aggregation (Bertelli, et al., 1995; Frankel, et al., 1993; Pace-Asciak, et al., 1995). It also has a vasodilatation activity (Fremont, 2000; Gusman, et al., 2001) with a marked dilation of retinal arterioles (Nagaoka, et al., 2007).

Numerous lines of evidence suggest that resveratrol also exhibits an antioxidative activity by inhibiting the oxidation of LDL and LDL peroxidative degradation (Aggarwal, et al., 2004; Kimura, et al., 1985).

Resveratrol was described as an anti-carcinogenic agent. *In vitro* studies have demonstrated that resveratrol has the ability to halt carcinogenesis at the initiation, promotion and progression stages and that it has great potential in the prevention and therapy of many tumors (Aggarwal, et al., 2004; Jang, et al., 1997).

Resveratrol can also enhances stress resistance and extend the lifespan of various organisms from yeast (Howitz, et al., 2003) to vertebrates (Valenzano, et al., 2006).

First International Conference on resveratrol and health was held in September of 2010 in Helsingor, Denmark. The purpose of that conference was to assess the current knowledge about resveratrol and to conclude recommendations for its human use and to direct the future studies about it (Smoliga, et al., 2012).

Syria is one of the countries that cultivate grape. The estimated cultured area is 46,000 hectare with an annual production of about 350,000 tons of grape fruit (Anonymous, 2011-2012 data). Since there is no data on resveratrol concentration in grape varieties cultivated in Syria, this study is the first to reports resveratrol concentration in dried seeds, fresh grape skin and pulp, and dried grape skin and pulp of four locally cultivated varieties.

MATERIALS AND METHODS

Sampling: Fruits samples of four Syrian grapes varieties: Hilwani, Zaini, Baladi, and Roumi, were collected in harvest season, September 2012, from Research Station of The Arab Centre for Studies of Arid Zones and Dry lands, located in south region of Syria. In addition, a seedless raisin samples included: Syrian, Iranian and American products were collected from Damascus market.

Sample preparation: The seeds from the fresh grape samples were manually separated from pulp, then the seeds and the seedless grape were dried on filter paper by oven at 60°C until constant weight.

Extraction: The samples were extracted according to Lacopini et al., (2008) with some modification. Briefly, for seeds samples, grind 10g of samples by laboratory grinder and weigh 2g of samples into 100ml flask, then add 25ml of mixture methanol: water: hydrochloric acid 0.12M (70:29:1 v/v/v) and extract the samples by using high speed homogenizer for 5 minutes, transfer the mixture to polypropylene

centrifuge tube, and centrifuged the sample at 3000 rpm for 10 minutes. The extract was collected in a flask and the precipitate was re-extracted using the same above procedure, to make up a final volume of 50ml. For the seedless grape samples, 25g of sample were extracted with 100ml according to the above procedure and the final extract volume was 200ml. All samples were extracted in triplicate.

Chemicals: Standard material of resveratrol (99%) was purchased from Sigma Chemical Co., and HPLC grade of Acetic acid, Methanol and water were obtained from Merck (Darmstadt, Germany).

Standards solution and calibration curve: A stock solution of 10mg/ml resveratrol was prepared in a Methanol. Immediately prior to analysis, a set of standards with 50, 70, 100 and 200μg/ml were prepared from the stock solution in a mixture of acetonitrile: methanol (1:1). Special care was taken in relation to the degradation of the standard solutions, keeping them protected from air and light exposure. The calibration curve was obtained by plotting four concentration (50, 70, 100, 200μg/ml) against peak area.

HPLC-DAD system for analysis of Resveratrol: The HPLC system used consisted of Agilent (Infinity 1260) series, equipped with a diode array detector (DAD), the column used was Eclipse RP- C_{18}, (150×4.6mm i.d.; 3.5μm), the column temperature was 35°C. The mobile phase consisted of Acetic acid: Water (60: 40), flow rate 0.8ml/min, the injection volume was 10μl, and UV detection at 306nm. The regression equations were obtained from calibration curve of resveratrol standard and used for the calculation of resveratrol quantity in samples.

Statistical Analysis: Sampling consisted on independent triplicates (n=3) for each variety and analytical parameter. Data were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used to assess the significance of differences among varieties. Multiple comparison using the least significant difference LSD test at *P* values less than 0.05 (SPSS, 17) was also applied. Microsoft Excel program was used to generate statistical histograms.

RESULTS

HPLC results of resveratrol concentrations in the acidic methanolic extracts of the four grape varieties are shown in table 1. Typical HPLC chromatograms of chemical standards and dried seeds, dried seedless grape are shown in Figure1. Retention times of A and B, C were 2.447-2.457min and 2.444min, 2.449min, respectively. In the range of 50–200μg/ml, good correlation of linearity has been achieved (n=3; r^2 = 0.99998).

Since many factors largely affects the determination of the phenolic content of samples, as described by other authors (Downey, et al., 2007), we tried to minimize these factors by collecting samples from plants at the same ripening stage and grown in the same field. Extraction procedures using acidic methanolic solvent and results should be read in relation to this important aspect.

Results showed that the total resveratrol content in fresh seedless grape ranged between 3.45 and 0.48mg/100g, where Baladi variety showed the highest value, and the Roumi variety showed the lowest one 0.48mg/100g. Resveratrol concentration measured in seedless raisins samples was higher than those determined in the fresh seedless grape samples. Resveratrol concentration in seedless raisins samples ranged between 1.21-8.2mg/100g. The highest concentration (8.2mg/100g), was found in the Baladi variety and the lowest concentration (1.23mg/100g), was in the Roumi variety. In dried seeds, resveratrol concentration ranged between 0.43-1.94mg/100g, where the highest value was also in the Baladi variety and the lowest was higher in the

Roumi variety. In commercial raisin varieties, resveratrol concentration was higher in the Iranian raisin followed by the Syrian raisin then the American raisin. The average concentration of resveratrol in those commercial raisins was 6.32±0.4 and 4.29 ±0.4, and 3.67±0.4mg/100g, respectively.

DISCUSSION

In all grape varieties and samples of commercial raisin grapes analyzed total resveratrol concentration was quite variable. Total resveratrol in seed extracts were lower than that in fresh seedless grape and raisin extracts. Analysis of variance using LSD test showed significant differences ($P<0.05$) in resveratrol concentration among dry and fresh seeds grapes and among seeds of different studied varieties. These differences are most likely due to the kind of variety, grape maturation stage, and ecological influences. A study by Melzoch et al., (2001) found that resveratrol content was largely dependent on grape variety. Lacopini et al., (2008) reported that resveratrol levels in some grape varieties' skin and seeds were extremely high and attributed that to kind of verity. Another study by Sun, et al., (2006), hypothesized that verity genotype largely affects its polyphenol content; and that stilbenes content in general is largely dependent on grape variety. On the other hand, Revilla and Ryan, (2000), found that variability results from differences in response to mold infections and physiological stresses. Those authors found that if such stresses are not found, the levels of stilbenes in grapes will remain low.

CONCLUSION

This study reports resveratrol concentration in four local grape varieties cultivated on large scale in Syria, namely: Hilwani, Zaini, Baladi, and Roumi. This concentration was within the range of resveratrol concentrations reported by others for different grape varieties.

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Table-1: Resveratrol content in grape varieties studied (mg/100g).

Varieties	fresh seedless grape	seedless raisin	dried seeds
Zaini	0.89 ±0.08	2.33±0.06	0.645±0.02
Hilwani	1.3±0.05	3.67±0.03	1.248±0.02
Baladi	3.45±0.02	8.2±0.02	1.94±0.03
Roumi	0.48±0.02	1.21±0.08	0.43±0.01

- Values are the means ± SD (n = 3)
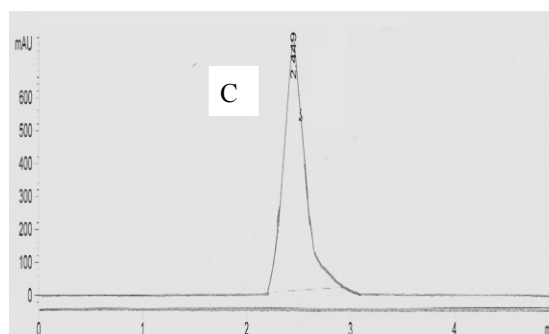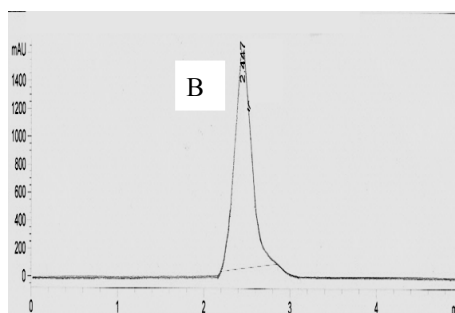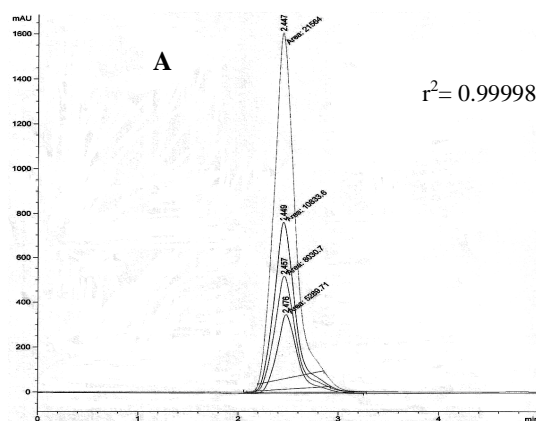
Retention time (min)

Figure-1: Typical HPLC chromatograms of chemical standards and samples.
- **A**: chemical standards; **B**: Chromatogram of resveratrol in the dried seed (Baladi variety); **C**: dried seedless grape (Baladi variety) (t_R = 2.447; 2.449 min).