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Short communication

Study of polyphenols in grape berries by reversed-phase high-performance liquid chromatography

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Abstract

Several polyphenols have been tested in grape berries from Spain. The flavonoid content is important because of the pharmacological properties of these compounds, whereas resveratrol has been proved to be an antifungal, antiinflammatory and an anticarcinogenic compound. A reversed-phase HPLC method has been developed and applied to determine resveratrol, quercetine, quercitrine and rutine content in several grape berries samples in a single analysis. Covering the grapes with a preservative paper yields a healthier product, but one which has a lower polyphenol content than unprotected grapes.

Keywords: Fruits; Food analysis; Polyphenols; Flavonoids

1. Introduction

Grape seeds are an important source of complex phenols. Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenol with two isomers. It is widely distributed in Gymnosperm and Dicotiledoneae [1]. The presence of resveratrol in grapes originates from a response to fungal infections, particularly of *Botrytis cinerea*, and enviromental stress. *trans*-Resveratrol was first isolated from *Vitisvinifera* [2], and later from those wines whose musts had been fermented together with the grape peel [3,4]. *cis*-Resveratrol was not detected in grapes, but its presence has been demonstrated in wine [5]. Resveratrol possesses antibacterial and antifungal activities and induces platelet hypoaggregation in rats; it protects liver from the lipidic peroxidation and inhibits the oxidation of low density lipoproteins (LDLs), modulating its metabolism, More recently, its carcinopreventive activity has been proved [6–9].

There exist commercial extracts of grape seeds with a high polyphenolic content; it was demonstrated that polyphenols are absorbed in humans and significantly decrease plasma cholesterol levels in higher cholesterol populations, and decrease LDL levels in all subjects.

Flavonoids are benzo-γ-pyrone derivatives which are ubiquitous in photosynthesizing cells. They have been used for years in folk medicine to treat human diseases such as inflammation, allergy, headache, parodentosis, virus and fungical infections, stomach or duodenal ulcers and even cancer [10]. Flavonoids are potent antioxidants that are present in plants and so in human diet; this fact is related with a reduced heart disease mortality.

In this work, we present a reversed-phase HPLC method that allows, in a single analysis, the de-

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termination and quantification of five polyphenols in grape berries: *cis*- and *trans*-resveratrol, quercetine, quercitrine and rutine.

2. Material and methods

2.1. Reference substances

trans-Resveratrol, rutine, quercetine and quercitrine were purchased from Sigma (St. Louis, MO, USA). *cis*-Resveratrol was obtained after *trans*-resveratrol isomerization at 360 nm for 24 h.

2.2. HPLC system

Varian modular chromatograph series 2510 with a Varian 9065 polychrom diode-array detection system. Experimental conditions: pump A, acetonitrile; pump B, water–acetic acid–acetonitrile (87:3:10) in a linear gradient elution as follows: 0 min: 5% A, 95% B; 25 min: 25% A, 75% B; flow-rate: 1.0 ml/min; detection at 306 nm; injection volume: 10 μl; column: Hypersil ODS 150×4.6 mm, 5 μm (Technochroma, Spain). Column oven: 40°C. t_0 was calculated by three successive injections of a KNO_3 solution in 100% MeOH, resulting 1.05 min. All solvents were of HPLC-grade and were filtered and degassed before their use.

2.3. Calibration graphs

Several aliquots of the solutions of every standard were diluted in the eluent to obtain reference solutions of decreasing concentration. These solutions were analyzed and the corresponding peak areas were plotted against the concentration of polyphenols injected. The concentrations of the components were calculated from the chromatogram peak areas using the normalization method. The identification of the different compounds was achieved by comparison of both t_R and the absorption spectra obtained for each eluted peak with those obtained for the standards.

2.4. Evaluation of the peak purity

To check the peak purity, the eluates were monitored with a photodiode array detector ($\lambda = 190–390$ nm). The three spectra corresponding to the upslope, apex and downslope of each peak were computer normalized and superimposed. Peaks were considered pure when there was exact coincidence between the three spectra (match factor ≥ 99.5).

2.5. Linearity, precision and accuracy

The linearity of the detector responses for the prepared standards was assesed by means of a linear regression analysis regarding the amounts of each standard (measured in mg) introduced in the loop of the chromatographic system and the area of the corresponding peak on the chromatogram. Accuracy and precision were evaluated by adding known escalating amounts of each standard to a solution of a known concentration whose analysis was replicated three times. The precision was expressed as relative standard deviation (RSD) and the accuracy as the amount found.

2.6. Sample preparation

Grape berries samples were purchased by the Origin Denomination Uva del Vinalopó (Alicante, Spain). They consisted of one variety of grapes which underwent two treatments. The first group (A) was covered with a preservant paper after collection, and the second one (B) was untreated. A and B groups were divided into two subgroups in order to analyze both the whole grape and the peel and pulp separately. After this clasification we had six samples to analyze: A (with preservative) A1: grape berries; A2: grape peels; A3: pulps. B (with no preservative): B1: grape berries; B2: grape peels; B3: pulps. Each sample was extracted according to the method of Waterhouse [10] and concentrated to dryness at low pressure and 35°C. An aliquot was then dissolved in HPLC-grade methanol for the analysis.

3. Results and discussion

Table 1 shows the polyphenol content of the samples.

Table 1
Polyphenol content of the samples (±RSD)

Polyphenol		Grape berries	Pulp	Peel
Rutine (mg/100 g)	A[a]	2.56±0.7	Trace	111.76±5.3
	B[b]	2.21±0.3	Trace	187.38±6.0
Quercitrine (mg/100 g)	A	Trace	–	Trace
	B	Trace	–	Trace
trans-Resveratrol (μg/100 g)	A	5.4±0.9	Trace	8.00±1.0
	B	9.6±0.5	Trace	15.00±1.5
Quercetine (mg/100 g)	A	0.47±0.02	–	13.23±1.3
	B	0.17±0.01	–	14.41±1.6
cis-Resveratrol (μg/100 g)	A	–	–	–
	B	–	–	–

[a] Grapes covered with a preservant paper after collection (with preservative).
[b] Grapes that suffered no treatment (no preservative).

3.1. Recovery

Grape berries samples containing known amounts of *cis*- and *trans*-resveratrol, quercetine, quercetrine and rutine were spiked with different levels of the standards to determine the recovery of the extraction procedure. The recovery of the polyphenols in grape berries was 94% or higher. The sensitivity of the method was found to be very good. On the basis of a 3:1 signal-to-noise ratio, the lower limits of detection for *cis*- and *trans*-resveratrol, quercetine, quercitrine and rutine were determined to be 1, 1, 2, 2 and 2 μg/l, respectively. The applicability of this method was tested by analysing a large number of grape berries samples obtained from the Origin denomination.

4. Conclusions

The effect of the use of a preservative paper on the polyphenol content of grape products is investigated. Covering the grapes with a preservative paper yields a healthier product, but this preservation allows no contact between the fruit and environmental agents such as fungi and stress. This fact is responsible for the poorer polyphenol content in the protected grapes. Those fruits that were not protected developed a higher content of these compounds. These data demonstrate the importance of including grapes in the diet as a polyphenolic source.

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