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SHORT COMMUNICATION

Searching *trans*-resveratrol in fruits and vegetables: a preliminary screening

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Abstract Resveratrol is a phytoalexin with potent anti-inflammatory, anti-oxidant and anti-carcinogenic effects. The object of this work was to determine whether this promising compound was present in the typical fruits and vegetables used in the Mediterranean diet. Our results indicated the presence of *trans*-resveratrol in concentrations ranging from 0.2 µg/g in tomatoes and 3 lg/g. in strawberries.

Keywords Resveratrol · Fruits · HPLC–UV

Introduction

Resveratrol, *trans*-3,5,4′-trihydroxy-trans-stilbene, is a phytoalexin produced by plants in response to infection by the pathogen *Botrytis cinerea* and also induced in response to a variety of stress conditions, such as changes in climate, exposure to ozone, sunlight and heavy metals (Athar et al. 2007). Resveratrol first aroused interest, when its presence in wine was reported in 1992 (Siemann and Creasy 1992). These authors suggested that this compound might be the biologically active ingredient in red wine. Much research has since been undertaken to highlight the beneficial properties of resveratrol providing evidence that this compound can act as an anticancer, platelet antiaggregation, antioxidant, antiaging, antifragilty, anti-inflammatory or antiallergenic agent (Gambini et al. 2015). Resveratrol exists in the natural world in both the *trans*- and *cis*-isomeric forms, which may in turn may have different biological effects. However, due to its stability, the *trans*-isomer is the most commonly used and in fact, *cis*-isomer is unavailable commercially (Huang and Mazza 2011). Physically, trans-isomer is a white powder with a slight yellow hue, which is soluble in ethanol and dimethyl sulfoxide and very slightly soluble in warm water (Rocha-González et al. 2008). There has been intensive research to identify the trans-isomer content in more than 70 plant species, including grapes, peanuts, berries, and pines (Szajdek and Borowska 2008; Athar et al. 2007; Sebastià et al. 2012; Arya et al. 2016). However, research is still ongoing and new sources of this promising molecule have been discovered such as in mulberry, Indian blackberry or Jackfruit of Indian origin (Shrikanta et al. 2015). The aim of this preliminary study is to analyze different fruits and vegetables present in the Mediterranean diet, in order to quantify the diet's *trans*-resveratrol content using liquid chromatography with the objective of finding new sources for this promising molecule.

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Materials and methods

Samples

Twenty-nine samples of different fruits and vegetables (Table 1) were available when the study was undertaken in the spring season. Samples were obtained over a period of several days from local markets and supermarkets in Valencia (Spain) and analyzed the same day they were bought.

Chemical and reagents

Resveratrol is obtained from Sigma-Aldrich (Taufkirchen, Germany). All solvents used were LC grade (Merck, Darmstadt, Germany) and LC-grade water was obtained by filtration of distilled water through a Milli-Q system (Millipore, Bedford, MA, USA). Solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corporation, Connecticut, USA) ultrasonic bath.

Table 1 *Trans*-resveratrol concentrations found in fruits and vegetables of analyzed samples

Sample	Incidence	*Trans*-resveratrol (μg/g)
Blueberry	0/1	–
Carrot	0/1	–
Cherry	0/1	–
D'Anjou pear	0/1	–
Dates	1/1	3.0
Flat peach	0/1	–
Golden apple	0/1	–
Lettuce	0/1	–
Macadamia nut	0/1	–
Nectarine	0/1	–
Onion	0/1	–
Peach	0/1	–
Peanut	0/1	–
Purple cabbage	0/1	–
Radish	0/1	–
Red grape	0/1	–
Red pepper	0/1	–
Strawberry	0/1	0.2
Tomato	3/10	0.2, 0.7, 2.1
Walnut	0/1	–
Watermelon	0/1	–
White grape	0/1	–
Wild asparagus	0/1	–

Resveratrol extraction and analysis

Resveratrol extraction and HPLC determinations were performed according to the method developed by Romero-Pérez et al. (2001) with a LOD of 0.003 mg/L and a LOQ of 0.01 mg/L. A liquid chromatography (LC) analysis of resveratrol was performed using a Jasco (Madrid, Spain) LC system PU-2089 Plus, equipped with a Quaternary Gradient Pump, a Rheodyne model 7725i injector (20 μL loop), and a UV detector L-7400 LaChrom from Merck (Darmstadt, Germany). The column used was the Gemini–NX C18 column (150 × 4.6 mm, 5 μm). The mobile phase consisted of a mixture of acetonitrile/water (20:80, v/v) at a flow rate of 1 mL/min. *Trans*-resveratrol identification was performed by comparing retention times of extracted samples to the pure standard. Quantification of *trans*-resveratrol was carried out by comparing peak areas of the analyzed samples to the calibration curve of peak areas obtained with the authentic *trans*-resveratrol standard. LC analysis of *trans*-resveratrol was carried out with a triple quadrupole mass spectrometer Quattro LC from Micromass (Manchester, UK), equipped with an LC Alliance 2690 system (Waters, Milford, MA, USA) consisting of an autosampler and a quaternary pump, a pneumatically assisted electrospray probe, a Z-spray interface, and using the Mass Lynx NT software 4.1 for data acquisition and processing. The autoinjector was programmed to inject 20 μL into the Gemini–NX C18 column (150 × 4.6 mm, 5 μm) maintained at 30 °C. The analytical separation for LC–MS/MS was performed using gradient elution with acetonitrile as the mobile phase A and water as the mobile phase B, both containing 0.01% glacial acetic acid. The gradient started with 70% of mobile phase A and 30% of phase B, with a linear increase to 37% of mobile phase B at 13 min. Flow rate was maintained at 0.2 mL/min. Analysis was performed in negative ion mode. The ESI source values were as follows: capillary voltage, 3.50 kV; source temperature, 120 °C; desolvation temperature, 400 °C; desolvation gas (nitrogen 99.99% purity) flow, 200 L/h. Cone voltages and collision energies were optimized during infusion of the pure standard and the most abundant ion fragment chosen for the selected reaction monitoring. The analyzer settings were: resolution 12.0 (unit resolution) for the first and third quadrupoles; ion energies, 1; entrance and exit energies, −3 and 1; multiplier, 500; collision gas (argon, 99.99% purity) pressure 3.74×10^{-3} mbar. For the detection of trans-resveratrol the precursor ion was *m/z* 227, and the product ions selected were *m/z* 185 and 143.

Results and discussion

Table 1 shows the presence of *trans*-resveratrol in three (tomato, strawberry and dates) out of twenty-nine fruits and vegetables analyzed. *Trans*-resveratrol concentrations varied from 0.2 µg/g (tomato and strawberry) to 3 µg/g in dates (*Phoenix dactylifera* L.). Figure 1B shows presence of resveratrol in tomato sample. Samples containing resveratrol from LC–UV were confirmed by LC–MS/MS is shown, in Fig. 1C, a chromatogram obtained in ESI positive ion mode of a positive sample, where the two *trans*-resveratrol product ions selected were *m/z* 185 and 143.

In tomato, Ragab et al. (2006) detected a *trans*-resveratrol concentration at relatively stable levels during fruit maturation, reaching a maximum concentration in the skin of 18.4 ± 1.6 µg/g dry weight at 4 weeks of post-harvest. Nicoletti et al. (2007) studied this compound in transgenic tomato plants, in which resveratrol synthase genes were introduced, revealing the genetic modification originating at different levels of accumulation of *trans*-resveratrol, among other stilbenes (*trans*- and *cis*-piceid and *cis*-resveratrol) in the fruit depending on the ripening stage. Moreover, they observed that the highest amount of *trans*-resveratrol was found in the peel of fruits harvested at maturity.

In strawberries, *trans*-resveratrol was detected in strawberry achenes (seeds) and pulp (receptacle tissue) at higher levels in achenes than in fruit pulp. In fact, a high growing temperature (25 and 30 °C), enriching CO_2 in the

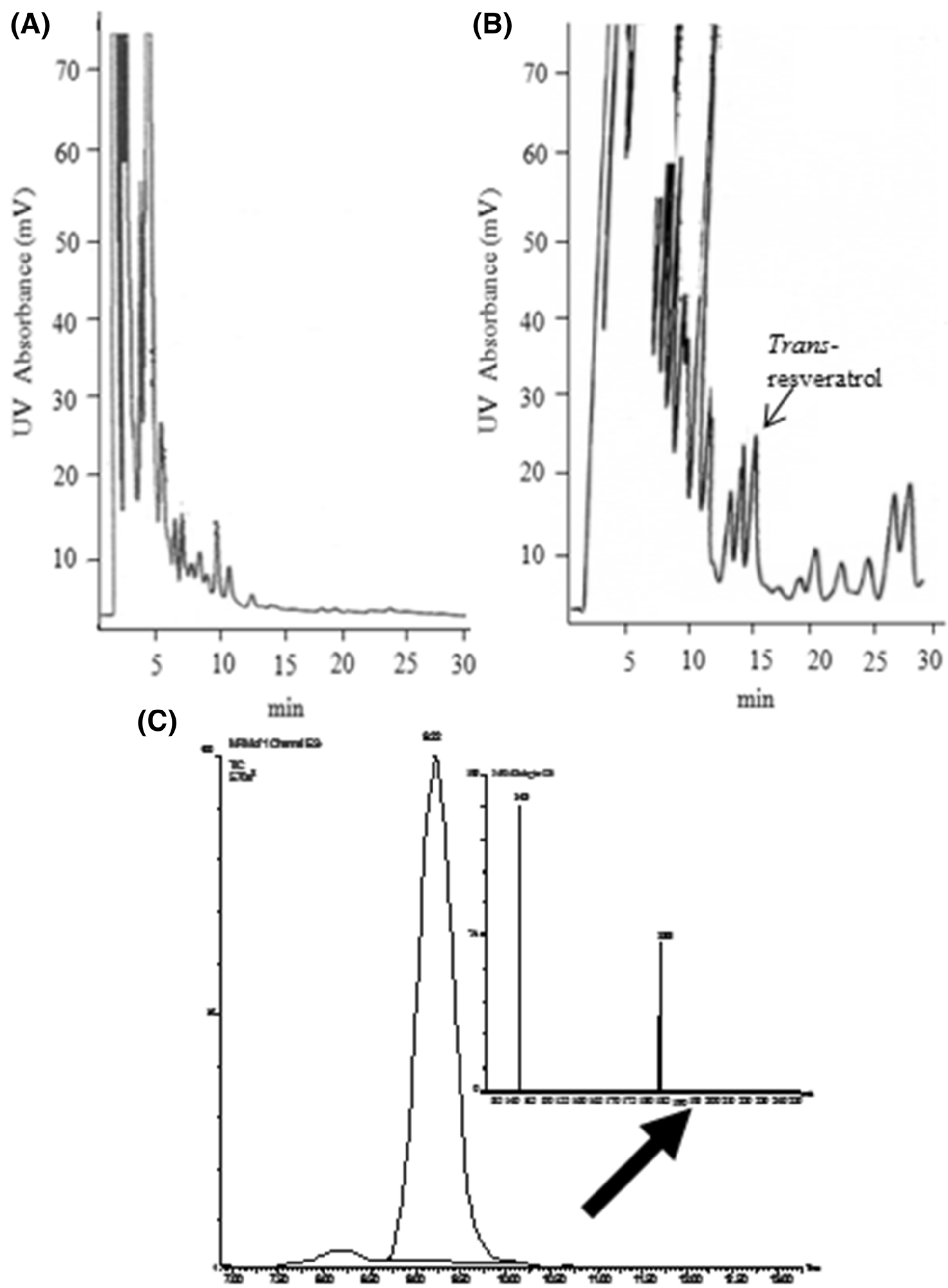

Fig. 1 LC–UV chromatograms of **a** negative fruit sample and **b** tomato sample and **c** the LC/MS/MS chromatogram of positive tomato sample with two resveratrol product ions selected, *m/z* 185 and 143

growing environment, adding compost as a soil supplement, applying methyl jasmonate prior to harvesting, hill plasticulture cultivation, advancing maturation or selecting mature pulp and achenes, significantly enhanced the *trans*-resveratrol content of strawberries (Wang et al. 2007). Seeram et al. (2006) demonstrated that strawberry extracts, which contained *trans*-resveratrol, had the most significant pro-apoptotic effects against the colon cancer cell line HT-29 in comparison with blackberries, blueberries, cranberries and red raspberry extracts.

Resveratrol content has also been detected in some underutilized fruits such as Jamun (*Syzygium cumini L.*), Jackfruit (*Artocarpus heterophyllus*) and Mulberry (*Morus rubra*. Shrikanta et al. (2015) showed the importance of extending the detection of this molecule in a wider range of fruits. In fact, for the first time to the best of our knowledge we found *trans*-resveratrol in dates. This finding revealed the possibility of considering dates as a new source of this molecule. Many countries in the Mediterranean area and Middle East are high consumers of dates (Al-Harrasi et al. 2014), eating raw or for dessert preparation, and so sources of *trans*-resveratrol, in their diet should not be underestimated, where dates are well-considered for their nutritional, economic and distinct medicinal properties (Vayalil 2012).

The present study has some limitations due the variety of fruits and vegetables available locally, since shops and supermarkets do not generally have a wide range of fruit, usually offering only those most commonly consumed and demanded by customers or an emphasis on local produce. Future studies will therefore be focused on the assessment of *trans*-resveratrol in fruits and vegetables of different varieties, as affected by season and origin.

We consider it important to quantify this polyphenol in a variety of fruits and vegetables in order to clarify whether diet can contribute to the ingestion of this promising molecule. The results highlight the possibility of considering dried dates as a new source for the *trans*-resveratrol molecule.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Human and animal rights statement This article does not contain any studies with human or animal subjects.

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