Antioxidant capacity of *trans*-resveratrol dietary supplements alone or combined with the mycotoxin beauvericin

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Abstract

Trans-resveratrol (*trans*-RSV) is a polyphenol with multiples biological properties, such as anti-inflammatory, antioxidant, anti-aging, anti-diabetic, and antiplatelet. It occurs naturally in grapes and derivate, peanuts and berries. Beauvericin (BEA) is a mycotoxin present in cereals that produces cytotoxicity, intracellular reactive oxygen species and lipid peroxidation. The general objective of this research was to evaluate whether *trans*-RSV could be used as a good polyphenol against damages produced by BEA. Because *trans*-RSV can be ingested through dietary supplements, to reach this goal, the following specific objectives were proposed: a) the *trans*-RSV content in different polyphenol dietary supplements by capillary electrophoresis, b) the antioxidant capacity of the *trans*-RSV in polyphenol supplements, and c) the influence of BEA in the antioxidant capacity of *trans*-RSV when they are in combination by photochemioluminiscence assay. The results obtained in this study showed that all polyphenol dietary supplements present higher RSV content that the content of the label. The polyphenol supplements present antioxidant capacity. And the combination of *trans*-RSV and BEA did not affect the antioxidant capacity of *trans*-RSV. Thus, RSV could contribute to decrease oxidant effects produced by BEA.

Keywords: dietary supplements, beauvericin, resveratrol, antioxidant capacity, photochemiluminescence, capillary electrophoresis.

1. INTRODUCTION

Some oxidants are formed in response to physiological processes. A disturbance between pro-oxidants and antioxidants defense system in favor of the oxidants is defined as oxidative stress, which can contributes to the development of chronic disease and ageing process (Davies, 2000; Halliwell, 2006; Lobo et al., 2010; Rahal et al., 2014).

Antioxidant compounds ingested through diet can scavenge free radicals and protect the organisms from oxidative stress. More than 8000 compounds have been identified with antioxidant properties. Polyphenolic compounds are a great class of antioxidants. They include phenolic acids, flavonoids, stilbenes and lignans (Pandei and Rizvi, 2009). Resveratrol (3, 5, 4'-trihydroxystilbene; RSV) is a stilbene abundant in grapes and grape products such as wines and grape juice. RSV exists in two diasteromic forms: *trans* and *cis* (Chen et al., 2007). *Trans*-RSV has biological properties such as antioxidant, anti-inflamatory, antiaging and antiplatelet activities among others, which prevent several human diseases (Fernández-Mar, et al., 2012; Li et al., 2012). This potential benefits resulted in increased consumption of *trans*-RSV supplements by several consumers. Many efforts have been made to provide a highly sensitive and selective analytical method for the determination and characterization of polyphenols in dietary supplements (Ignat et al., 2011). The polyphenol content has been determined in different food matrices by spectrophotometry (Camont et al., 2009), high-performance liquid chromatography (HPLC; Mark et al., 2005), gases chromatography (CG; Goldberg et al., 1995) and capillary electrophoresis (CE; Brandolini et al, 2002; Arribas et al., 2014; Gatea et al., 2015).

Moreover, antioxidant capacity can be determined by Trolox Equivalent Antioxidant Capacity (TEAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

(ABTS) method, oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), total radical-trapping antioxidant parameter (TRAP), ferric reducing antioxidant power (FRAP), photochemiluminescence (PCL) and thiobarbituric acid (TBA), among others (Prior et al., 2005; Alam et al., 2013).

Beauvericin (BEA) is a mycotoxin synthetized by many species of *Fusarium* fungi. BEA is a contaminant of cereals and product composed by cereals (Mahnine et al., 2011; Juan et al., 2012. It has been demonstrated that BEA is cytotoxic, decreases mitochondrial membrane potential, produces lipid peroxidation, DNA damage and cell death (Ruiz et al., 2011; Prosperini et al., 2013; Mallebrera et al., 2016), which could be related with oxidative stress produced by BEA in several cell lines (Ferrer et al., 2009; Prosperini et al., 2013; Mallebrera et al., 2015). Because of resveratrol has multiple biological properties and it can be ingested through dietary supplements, this could be used to mitigate the oxidative damage caused by beauvericin.

The general objective of this research was to evaluate whether *trans*-RSV could be used as a good polyphenol against damages produced by BEA. Because *trans*-RSV can be ingested through dietary supplements, to reach this goal, the following specific objectives were proposed: a) *trans*-RSV content in dietary supplements by CE, b) the antioxidant capacity of *trans*-RSV in dietary supplements and c) the influence of BEA in the antioxidant capacity of *trans*-RSV when they are in combination with BEA by PCL.

2. MATERIAL AND METHODS

2.1. Reagents

All reagents were purchased from Sigma-Aldrich (Milan, Italy). The luminol PCL assay was carried out using the Photochem® instrument with the ACL kit (Analytikjena, Jena, Germany).

2.2. Samples

Commercial samples of *trans*-RSV dietary supplements (n=4) were collected during 2015 from different pharmacies in Italy. Table 1 shows the samples analyzed and content of *trans*-RSV in each of them according to the nutritional label.

2.3. Sample preparation

Briefly, 0.30 g of each sample, were extracted with 5 mL of methanol (MeOH) and mixed using a vortex every 5 min for 15 min. Then, it was centrifuged at 5000 rpm during 5 min and finally the supernatant were collected in a flask of 20 mL. This procedure was performed four times. Then, the extracts were completed to 20 mL with MeOH. Three independent extractions were performed for each sample.

2.4. Capillary electrophoresis

CE analyses were performed according to Brandolini et al. (2002) using a CE Beckman MDQ equipped with a diode array detector (Beckman, Fullerton, CA). The separation was obtained by 75 mm i.d. and 57 cm total length fused silica capillary column maintained in a cartridge with a detector window of 100 µm x 800 µm. The capillary was conditioned before use by flushing 0.1 M NaOH for 1 min, then with water and, finally with buffer (20 mM $Na_2B_4O_7$ and 50 mM PEG 400, 10% MeOH) for

3 min. The sample was injected into the capillary by pressure injection for 5 s. Separation was obtained at 25 kV and 25 °C for 15 min at 315 nm. After each separation the capillary was rinsed sequentially with NaOH 0.1M for 2 min. and buffer analysis for 3 min. All analyses were performed in three independent assays. Data are analyzed using the Karat 32 software (Beckman Coulter, Fullerton, CA).

2.5. Antioxidant activity

2.5.1. Antioxidant activity of *trans*-RSV dietary supplements

The antioxidant capacities of *trans*-RSV dietary supplements were determinated using a PCL technique, namely, the luminol PCL assay. The determination was carried out using the Photochem® instrument with the ACL kit (Analytikjena, Jena, Germany), and following the procedure described by Popov and Lewin (1999). Two or three mL reagent 1 (solvent and dilution reagent), 200 µL reagent 2 (buffer solution), 25 µL reagent 3 (photosensitizer) and 10 µL of standard or solution were mixed and measured. Trolox was used as standard to obtain a calibration curve (0.5–2 nM). The light emission curve was measured at λ_{max}=350nm during 180 s, using the inhibition of superoxide anion radicals as the parameter to evaluate antioxidant effect. The antioxidant capacity was determined by using the area under the curve. The results were expressed as µmol Trolox equivalents (TEs) per g *trans*-RSV. Antioxidant capacity of supplements was determined replacing standard by diluted samples. Determinations were performed with 6 replicates of each sample.

2.5.2. Antioxidant activity of *trans*-RSV when combined with BEA.

Considering that *trans*-RSV possesses antioxidant properties and BEA increases ROS production, the *trans*-RSV antioxidant capacity against oxidant activity of BEA is

an objective of interest. Thus, the antioxidant activity of *trans*-RSV, BEA and four combinations of BEA+*trans*-RSV with 1:2.5; 1:5; 2:1 and 1:1 ratio were determined using the PCL technique describe previously.

3. RESULTS AND DISCUSSION

During the last years, many *trans*-RSV dietary supplements have been investigated to determine their biological properties. RSV is related with French Paradox, and low incidence of cardiovascular diseases may co-exist with a high-fat diet intake and moderate consumption of red wine (de la Lastra and Villegas, 2007).

In Table 2 is shown the *trans*-RSV content in each sample analyzed by CE. As can be observed, the *trans*-RSV content was higher than the *trans*-RSV content in the label for samples 1,2,3 (Table 1). Sample 4 contains the highest *trans*-RSV content of all the samples. The *trans*-RSV content of sample 4 is not on the label. Nutritional values of dietary supplements found on the labels are expressed as mean of several batches selected. In this respect, according to Italian legislation, the polyphenols content can ranged from ±30% on the labeled content of the product (Circolare n7, 2002).

The measurement of the antioxidant capacity of dietary supplements is an interesting matter of health. On the other hand, consumers want to know if the consumption of dietary supplementation can protect them against oxidative stress or not.

The antioxidant capacity of *trans*-RSV dietary supplements expressed as μmol TEs/g of sample is shown in Table 3. Antioxidant capacity of the sample was from highest to lowest in the following order: 3>4>2>1.

The antioxidant capacity of *trans*-RSV measured by Photochem® is 0.5 μmol TEs/mg. The theoretical data corresponding to *trans*-RSV antioxidant capacity was verified for each dietary supplement and shown in Table 3. Sample 1 shown lower

antioxidant capacity that those of the theoretical data, and samples 2, 3 and 4 were significantly higher. Moreover, the antioxidant capacity observed is not correlated with the *trans*-RSV content. So, the results obtained in this study are acceptable if we consider the content of other polyphenols in the samples (Table 4). In conclusion, we suggest that the antioxidant effect of each dietary supplement is not given by the *trans*-RSV content; this is due to the combination of antioxidant compounds in the samples. Moreover, it can be observed that *trans*-RSV content is present in low quantity (Table 1 and Table 2) respect to the content of other antioxidant compounds (Table 4) in the dietary supplement.

Results obtained in this study demonstrated that *trans*-RSV showed 0.5 µmol TEs/mg antioxidant capacity. It has been demonstrated by other methods that *trans*-RSV possess antioxidant capacity (Gülçin, 2010; Lucas-Abellán et al., 2011). *Trans*-RSV has effective $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $O2^{\bullet-}$ and H_2O_2 scavenging activities (Gülçin, 2010). Moreover, a comparative study between ORAC, ABTS and ORAC assays corroborates that RSV has antioxidant capacity (Lucas-Abellán et al., 2011). The highest RSV antioxidant activity was observed with the ORAC method. The ORAC assay measures low RSV concentrations with high precision. Conversely, DPPH assay measures high RSV concentrations (5–90 µM), but lower than 5 µM cannot be quantified. Due to RSV concentration in food is very low; ORAC method was considered the best for determining the antioxidant capacity of RSV (Lucas-Abellán et al., 2011).

On the other hand, it has been demonstrated that BEA increases intracellular ROS generation and LPO production by *in vitro* methods with culture cells (Ferrer et al., 2009; Prosperini et al., 2013; Mallebrera et al., 2015). Moreover, an excessive ROS accumulation may induce intracellular oxidative damages. BEA antioxidant activity was also assayed by PCL method. However, BEA did not show antioxidant capacity (data

not shown). According to Wätjen et al. (2014), BEA had no effect as radical-scavenging activity in a cell-free assay.

Moreover, to evaluate *trans*-RSV antioxidant capacity in presence of BEA, the combination of BEA+*trans*-RSV was evaluated by PCL method. The results obtained in this study showed that BEA did not show antioxidant capacity when it is evaluated alone. In addition, not significant differences were observed in the antioxidant capacity of *trans*-RSV when it was evaluated alone and in combination with BEA. Due to the presence of beauvericin does not affect the antioxidant capacity of *trans*-RSV, this polyphenol could be used to evaluate the mitigation effect against BEA damages. Therefore, more studies related the mechanism of BEA and *trans*-RSV antioxidant effect in culture cells or/and in vivo assays are required.

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Legend of Tables and Figures

Table 1: *Trans*-RSV content and components in the label differents to *trans*-RSV.in commercial dietary supplements samples.

Table 2: *Trans*-RSV content (mg/g) in the dietary supplements analysed by CE. Data are expressed as mean ±SD (n=6).

Table 3: Antioxidant capacity of *trans*-RSV supplements analyzed by PCL and theoretical antioxidant capacity of each supplement. Data are expressed as mean ±SD of different samples (n=6).

Table 1: *Trans*-RSV content and components in the label differents to *trans*-RSV.in commercial dietary supplements samples.

Sample	***Trans*-RSV content in the label (mg/g)**	**Other compounds in the label**
1	3.4	*Monascus purpureus*, octacosanol, vitamin B3, alpha lipoic acid, omega-3 fatty acid, chrome, pantothenic acid, vitamin B12 and folic acid.
2	1.23	Vitamin C (74.1 mg/g), vitamin E (12.3 mg/g), zinc, copper, selenium, omega-3 fatty acid, lutein and zeaxanthin
3	8.62	Soy isoflavones, vitamin K, vitamin D, quercetin (129.3 mg/g), calcium, magnesium and isovitexin.
4	Not declared	Stilvid® (443.3 mg/g), *Punic granatum*, selenium, vitamin C (416.6 mg/g), zinc and vitamin B2 (40 mg/g)

Table 2: *Trans*-RSV content (mg/g) in the dietary supplements analysed by CE. Data are expressed as mean ±SD (n=6)

Sample	**Content (mg/g ± SD)**
1	4.44±0.52
2	2.41±0.15
3	10.75±0.48
4	24.79±0.89

Table 3: Antioxidant capacity of *trans*-RSV supplements analyzed by PCL and theoretical antioxidant capacity of each supplement. Data are expressed as mean ±SD of different samples (n=6).

Sample	***trans*-RSV antioxidant capacity (*) (µmol TEs/g)**	**Theoretical antioxidant capacity (**) (µmol TEs/g)**
1	1.54±0.09	2.2
2	577.11±30,47	1.2
3	1054.75±30,08	5.37
4	203.53±69,82	12.39

*Antioxidant capacity of *trans*-RSV supplement assayed by PCL.
**Theoretical antioxidant capacity produced in 1 mg of *trans*-RSV from each sample.