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STABILITY EVALUATION OF RESVERATROL SUBMITTED TO IONIZING RADIATION

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ABSTRACT

The polyphenol trans-resveratrol (trans-3, 4',5-thihydroxystilbene) is a natural phytoalexin, reported to exert different biological activities, such as antioxidant properties. In the attempt to make possible the topic administration of resveratrol it will be immobilized in a hydrogel matrix obtained by gamma radiation crosslinking process which can cause undesirable hydrolysis reactions in the active compound. The aim of this work was to verify the aqueous/ethanol resveratrol solution stability and antioxidant activity after irradiation at 20kGy. The integrity and stability were compared with nature one by High Performance Liquid Chromatography (HPLC) technique. The antioxidant activity was determined by the free radical scavenging method, using 2,2-Diphenyl-1-picrylhydrazyl (DPPH.) as free radical. The results demonstrated the decomposition of resveratrol and reduction of antioxidant capacity after irradiation at 20 kGy dose.

1. INTRODUCTION

The polyphenol trans-resveratrol is a naturally occurring phytoalexin produced by some spermatophytes in response to injury. This compound is found in red wine and in a wide variety of plant species, including mulberries, peanuts and grapes [1].

Resveratrol has been shown to possess exceptionally benefits for the skin, such as antiproliferative and chemopreventive properties against skin carcinogenesis and prevention of premature aging, partly of these properties could be attributed to the resveratrol antioxidant activity [1, 2].

Physiological processes related to absorption, solubility and transport limit the amount of orally administrated resveratrol that can be delivered into skin [3]. In this context, this active compound immobilized in hydrogels could be very interesting to a topical administration. However, the crosslinking process by gamma radiation in aqueous solution, used to obtain some types of hydrogel membranes, can cause undesirable hydrolysis reactions modifying the activity of the active compound [4].

The aim of this work was to verify the resveratrol stability and the antioxidant activity after irradiation at 20kGy to be immobilized in a hydrogel matrix.

2. MATERIALS AND METHODS

Trans-resveratrol was purchased from Attivos Magistrais, spectrophotometric grade ethanol and HPLC grade Acetonitrile from Vetec, phosphoric acid (H_3PO_4) from Merck and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich. The solutions and eluents were prepared using Milli-Q water.

2.1. Preparation of Resveratrol solutions

Resveratrol solutions (0.1mg.mL^{-1}) were prepared in ethanol/water (50:50, v:v) and irradiated in Gammacell 220 source (Atomic Energy of Canada Limited, Ottawa Canada) ^{60}Co gamma ray source with 2.28 kGy.h^{-1} dose rate at 0 and 20 kGy dose.

The stability of resveratrol was evaluated by high performance liquid chromatography (HPLC). Before the analyses of resveratrol solutions it was run only ethanol/water (50:50, v:v) solution irradiated at 20 kGy and non irradiated as control.

2.2. Stability evaluation of resveratrol

The HPLC system used for the analyses was a Shimatzu LC-20AT and the quantifications were performed on a C_{18} column, 250 x 4.6 mm, 5-µm particle size, from Chrompack. Measurements were carried out at a constant column temperature (25ºC) and constant flow rate (1 mL.min^{-1}).

The analysis was performed with an isocratic elution using a Water:Acetonitrile (75:25) solution as eluent. The pH of the solution was adjusted to 3.0 by using concentrated H_3PO_4 [5].

The injected sample volume was 10 µL and the chromatograms were recorded at 306 nm using a UV detector.

2.3. Determination of free radical scavenging capacity

The antioxidant capacity of resveratrol solutions was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a free radical and the DPPH method reported by Brand-Williams *et al.* (1995) was used as a reference [6].

An ethanolic solution of the free radical (3.9 mL of 4.15 x 10^{-2} mg.mL^{-1}) was added to 0.1 mL of the Resveratrol solution and the absorbance was monitored spectrophotometrically at 515 nm, every 10 min., during 190 min., using a Cary 300 spectrophotometer.

The DPPH concentration (C_{DPPH}) in the reaction medium was calculated with the equation 1 obtained from a calibration curve made with an ethanolic solution of DPPH between 4.15x10^{-2} mg.mL^{-1} and 4.15x10^{-4} mg.mL^{-1}:

$$Abs_{515nm} = 0{,}0389 \times (C_{DPPH}) - 0{,}0001 \quad (1)$$

The free radical scavenging capacity of the resveratrol solutions was determined comparing the percentage of remaining DPPH at different times. The percentage of remaining DPPH (%DPPHrem) was calculated as follow:

$$\%DPPHrem = \frac{[DPPH]}{[DPPH]t = 0} \times 100 \quad (2)$$

3. RESULTS

The chromatograms of the HPLC analyses of resveratrol solutions irradiated with a dose of 0 and 20 kGy are shown in Fig. 1 and Fig. 2, respectively. We can observe almost complete degradation of resveratrol and the formation of decomposition products after irradiation. These results are similar to that presented by Bader *et. al.* (2007) which detected the formation of products and structural decomposition of aqueous resveratrol solutions saturated with pure gases and irradiated with a dose of 1 kGy [7].

The radiolysis of water by ionizing radiation can result in the production of substantial quantities of oxidizing species [8] which could attack resveratrol by indirect effect of radiation.

In the chromatograms of ethanol/water solutions no peaks were observed.

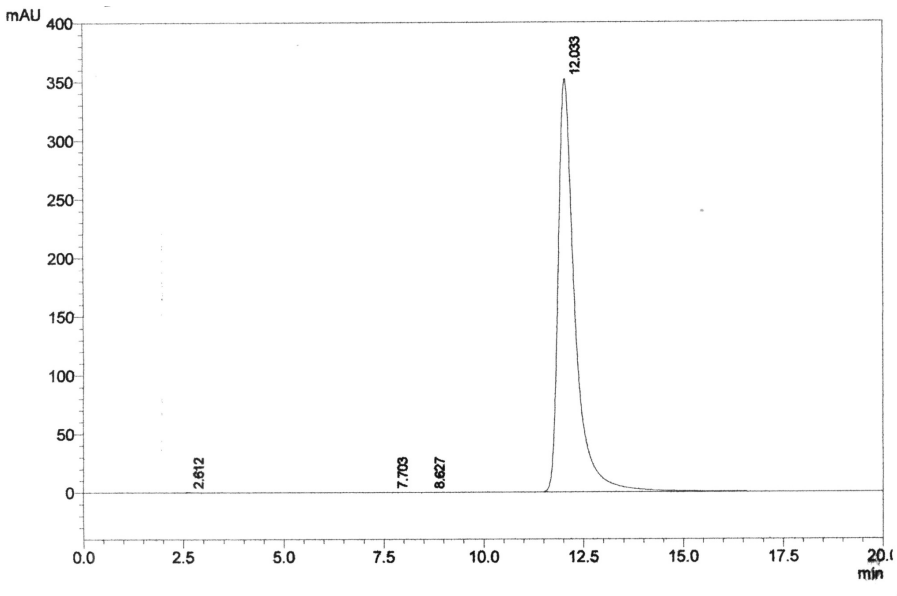

Figure 1. HPLC chromatogram of non irradiated resveratrol solution.

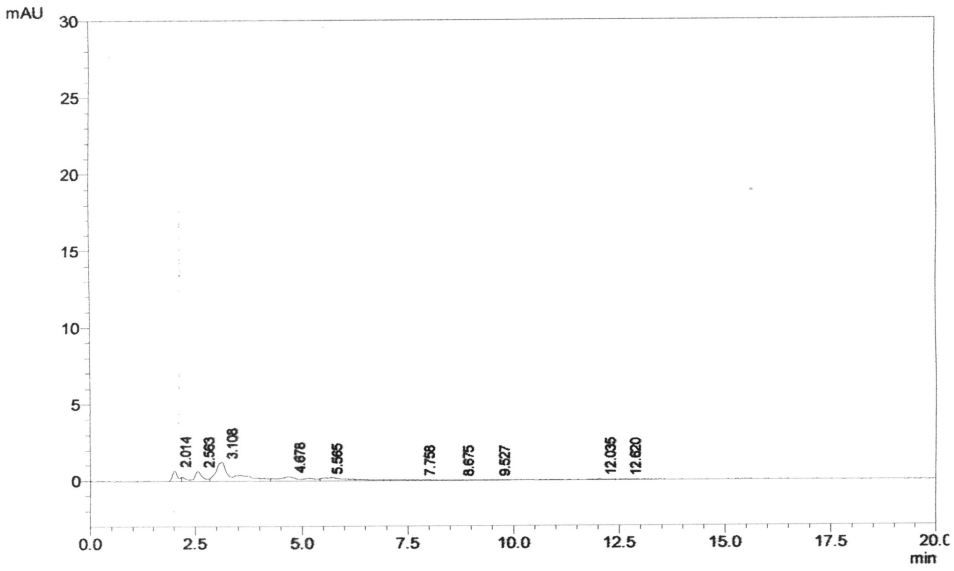

Figure 2. HPLC chromatogram of resveratrol solution irradiated at 20 kGy dose.

In Table 1 are presented the retention times and peak area percentages of resveratrol solutions. The resveratrol retention time was 12 minutes and the percentage areas were approximately 99.9% and 2.5% for irradiated resveratrol at 0 and 20 kGy, respectively.

The detection of six new peaks in irradiated resveratrol solutions at 20 kGy suggests the formation of decomposition products.

Table 1. Retention times and peak areas of Resveratrol solutions irradiated at 0 and 20 kGy.

Dose (kGy)	Peak	Retention time (min)	Area (%)
0	1	2.612	0.049
	2	7.703	0.010
	3	8.627	0.010
	4	**12.033**	**99.917**
20	1	2.014	7.390
	2	2.563	12.260
	3	3.108	41.870
	4	4.678	14.085
	5	5.565	12.389
	6	7.758	4.153
	7	8.675	1.332
	8	9.527	2.446
	9	**12.035**	**2.505**
	10	12.620	1.570

In the DPPH assay the antioxidant activity of resveratrol solutions were determined as their capacity of scavenge free radical. The percentage of remaining DPPH was determined in different times and the reaction kinetics was presented in Fig. 3.

The percentage of remaining DPPH after 190 minutes was near to 72% and 85% in resveratrol solutions irradiated at 0 and 20kGy dose, respectively. The free radical scavenging presented by the irradiated resveratrol at 20 kGy could be a result of decomposition product formed after irradiation.

In Fig. 3 we can observe that the percentage of remaining DPPH after 20 minutes was near to 85% in non irradiated resveratrol and this value was reached only after 3 hours of reaction by the irradiated one, suggesting that the free radical scavenging capacity was reduced after irradiation.

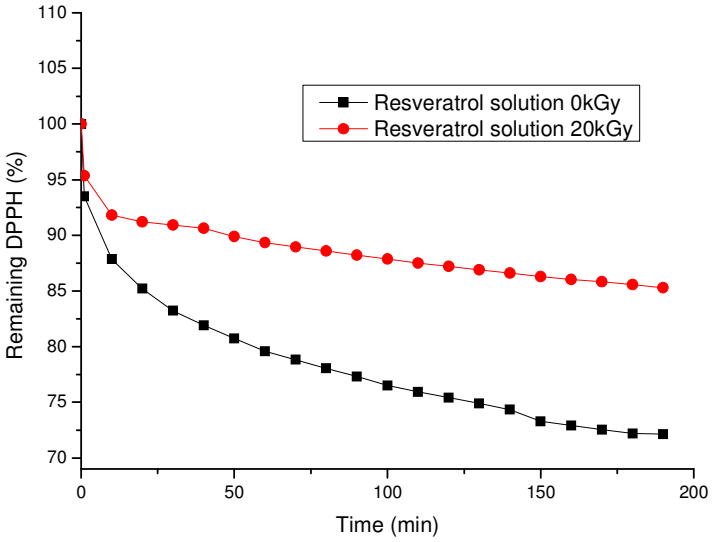

Figure 3. DPPH kinetic curves. Resveratrol solutions irradiated at 0 and 20 kGy.

4. CONCLUSIONS

Resveratrol showed loss of stability and integrity after irradiation at 20 kGy dose, presenting degradation products and a reduction in the antioxidant activity based on free radical scavenging capacity.
The study might be continued with the detection of the formed degradation products and the resveratrol incorporation in PVP hidrogel matrix before crosslinking irradiation.

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