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Radiation-induced enhancement of antioxidant activity in extracts of rosemary (*Rosmarinus officinalis* L.)

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Abstract

Dry rosemary leaf powder was subjected to 30 kGy of gamma ray irradiation, followed by solvent extraction with methanol, ethanol or water. The antioxidant activity of the extracts was assessed using the DPPH radical-scavenging method and the reducing power test. EC_{50} values, using the radical-scavenging method, indicate a 22% increase in the antioxidant activity of ethanol and water extracts as a result of irradiation treatment. EC_{50} values in the reducing power test show an increase of 45% and 28% for the ethanol and water extracts, respectively. The antioxidant activity of methanol extracts of irradiated rosemary remained the same as in the controls in both types of test. A high correlation was found between the EC_{50} values obtained in the DPPH radical test and those from the reducing power test. Total phenolic content (Folin–Denis test) increased by 35% in the water extracts as a result of irradiation but remained the same in the methanol and ethanol extracts. The methanol extract showed the highest antioxidant activity and the highest amount of total phenolic compounds. Radiation reduced the good correlation between antioxidant activity and total phenolic content.

Keywords: Rosemary; Gamma irradiation; Solvent extraction; Antioxidant activity; DPPH radical; Reducing power; Total phenolic content

1. Introduction

Rosemary (*Rosmarinus officinalis* L.) belongs to the Lamiaceae family of herbs which, in addition to being used as a food flavouring, is also known medicinally for its powerful antioxidant activity, its antibacterial and antimutagenic properties, and as a chemopreventive agent (Oluwatuyi, Kaatz, & Gibbons, 2004). Since the use of synthetic antioxidants in the food industry is currently being severely questioned, antioxidants from natural sources are in increasing demand. Owing to its antioxidant properties, rosemary is widely used today as a food preservative, either in ground form or as an extract (Peng, Yuan, Liu, & Ye, 2005). The main compounds responsible for rosemary's antioxidant properties have been identified as phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epi- and iso-rosmanol, rosmadial and methyl carnosate (Ibañez et al., 2003). Other compounds, such as rosmarinic acid, caffeic acid and flavonoids, have also been associated with the antioxidant activity of rosemary (Del Baño et al., 2003; Suhaj, 2006).

Especially during picking, processing and packing, rosemary is susceptible to contamination by pathogenic microorganisms (Legnani, Leoni, Righi, & Zarabini, 2001). Gamma radiation is a highly effective means of inhibiting the growth of undesirable microbes and avoiding the occurrence of food-transmitted diseases. This is substantiated by the fact that an increasing number of countries have adopted irradiation as a way to ensure the hygienic quality of dehydrated foods (IAEA, 2006). The international safe dose clearance is up to 10 kGy, though some countries, including Argentina, have increased this level to 30 kGy without any harmful effects being observed.

There is growing scientific interest in the influence of irradiation processes on antioxidant activity and the compounds responsible for such activity. There has been a marked increase in the literature on the subject since

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2000 in relation to foods of plant origin, including studies on irradiation-induced modifications in antioxidant compounds and the antioxidant properties of herbs and spices (Byun, Yook, Kim, & Chung, 1999; Polovka et al., 2006; Suhaj, Rácová, Polovka, & Brezová, 2006; Topuz & Ozdemir, 2004; Variyar, Bandyopadhyay, & Thomas, 1998), mushrooms (Huang & Mau, 2006), sorghum flour (Fombang, Taylor, Mbofung, & Minnaar, 2005), chinese cabbage (Ahn et al., 2005), green tea extracts (Jo, Son, Lee, & Byun, 2003) and phytic acid (Ahn, Kim, Jo, Kim, & Byun, 2004). The effect of irradiation on some of the compounds responsible for antioxidant activity in rosemary has been reported by Calucci et al. (2003) and Koseki et al. (2002). However, the influence of gamma radiation on the antioxidant activity of rosemary has not, as yet, been studied in vitro. The objective of the current study, is therefore to evaluate the impact of a 30 kGy dose of gamma rays on dry rosemary leaves in terms of: (i) antioxidant activity (DPPH radical scavenging ability and reducing power) of methanol, ethanol and water extracts and (ii) the amount of total phenolic compounds in these extracts. The study also aims at establishing whether there is a correlation between DPPH radical-scavenging ability and the reducing power of the extracts and between antioxidant activity and total phenolic content.

2. Materials and methods

2.1. Samples

The rosemary was harvested in Bahía Blanca, Argentina, between the months of August and October, at which time it was in full bloom. The fresh leaves were carefully washed with de-ionized water, dried immediately thereafter in an oven at 37 °C until they achieved a constant weight, and then ground in a mortar.

2.2. Irradiation

Three samples of dry rosemary leaf powder (~70 g each) were placed in plastic bottles and irradiated at the facilities of the Comisión Nacional de Energía Atómica in Ezeiza Atomic Center, Buenos Aires Province, Argentina. The samples were treated under air conditions at 20 °C with a dose of 30 kGy using ^{60}Co gamma rays. The dose-rate was 5.5 Gy/s, as determined by red Perspex dosimeter, and the dose uniformity ratio was 1.25. The control and irradiated samples were kept in a desiccator in the dark at room temperature until used. Samples were analyzed one month after irradiation.

2.3. Preparation of extracts

Rosemary leaf extracts were obtained using methanol, ethanol or water. The dry powder (0.025–1.5 g) was mixed with 50 ml of solvent or of water and placed in an ultrasound bath for 2 h at 40 °C. The extracts were then centrifuged at 2000 rpm for 10 min and the supernatant used immediately for the experiments.

2.4. Evaluation of antioxidant activity

2.4.1. Scavenging activity on the 2,2-diphenyl-1-picryl hydrazyl radical (DPPH· test)

The relative stability of the DPPH· radical has been widely used to evaluate the antioxidant activity of various plant extracts and pure compounds (Yen & Duh, 1994). The method is based on the reduction of an alcoholic solution of DPPH· owing to the donation of a hydrogen by an antioxidant compound. DPPH· solutions have an intense violet colour and show a strong absorption band at 517 nm. The DPPH radical reduction makes itself apparent by a change in colour from intense violet to light orange, with a consequent decrease in absorbance. The DPPH· remaining after a certain time corresponds inversely to the scavenging activity of radicals by the antioxidant.

DPPH radical-scavenging by rosemary leaf extracts was measured according to the method of Brand-Williams, Cuvelier, and Berset (1995) with some modifications. Twenty milliliter of extract were mixed with 3 ml of a 60 μM solution of DPPH· in ethanol. The mixture was shaken vigorously and kept in the dark at room temperature until the measurements were taken. Absorbance was measured at 517 nm in a Metrolab RC 325 spectrophotometer. Ethanol was used to zero the spectrophotometer. The DPPH· solution was prepared daily, stored in flasks, covered, and kept in the dark at 4 °C until the measurements were taken.

The scavenging activity of the extracts was expressed as the percentage of inhibition of the DPPH radical, defined as (Yen & Duh, 1994)

$$\text{Inhibition percentage (IP)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where A_{control} is the absorbance of the control (containing all reagents except the sample), A_{sample} is the absorbance of the sample, both measured at 517 nm in a steady state.

The $EC_{50(DPPH)}$ value, which represents the concentration of extract that gives rise to a 50% reduction in DPPH· absorbance, was determined by linear regression analysis.

2.4.2. Reducing power

The reducing power of rosemary leaf extract was determined by evaluating the transformation of Fe^{3+}–Fe^{2+} according to the method of Oyaizu (1986). One milliliter of extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (1%, p/v). The mixture was incubated for 20 min at 50 °C. After rapid cooling, 2.5 ml of trichloroacetic acid were added (10%, p/v) and the mixture was centrifuged for 10 min at 2000 rpm. Finally, 5 ml from the upper layer was mixed with 5 ml of distilled water and 1 ml of ferric chloride (0.1%). After vigorous shaking,