

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

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Received 20 September 2006; received in revised form 18 October 2006; accepted 1 December 2006

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. EC₅₀ 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. EC₅₀ 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 EC₅₀ 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. © 2007 Elsevier Ltd. All rights reserved.

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 ⁶⁰Co 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 centri-

fuged 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-picrylhydrazyl 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 $EC_{50(\text{DPPH})}$ 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,

the absorbance of the resulting solution was measured at 700 nm in a Metrolab RC 325 spectrophotometer. The higher the absorbance, the greater was the reducing power. The $EC_{50(RP)}$ value, which represents the concentration of extract at which absorbance is 0.5 for the reducing power, was obtained by linear regression analysis.

2.5. Determination of total phenolic compounds

Total phenolic compounds were determined by spectrophotometer, using the Folin–Denis reagent (AOAC, 2000). One milliliter samples of rosemary leaf extracts were placed in 50 ml flasks containing 35 ml of distilled water; 2.5 ml of Folin–Denis reagent were added and, after 3 min, 5 ml of a solution of sodium carbonate (35%, w/v) were added. The mixture was diluted with distilled water, mixed, and after 30 min the absorbance was measured at 760 nm in a Metrolab RC 325 spectrophotometer. The amount of total phenolic compounds was determined by a calibration curve, using tannic acid as standard. The results were expressed in mg tannic acid/g dry rosemary.

2.6. Statistical analysis

For statistical purposes, the effects of the treatment (control and irradiated), the extraction solvent (methanol, ethanol and water) and the extract concentration (0.5, 1, 2, 5, 10, 15, 20, 30 mg dry rosemary/ml solvent) on the scavenging ability of DPPH \cdot and the reducing power were analyzed by triple ANOVA (Zar, 1999). Double ANOVA was used to assess the effect of the treatment and the solvent on EC_{50} values and total phenolic content. All data are expressed as means \pm standard deviation of triplicate measurements. Differences between the means were analyzed by Fisher's least significant test at $\alpha = 0.05$.

3. Results and discussion

3.1. Evaluation of antioxidant activity

3.1.1. DPPH \cdot test

The scavenging activity of control and irradiated rosemary leaf extracts, expressed as inhibition percentage (IP) of DPPH radical, was analyzed in a range of concentrations between 0.5 mg and 30 mg dry rosemary/ml methanol, ethanol or water (Fig. 1). For the control, the IP of the methanol and ethanol extracts increased with concentration between 0.5 mg/ml and 10 mg/ml, whereas, for the water extract, this increase continued up to 15 mg/ml. A comparison of the three solvents shows significant differences in IP only at 2 and 5 mg/ml, in decreasing order methanol > ethanol > water. At extract concentrations of 15 mg/ml, IP was practically constant at its maximum value and no significant differences were observed due to the effect of the solvent ($p > 0.05$). After the application of a 30 kGy dose of gamma radiation, the pattern of change in IP as a function of concentration was similar to that in the control (Fig. 1). However, the IP values of methanol, ethanol and water extracts of irradiated rosemary were higher than those of the control. The analysis of the differences between irradiated and control IP ($IP_I - IP_C$) for the three solvents shows statistically significant increases as a result of treatment for extract concentrations between 15 mg/ml and 30 mg/ml (Table 1). The effect of gamma radiation on the scavenging activity of the DPPH radical has been studied in other products of low aqueous activity. For black pepper treated with doses of between 5 kGy and 30 kGy, the scavenging activity of methanol extracts tended to decrease, mainly immediately after irradiation and one month after treatment (Suhaj et al., 2006). Similarly, pepper ethanol extracts showed a decrease in their DPPH-scavenging ability after application of doses of 20 and 30 kGy (Polovka et al., 2006). The radical-scavenging activity of ethanol and water extracts of non-irradiated Korean medicinal herbs was,

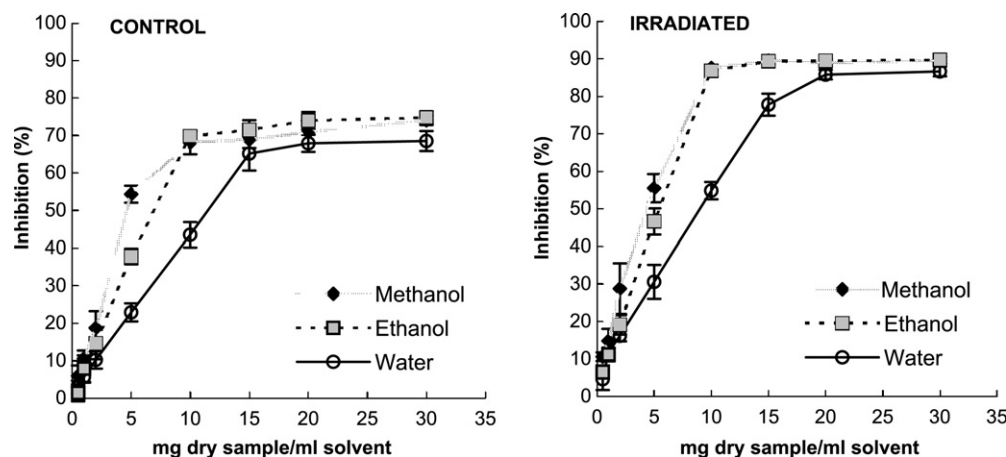


Fig. 1. Scavenging activity of rosemary leaf extracts on the DPPH radical. Values expressed as means \pm SD ($n = 3$).

Table 1
Differences in the inhibition percentage (IP) of extracts of control (C) and irradiated (I) rosemary leaf

mg dry rosemary/ml solvent	IP _I – IP _C		
	Methanol	Ethanol	Water
0.5	4.65	5.09	1.68
1	4.25	3.31	6.10
2	10.0	4.47	5.98
5	1.13	8.80	7.67
10	19.4 ^a	17.0 ^a	11.3
15	20.6 ^a	17.9 ^a	12.6 ^a
20	18.0 ^a	15.5 ^a	17.9 ^a
30	15.5 ^a	14.9 ^a	18.1 ^a

^a (IP_I – IP_C) ≥ 11.4 indicates a significant difference between control and irradiated ($p < 0.05$).

however, indistinguishable from that of samples treated with a dose of 10 kGy gamma irradiation (Byun et al., 1999). According to another report, methanol extracts of irradiated freeze-dried mushrooms did not show significant modifications in their scavenging activity as a result of irradiation doses between 2.5 kGy and 20 kGy (Huang & Mau, 2006). Jo et al. (2003) reported that doses between 10 kGy and 20 kGy applied to ethanol extracts of green tea leaves gave rise to a significant increase in DPPH-scavenging ability immediately after treatment.

3.1.2. Reducing power

The reducing powers of methanol, ethanol and water extracts of control and irradiated rosemary leaf were assessed by the potassium ferricyanide reduction method (Fig. 2). For a range of concentrations between 0.5 mg and 30 mg of dry rosemary/ml of solvent, the reducing power behaviour resembled that observed for IP (Fig. 1). For control rosemary, the reducing power of the extracts increased with concentrations between 0.5 mg/ml and 15 mg/ml. Within this range, the reducing power was affected by the extraction solvent in the order: methanol ≥ ethanol > water. For concentrations above 15 mg/ml, the reducing powers of the three extracts

remained practically constant around their maximum value and no significant differences were observed as a result of the solvents ($p > 0.05$). The reducing power of dry rosemary leaves, as a function of concentration, was studied by Dorman, Peltoketo, Hiltunen, and Tikkanen (2003) only for water extraction. A linear increase ($R^2 = 0.996$) of absorbance at 700 nm was observed for a range of concentrations between 0 mg/ml and 1 mg/ml. Our experimental evidence clearly shows that this linearity is maintained up to 15 mg of dry rosemary/ml of water ($R^2 = 0.995$) and that a 30 kGy dose of radiation produces an increase in reducing power for the whole range of concentrations analyzed (Fig. 2). Table 2 shows the differences between the reducing power of irradiated and control rosemary extracts (RP_I – RP_C), where it can be observed that the reducing power enhancement by the treatment is only significant for the three extracts at concentrations between 10 mg/ml and 30 mg/ml. Increased reducing power, as a result of irradiation, has also been reported for pure compounds, such as phytic acid (Ahn et al., 2004) and for foodstuffs, such as Chinese cabbage (Ahn et al., 2005).

Table 2
Differences in reducing power (RP) of extracts of control (C) and irradiated (I) rosemary leaf

mg dry rosemary/ml solvent	RP _I – RP _C		
	Methanol	Ethanol	Water
0.5	0.034	0.122	0.083
1	0.127	0.244	0.04
2	0.194	0.530 ^a	0.093
5	0.133	0.775 ^a	0.296 ^a
10	0.799 ^a	0.501 ^a	0.545 ^a
15	0.663 ^a	0.821 ^a	0.410 ^a
20	0.864 ^a	0.846 ^a	0.552 ^a
30	0.933 ^a	0.763 ^a	0.777 ^a

^a (RP_I – RP_C) ≥ 0.296 indicates a significant difference between control and irradiated ($p < 0.05$).

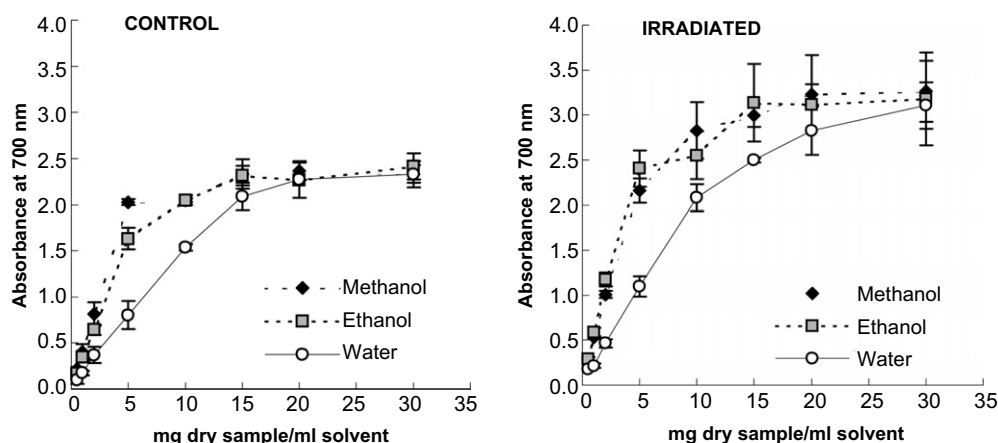


Fig. 2. Reducing power of methanol, ethanol and aqueous rosemary leaf extracts. Values expressed as means ± SD ($n = 3$).

3.1.3. EC_{50} values in antioxidant activity

For better comparison of the antioxidant activity of the rosemary leaf extracts, the results obtained from the DPPH radical experiments and the reducing power experiments were expressed as EC_{50} values. A low EC_{50} value is indicative of strong antioxidant activity.

The $EC_{50(DPPH)}$ values for control and irradiated rosemary were affected by the extraction solvent (Fig. 3). For the control rosemary extracts, the $EC_{50(DPPH)}$ value was significantly lower for methanol (4.63 mg dry rosemary/ml of solvent) than for ethanol and water, whose values were 6.90 and 11.5 mg/ml, respectively. Other studies on the DPPH-scavenging activity of rosemary extracts show that the concentration of extract necessary for a 50% reduction in the concentration of DPPH \cdot is approximately 0.2 mg extract/ml for water and methanol extracts of rosemary leaf (Dorman et al., 2003; Kosar, Dorman, & Hiltunen, 2005). Fig. 3 shows that the effect of 30 kGy gamma rays on $EC_{50(DPPH)}$ was different for each solvent used. Whereas, for water and ethanol rosemary extracts, the $EC_{50(DPPH)}$ values diminished by approximately 22% with respect to the control, the effect was not significant in the methanol extract ($p > 0.05$). Similarly, methanol extracts of mushrooms did not alter their EC_{50} values in the scavenging ability against DPPH radicals after irradiation with doses of 2.5–20 kGy (Huang & Mau, 2006).

The $EC_{50(RP)}$ values for control and irradiated methanol, ethanol and water rosemary extracts are shown in Fig. 3.

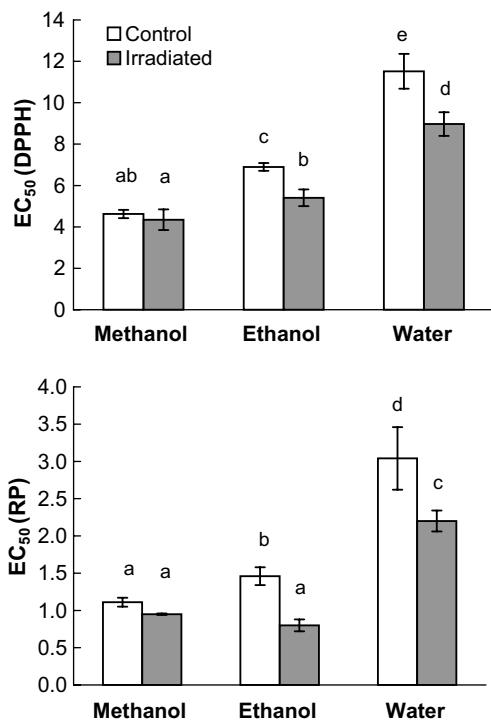


Fig. 3. EC_{50} values (mg dry rosemary/ml solvent) for rosemary extracts according to the DPPH radical test and the reducing power (RP) test. Values expressed as means \pm SD ($n = 3$). Two treatments with the same letter signify that there is no difference at a 5% confidence level.

The control $EC_{50(RP)}$ values differed significantly according to the solvent used for the extraction and showed the following order: methanol (1.11 mg/ml) < ethanol (1.46 mg/ml) < water (3.04 mg/ml). Irradiation significantly affected $EC_{50(RP)}$ values for the ethanol and aqueous extracts, which decreased by 45% and 28%, respectively, with respect to the controls. These results are in agreement with the data reported by Huang and Mau (2006) for gamma-irradiated *Agaricus blazei*. The decrease in the methanol extracts as a result of irradiation was not significant ($p > 0.05$).

On the basis of the results presented here and given the inverse correlation between antioxidant activity and the value of EC_{50} , it can be concluded that irradiation increases the antioxidant activity of ethanol and water extracts of rosemary leaf. A likely explanation for this is that gamma irradiation in dry rosemary induces the formation of new compounds with higher antioxidant activity and which are more soluble in ethanol and water than in methanol. It is known that irradiation produces free radicals with oxidizing capacity (Thakur & Singh, 1994) that could oxidize polyphenols present in rosemary, the latter showing higher antioxidant activity than non-oxidized ones (Kaur & Kapoor, 2001). The presence of such compounds in the ethanol and water extracts of rosemary leaf could partially explain the increase in antioxidant activity observed after treatment with a gamma radiation dose of 30 kGy. Further, studies are required on the composition of the three extracts in order to elucidate the observed differences in antioxidant activity.

3.1.4. Comparison of methods

In order to find out the degree of correlation between the results obtained by the two methods used in the present study to assess antioxidant activity, the EC_{50} values of the DPPH free radical experiment were graphically represented versus the results of the reducing power experiment. As shown in Fig. 4, there is a high correlation between the two methods, both for control and irradiated rosemary ($R^2 > 0.8$ in both cases). This means that the radical-scavenging activity and reducing power trends were similar in methanol, ethanol and water extracts of rosemary leaf.

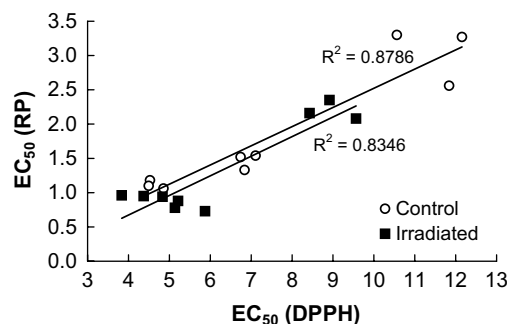


Fig. 4. Correlation between the DPPH \cdot results and the reducing power (RP) results, expressed as EC_{50} values (mg/ml of solvent) of methanol, ethanol and aqueous rosemary extracts.

Although commonly used to determine antioxidant activity *in vitro*, the DPPH[•] test does not closely resemble the biological situation since it is an exogenous radical (Chen, Wu, Shieh, Kuo, & Hsieh, 2006). The reducing power experiment, on the other hand, in terms of the reduction of Fe³⁺–Fe²⁺, appears to be a useful method of assessing antioxidant activity in biological systems, since Fe is a natural metabolic component. Nonetheless, both methods are useful for gaining preliminary information on antioxidant activity, *in vitro*, of rosemary leaf extracts.

3.2. Total phenolic compounds

There are differing reports in the literature on the amount of total phenolic compounds in rosemary. In addition to intrinsic factors in the plant (Del Baño et al., 2003; Hidalgo, Uebera, Tena, & Valcárcel, 1998; Ibañez et al., 2003), other considerations, such as the pre-extraction processing and extraction conditions (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004; Wada et al., 2004) directly affect the concentration of these compounds in the final extract. For dry rosemary leaves, Dorman et al. (2003) reported the concentration in water extracts to be 185 mg gallic acid equivalents/g of extract. Similar values were reported by Kosar et al. (2005) for methanol extracts. Working with methanol extracts of herb leaves and stems, Shan, Cai, Sun, and Corke (2005) found a phenolic concentration of 5.07 g gallic acid equivalents/100 g of herb (dry weight). For fresh rosemary extracts obtained with phosphate buffer, Zheng and Wang (2001) reported a concentration of 2.19 mg gallic acid equivalents/g herb (fresh weight). According to Rababah, Hettiarachchy, and Horax (2004), phenolic extraction with a mixture of acetone:methanol:water:formic acid gives a concentration of 92.5 mg chlorogenic acid equivalents/g herb (dry weight).

The results of the current paper, show the effect of solvent extraction and ionizing irradiation on the concentration of total phenolic compounds in rosemary leaf extracts, expressed as mg equivalents of tannic acid/g dry rosemary (Fig. 5). Solvent extraction significantly affected the concentration of total phenols in control and irradiated

rosemary. In both cases, the concentration decreased in the order: methanol > ethanol > water. It can be inferred from these results that methanol is the most efficient solvent for extracting phenolic compounds from control rosemary leaves and from those decontaminated by gamma irradiation. Little information is available in the literature on the effect of ionizing radiation on rosemary phenolic compounds. Koseki et al. (2002) reported a decrease, with respect to controls, of the amount of total phenolic compounds in dehydrated rosemary after irradiation doses of between 10 kGy and 30 kGy, although details of the extraction methodology were not given. From the analysis of our results (Fig. 5) it can be concluded that a 30 kGy dose of gamma radiation applied to dry rosemary leaves does not significantly affect total phenolic content in the methanol and ethanol extracts. In the water extract, however, a significant increase, in the order of 35%, was observed in the concentrations of these compounds after treatment. Taking into account the chemical composition of rosemary (Del Baño et al., 2003; Ibañez et al., 2003; Suhaj, 2006), we can hypothesize that this increase in total phenolic content is due to the presence of water-soluble quinone-type compounds, resulting from the direct effect of gamma rays on the characteristic phenolic diterpenes of rosemary. The increase in quinone radical observed by Calucci et al. (2003) in aromatic spices and herbs irradiated with 10 kGy, including Lamiaceae herbs, supports this hypothesis. Increases in phenolic compound content after irradiation with sanitization doses have been reported for other dehydrated plant materials. Huang and Mau (2006) reported a higher content of tocopherols in irradiated than in non-irradiated lyophilized mushrooms. Variyar et al. (1998) found increased amounts of phenolic acids in irradiated cloves and nutmeg, and Topuz and Ozdemir (2004) reported an increase in capsaicinoid content in irradiated paprika. These increases were associated with the degradation of tannins (Variyar et al., 1998) and changes in the conformation of the molecules (Topuz & Ozdemir, 2004) as a result of the irradiation treatment, with a consequent improvement in the extraction yield of the phenolic compounds analyzed.

Various authors have reported a correlation between phenolic content and antioxidant activity. Velioglu, Mazza, Gao, and Oomah (1998) reported a significant correlation coefficient between total phenolic content and antioxidant activity in selected fruits, vegetables and grains. Kähkönen et al. (1999), on the other hand, found that the antioxidant capacity of vegetable extracts is not necessarily related to a high phenolic compound content. Our results suggest, that for control rosemary leaf extracts, there is a good correlation between total phenolic content and antioxidant activity ($R^2 = 0.844$ in the DPPH[•] test and $R^2 = 0.720$ in the reducing power test). However, in the case of irradiated rosemary, phenolic compounds appear to be not the only compounds responsible for the antioxidant activity of the extracts ($R^2 = 0.638$ in the DPPH[•] test and $R^2 = 0.409$ in the reducing power test).

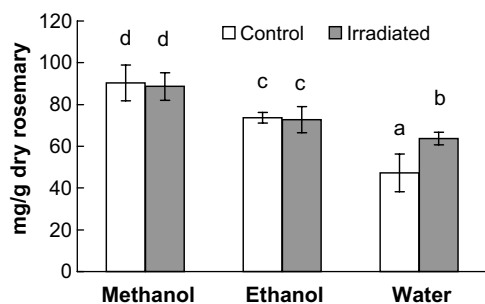


Fig. 5. Effect of irradiation (30 kGy) and extraction solvent on the total phenolic content in extracts from rosemary. Values expressed as means \pm SD ($n = 3$). Two treatments with the same letter signify that there is no difference at a 5% confidence level.

4. Conclusions

The antioxidant activity in vitro and total phenolic content were assessed in methanol, ethanol and water extracts of control and irradiated (30 kGy) dry rosemary leaves. It was confirmed that rosemary extracts can act as hydrogen and/or electron donors and react with free radicals, converting them into more stable products and in this way terminating radical chain reactions. The methanol extracts exhibit a higher level of antioxidant activity and a higher phenolic content, whether they are irradiated or not. Gamma radiation improved antioxidant activity in ethanol and water extracts and increased total phenolic content in water extracts of rosemary leaf, whereas irradiation treatment had no significant effect on the methanol extracts. Therefore, the type of solvent used in the extraction process plays a key role in assessing the impact of irradiation on antioxidant activity and total phenolic content of rosemary leaf extracts. Gamma radiation reduces the degree of correlation between antioxidant activity and total phenolic content of the extracts, indicating that phenolic compounds are not solely responsible for the antioxidant activity of rosemary leaf extracts.

Aside from being safe from the microbiological point of view, sanitizing dry rosemary with gamma radiation gives rise to extracts with improved antioxidant properties which could be of use in the food and pharmaceutical industries.

Acknowledgements

The authors are indebted to Mr. R. Camina for carrying out the statistical analyses. Thanks are due to the Comisión Nacional de Energía Atómica for carrying out the processing. This work was supported, in part, by the Secretaría de Ciencia y Tecnología of the Universidad Nacional del Sur.

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