The effects of γ-irradiation on the antioxidant activity of rosemary extract

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The effect of high energy radiation (γ-rays) on rosemary plant extract activity is presented. The increase in the stabilization action of this natural extract was revealed by isothermal chemiluminescence procedure. The moderate dose applied to rosemary extract promoted significant growing in the total antioxidant activity and the improvement in the stabilization efficiency found in rosemary extract/paraffin system. The comparison between γ-irradiated rosemary extract (soon from irradiation and after 30 days of storage) and some commercial phenolic compounds (BHT and IONOX 100) regarding the progress of oxidative protection occurred in organic substrate has been emphasized the elevated protection in the radiation treatment promoted by natural antioxidant products. For the thermal oxidation of paraffin the improvement in the oxidation induction time of 4.8 times (additive concentration of 0.25 %) and of 10.8 times (additive concentration of 1.50 %) and the diminution by 1.35 to 3.25 times for the 0.25 % and 1.50 % respectively, of additive concentrations were obtained.

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1. Introduction

Gamma irradiation induces oxidative stress in organic substrate by generating reactive oxygen species such as hydroxyl ($HO^{\cdot}$), peroxyl ($RO_2^{\cdot}$) and superoxid anion ($O_2^{\cdot-}$) radicals which react rapidly with macromolecules such as proteins, lipids and nucleic acids damaging of wide range of essential components [1]. Numerous studies have examined the radioprotective effects of antioxidant compounds known as free radical scavengers. These compounds protect cells and their organic constituent molecules against free radical damage [2-4]. The appropriate references reveal enormous interest on the radioprotecting property of herbal extracts because of their ability in the scavenging of free radicals [5-9].

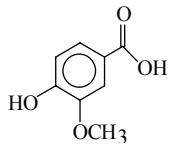
Rosemary is reputed to be one of the richest sources of potent antioxidants consisting in phenolic acids (e.g. vanillic, caffeic, chlorogenic, rosmarinic acids), phenolic diterpenes (e.g. carnosol, rosmanol, isorosmanol, carnosic acid) and flavonoids (e.g. naringenin, apigenin, luteolin, diosmin, hispidulin, genkwanin, cirsimaritin). The compounds of interest in the present study are presented in Fig 1. The general view on the composition of rosemary extract reveals the main constituents expressed as % by weight of dry vegetal matter: carnosic acid: 2.33, carnosol: 3.16, rosmarinic acid: 2.59 [10-15].

Various studies have been performed on the radical – scavenging activity of rosemary and on the influence of gamma irradiation upon antioxidant content [16-18]. Haraguchi et al [19] reported the inhibition of superoxide and lipid peroxidation by four diterpenoids from rosemary, *i. e.* carnosic acid, carnosol, rosmanol and epirosmanol. Del Bano et al [20] investigated the efficiency of carnosic acid, carnosol and rosmarinic acid as radioprotectors against damage induced by gamma radiation [21]. The mechanism under which rosemary act in the screening of human body against high energy exposure was not elucidated.

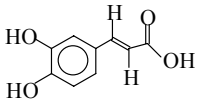
Our previous investigation on the antioxidant activity of carnosic acid and some of its derivatives playing the role of very efficient protectors has pointed out the significant improvement in stabilization of low density polyethylene during thermal ageing [22].

The purpose of this study is the exploration on the antioxidant property of rosemary extract after γ-irradiation using isothermal chemiluminescence method. The changes occurred in powder rosemary extract by gamma irradiation and the consequence of subsequent storage after exposure were envisaged. Previously reported results on the radiation effects on plant materials [23, 24] have highlighted the requirement of radiochemical studies on plant products for assessment of effect scale. In alive organisms subjected to ionizing radiation, acute and chronic diseases accompanying the damage in tissue and cell are developed depending on dose and exposure time. Antioxidants are known for their ability to scavenge the free radicals and to protect living systems during radiooxidative processes [2].

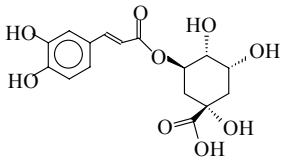
Phenolic acids



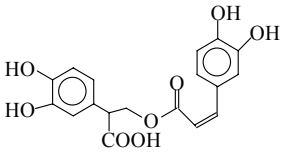
Vanillic acid



Caffeic acid

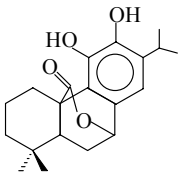


Chlorogenic acid

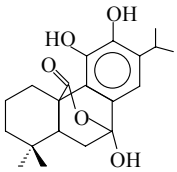


Rosmarinic acid

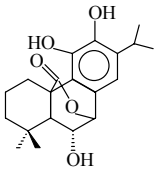
Phenolic diterpenes



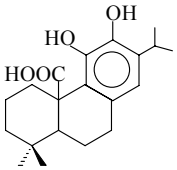
Carnosol



Rosmanol

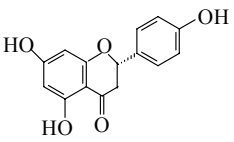


Isorosmanol

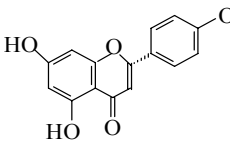


Carnosic acid

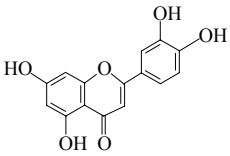
Flavonoids



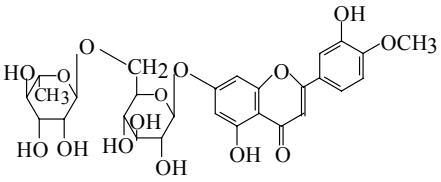
Naringenin



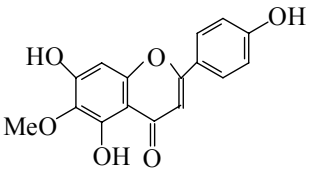
Apigenin



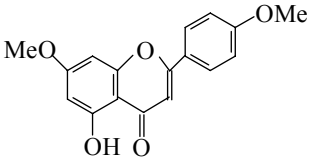
Luteolin



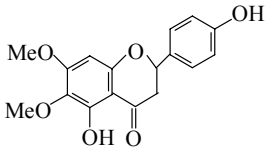
Diosmin



Hispidulin



Genkwanin



Cirsimaritin

Fig. 1. Structures of the most important natural antioxidants involved in the protection of alive bodies.

2. Experimental

Fresh rosemary leaves were collected, cleaned, dried and powdered in a grinder. The vegetal material (each charge weighting 50 g of dry matter) was then refluxed in 2 liters of ethanol at 65 – 75 ^{0}C for 10 h during continuous extraction in Soxhlet apparatus. The ethanolic extract (ethanol of analytical grade purity) was subjected to precipitation by non-solvent method and solid phase was finally filtered and dried under vacuum. The dry rosemary solid was added into neat paraffin at a concentration of 0.25 % w/w. All reagents were of analytical grade purity.

Isothermal oxyluminescence determinations were performed in air at 165 ^{0}C in an oxyluminograph OL-94 instrument. Details on this equipment and on the measurement procedure have been previously presented [25]. The meanings of kinetic parameters that are evaluated in this paper are presented in Fig. 2.

The total antioxidant activity of the irradiated extract samples was measured using a flow injection analysis

method based on the chemiluminescence (CL) reaction of luminol with H_2O_2 in the presence of Co (II) ions and EDTA [Pajero et al., 2000; Giokas et al., 2007].

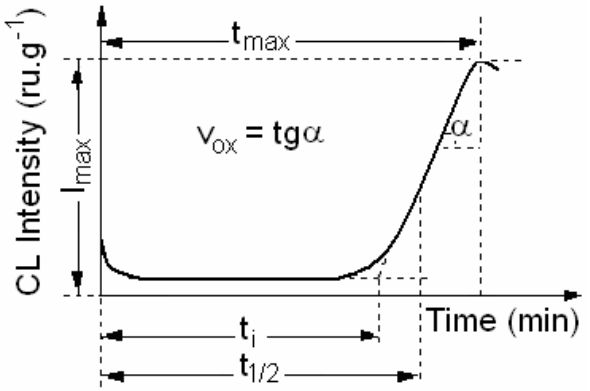


Fig. 2. The main kinetic parameters obtainable from chemiluminescence diagram: t_i, oxidation induction time; $t_{1/2}$, time required for attending half of maximum CL intensity; v_{ox}, oxidation rate in the propagation stage of degradation; t_{max}, time elapsed from the start of CL measurement until the maximum intensity is achieved; I_{max}, maximum CL intensity.

Under these reaction conditions the concentration of the catalyst involved in the chemiluminescence emission, Co (II) ions, was very low owing to complexation. Thus, it was possible to record a not too high, but constant chemiluminescence signal. In Fig. 3 the flow injection (FI) assembly for chemiluminescence determination of antioxidant activity is presented. The analyzed sample was injected into a carrier of borate buffer which joints a flux of hydrogen peroxide and Co (II)/EDTA/luminol solution in the chemiluminescence flow cell placed just in front of the photomultiplier tube.

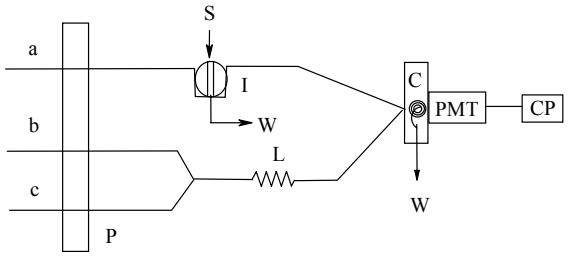


Fig. 3. Experimental set-up for flow injection – chemiluminescence determination. tracks: (a) carrier solution (0.05 M sodium borate buffer, pH 9); (b) H_2O_2 solution (2.10^{-4} M); (c) Co (II)/EDTA/luminol solution.

By pumping these solutions through the channels (a), (b) and (c) of the assembly (Fig. 3) a constant chemiluminescence emission could be recorded. When a sample containing an antioxidant was injected into the flux (a), the recorded chemiluminescence signal decreased. The level of the diminishing in CL intensity was directly related to the concentration of oxidation inhibitor and to the antioxidant activity of the sample components. A calibration curve representing the percentage decrease of chemiluminescence signal *versus* the concentration of antioxidant was drawn. A solution of caffeic acid in 80 % ethanol was used as an antioxidant standard. The method allows the determination of caffeic acid in the domain 2.5 – 300 μM. The antioxidant capacity of the analyzed samples has been reported in caffeic acid equivalents.

Irradiation was carried out in air by γ-ray exposure in a ^{137}Cs GAMMATOR M-38-2 installation. The dose rate was 0.4 kGy/h.

3. Results and discussion

Fig. 4 shows the chemiluminescence (CL) spectra recorded for irradiated rosemary extract in paraffin at different concentration after irradiation at 40 kGy. The kinetic analysis of chemiluminescence data was done on the basis of following parameters (Table): induction time (t_i), time required for attending half of maximum CL intensity ($t_{1/2}$), maximum oxidation rate (V_{ox}^{max}), maximum CL emission intensity (I_{max}) and the time corresponding to the maximum CL emission value (t_{max}).

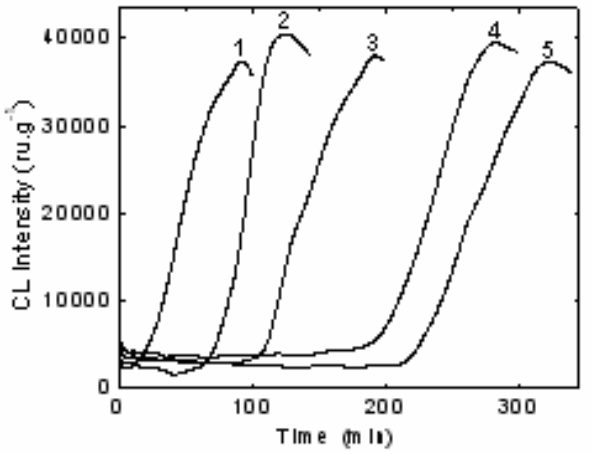


Fig. 4. CL spectra of thermally degraded paraffin (180^0C, air) in the presence of different concentration of irradiated rosemary extract (40 kGy). (1) pristine paraffin; (2) 0.25 wt %; (3) 0.50 wt %; (4) 0.75 wt % ; (5) 1.50 wt %.

Zlatkevich [28] pointed out that the critical hydroperoxide concentration is reached when 50 % of compound would be oxidized. Starting from this assumption, the time corresponding to $I_{CL} = 0.5\ I_{max}$ was also determined. In Table this parameter ($t_{1/2}$) is included.

As it was expected, the rosemary extract inhibited paraffin degradation at 168^0C by trapping radicals existing in the oxidizing system. This action is proved by the increase values of t_i, $t_{1/2}$ or t_{max} and the diminution in V_{ox}^{max} relative to blank sample. Therefore, the improvement in the antioxidant features is highlighted by the enhance in the oxidation induction time of 4.8 times

(additive concentration of 0.25 %) and of 10.8 times (additive concentration of 1.50 %) in respect to pristine paraffin. Under similar experimental conditions, the oxidation rate of paraffin modified with rosemary extract was diminished by 1.35 to 3.25 times for different additive concentrations (0.25 % and 1.50 % respectively). The comparison between the maximum intensities in the time dependencies of chemiluminescence signal recorded for paraffin stabilized with 0.25 % and 1.50 % rosemary extract has pointed out the protector role of additive in its action on the inhibition of oxidation lowing them down to 1.32 and 1.81 times, respectively. This behavior illustrates the falling down of hydroperoxide level. Rosemary extract acts as peroxyl radical chain interrupter and it may be proposed as potential substitute of chemically synthesized antioxidants. Fig. 5 illustrates the real benefit in the stabilization of organic materials by natural antioxidants at the increasing amounts of additive.

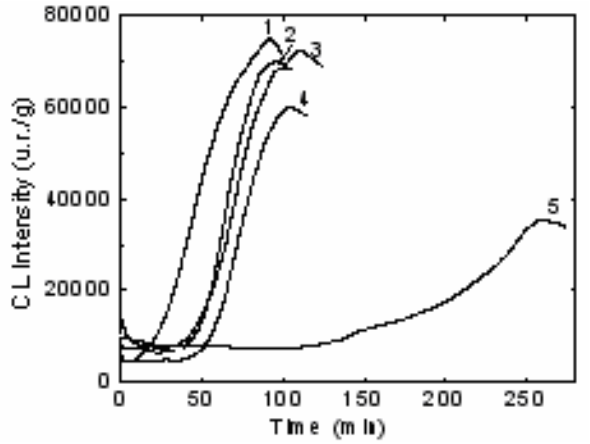


Fig. 5. CL spectra of thermal degraded paraffin (180^{0}C, air): free of additive (1) and substrate containing 0.25 wt % of BTH (2), unirradiated rosemary extract (3); IONOX – 100 (4) and 2.5 kGy γ-irradiated rosemary extract (30 days of storage). Concentration 0.25 wt %.

The relevant efficiency for oxidative stabilization of 30 kGy irradiated rosemary attended 59 % and 120 % for the exposures to 15 and 30 days, respectively.

The antioxidant activity in rosemary has been ascribed to different diterpenes such as carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol and methyl carnosate, as well as to some flavonoids and other phenolic compounds (Fig. 1) [11, 29-32]. It was reported that carnosic acid is associated with the highest antioxidant activity of rosemary [21].

The effect of storage on the chemiluminescence response of irradiated rosemary extract added to paraffin was followed. The dependence of CL response on absorbed dose reveals the beneficial consequence of storage upon the oxidation induction time for the rosemary extract exposed to other doses exceeding 2.5 kGy (Fig. 6). This storage period produced an enhancement in the CL response, which confirms the contribution of high energy irradiation to the enhancement of antioxidant activity of rosemary extract.

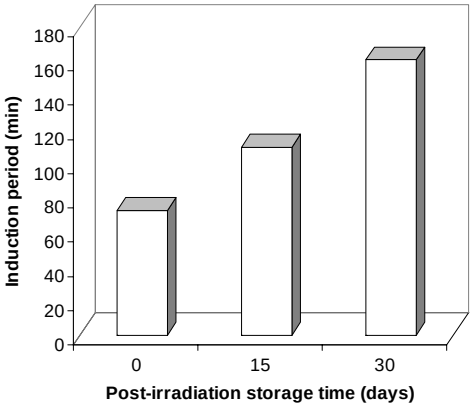


Fig. 6. Effect of storage time on CL induction period for 5 kGy γ-irradiated rosemary extract. (additive concentration 0.25 % w/w).

As a confirmation of helpful effect of radiation treatment applied to spice extracts, our preliminary extended investigations on the increase in the inhibitive activity of γ-irradiated sage extract have revealed the longer oxidation induction times (OITs) obtained for the exposure to 2.5 and 5 kGy. This kinetic parameters becomes 70 % and 340 % higher in comparison with the OIT value of unexposed material.

The maximum increase in the total oxidative capacity (TAC) was observed at the dose of 5 kGy (Fig. 7). The slight descended values of TAC emphasize that the equivalent antioxidant activity for the irradiation at 2.5 can be obtained for doses closed to 40 kGy.

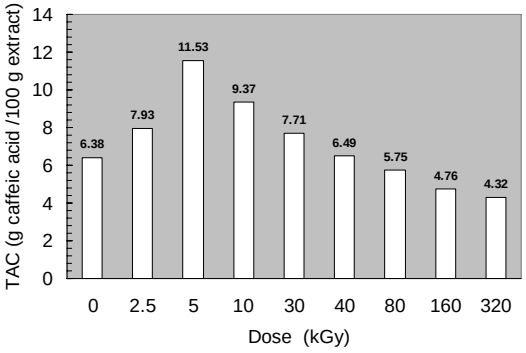


Fig. 7. Dose dependence of total antioxidant capacity (TAC) of γ-irradiated rosemary extract.

The existence of various molecular configurations with antioxidant features can be supposed because the remanent stabilization behavior validates the hypothesis of the existence of active intermediates starting from initial antioxidant structures. The improvement in the total antioxidant capacity (TAC) of irradiated rosemary extract on lower dose range would be the consequence of modifications in molecular functionality, which accompany other induced effects like the low proportion of structure damage. However, the following smooth

diminishing occurred in the antioxidant capacity value over 5 kGy would be explained by the higher rate of damage in respect with the contribution of favorable changes.

The antioxidant has previously reported for this kind of protection system [33]. It is very interesting from the practical viewpoint of the efficiency in the antioxidant activity of the plants, because new antioxidative compounds such as carnosol are produced during cascade evolution (Fig. 8) [34]. Carnosol also blocks a free radical becoming rosmanol. Rosmanol continues the free radical scavenging untill gradosol is created and the later continues the protection process by radical scavenging. The carnosic acid quinone has not antioxidant activity [35]. The presence of such compounds in the extracts of rosemary would explain the increase in activity of oxidation inhibition promoted by rosemary extract, which was observed after exposure to gamma radiation.

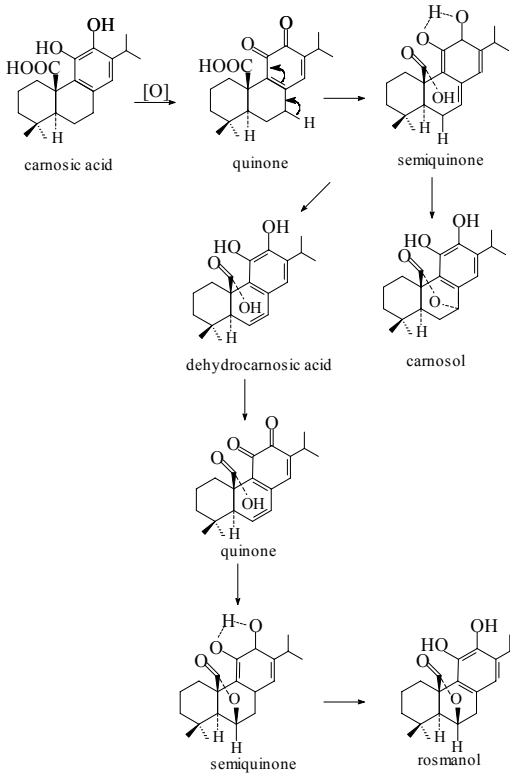


Fig. 8. Cascade mechanism in the oxidation reactions of carnosic acid.

The cascade mechanism can be successfully applied for other natural antioxidant compounds belonging to the class of diterpenoids, for example: rosmarinic acid, vanillic acid, chlorogenic acid and many other similar structures.

The correlation between the favorable consequences of neat rosemary extract in the treatment of cancer [36, 37] and antirad action of this powder [8, 9] allows the significant diminishing of radiation effects on the irradiated carcinogenic patients.

4. Conclusions

The exposure to γ-radiation at low doses induces an increase in the antioxidant activity of rosemary extract. The intermediates formed in the oxidation chain of primary compounds through cascade mechanism bring about additional protection enhancing the most important kinetic parameter, for example, oxidation induction time with 59 % and 120 % for 15 and 30 days of storage the after exposure to 2.5 kGy, respectively in the case of rosemary extract and with 70 % and 340 % for the exposures to 2.5 and 5 kGy, respectively in the case of sage extract. The differences between the extracts subjected to γ-irradiation and pristine additive can be ascribed to the unlike proportion between these protective blends constituents of natural antioxidant systems, which ensure high level of radical scavenging. By □-irradiation, rosemary extract can provide real protection against the oxidation of organic environment, where it plays the role of degradation inhibitor. On the other hand, the most important application of this rosemary extract modified by high energy irradiation is the protection of cancer patients' irradiated tissues during their radiation therapy The irradiation stage foreseen in the production technology of more efficient rosemary extract may be included. In the protection offered by irradiated rosemary extract even at 30 kGy, the total capacity reached similar value corresponding for 5 kGy. It means that on the dose range of 2.5 – 30 kGy, γ-irradiated rosemary extract is characterized by improved stabilization efficiency in respect with untreated material.

Rosemary extract is one of the best natural antioxidant systems that manage very efficient defense in the delaying oxidative ageing caused by various energetic and environmental agents in living world.

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