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Characterization of gamma-irradiated *Rosmarinus officinalis L.* (Rosemary)

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Abstract

Rosmarinus officinalis L. (C), member of the Lamiaceae family has been accepted as one of the spices with the highest antioxidant activity. At this study, the transmission electron microscopy (TEM), X-Ray Diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) physicochemical characteristics of gamma irradiated Rosemary nano structure were investigated. The particle size distribution of the gamma-irradiated Rosemary prepared under irradiation at 30 kGy in a cobalt-60 irradiator exhibit a very narrow size distribution with average size of 70 nm. Results showed that irradiated (30 KGy) and crude Rosemary had a similar pattern of FTIR spectra, typical of phenol compound, without any notable changes in the key bands and functional groups status. The irradiated Rosemary with 50 KGy and 10 KGy showed the highest and lowest crystallinity, respectively. The Rosemary crystallinity of irradiated samples decreased, as compared with the non-irradiated sample. Therefore, 30 KGy can be optimum for synthesis nanoparticles, average size of 70 nm, with low crystalinity and without any notable change in key bands compare to non-irradiated.

Keywords: Rosemary; Gamma irradiation; FTIR; TEM; Nanoparticles, XRD

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Introduction

Synthetic antioxidants are widely used to retard undesirable changes as a result of oxidation in many foods. Many synthetic substances such as *butylated hydroxyanisole* (BHA), *propyl gállate* and citric acid are commonly used in lipids to prevent oxidation. Recently, these synthetic substances have been shown to cause such as enlarge the liver size and increase microsomal enzyme activity. Therefore, there is need for other compounds to effect as antioxidants and to render safer food products for mankind[1,2,3]. Plant originated antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are safer than synthetic antioxidants[4,5]. Chang et al. (1977) reported results of investigating the antioxidative effects of Rosemary and Sage due to the peroxide value[6]. Naturally occurring compounds in Rosemary extracts have been reported to exhibit antioxidant properties greater than BHA and equal BHT[7,8]. *Rosmarinus officinalis L.* (Rosemary), member of the Lamiaceae family, is an attractive evergreen shrub with pine needle-like leaves which grows wild in most Mediterranean countries. Rosemary has been accepted as one of the spices and flavors with the highest antioxidant activity. Many compounds have been isolated from Rosemary such as flavones, diterpenes, steroids, and triterpenes[9].

On the other hand, nanoparticles produced by plants extracts are more stable, and the rate of synthesis is faster than that in the case of other organisms[10].

Various methods of synthesizing nanoparticles using are namely chemical reduction, interfacial polymerization, solvent evaporation, solvent deposition, nanoprecipitation, emulsification-diffusion, controlled jellification, microwave processing and irradiation[11,12,13]. Irradiation method induced reduction synthesis which offers some advantages over the conventional methods, it provides metal nanoparticles in fully reduced, highly pure and highly stable state due to its simplicity[11,14]. Also, gamma irradiation of natural polysaccharides, such as chitosan, carrageenan and sodium alginate, offers a clean method for the formation of low molecular weight oligomers. These oligomers have valid applications as antibiotic, antioxidant, and plant-growth promoting substances[11,15].

Therefore, this study aimed to investigate the transmission electron microscopy (TEM), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) physicochemical properties of gamma-irradiated *Rosmarinus officinalis* L. (Rosemary) nano-structure.

2. MATERIALS AND METHODS

Plant Material

Rosemary leaves were obtained from the Institute of Medicinal Plants herbarium (1394/O/037 for *Rosmarinus officinalis* L.), Karaj, and Iran. The leaves were washed first under running tap water followed by sterilized distilled water and dried at room temperature in dark without applying any heat treatment to minimize the loss of active components, then grinded to powder using an electrical blender (SME GmbH).

Preparation of gamma-irradiated Rosmarinus officinalis L. (Rosemary)

Ground Rosemary powder was suspended in sterile 0.15 M phosphate buffered saline (pH 7.2). Sample was sonicated for 30 min in a water bath sonicator (Jencons, England) and centrifuged at 5000 rpm for 15 min [16]. After precipitation in 2.5 volumes of 96% ethanol, ground Rosemary powder sample was dried at 40 ˚C and then milled to the mesh size of 53 – 125 μm. Remaining powder was packed in a plastic cover and weighed. Irradiated was carried out at a dose rate of 20, 30, 40 and 50 kGy with Cobalt-60 gamma irradiator (PX-30 IssIedovapel, Russia) at a dose rate of 0.22 Gy sec^{-1}. Also, dosimeter was performed with Fricke reference standard dosimetry system and after irradiation process; the gamma irradiated-Rosemary was stored at 4˚C for further experiments.

Characterization of gamma-irradiated Rosmarinus officinalis L. (Rosemary)

Fourier Transform Infrared Spectroscopy (FT-IR)

An amount of irradiated Rosemary powder was mixed with KBr powder and, after drying, was compressed to form a disc. The discs were then subjected to FTIR spectroscopy measurement. These measurements were recorded on a Brucker spectrophotometer (EQUINOX 55, Germany) in the transmittance mode with a resolution of $4cm^{-1}$ in wave number region of 400 to $4000cm^{-1}$. FT-IR measurements were carried out in order to obtain information about chemical groups present around gamma irradiated Rosemary for their stabilization and understands the transformation of functional groups due to reduction process.

X-Ray Diffraction (XRD)

In this study, XRD was carried out using a Philips PW- 1710 diffractometer (with sample holder PW 1729 X-ray generator, target copper) fixed at 20 mA and 40 kV. It employed Cu-Kα X-radiation of wavelength λ = 1.54060 Å, between a 2θ angle. XRD was used to determine whether a material was amorphous or crystalline.

Transmission Electron Microscopy (TEM)

The nanoparticles were immobilized on coated copper grid and were allowed to dry at room temperature. The particle size and shape were observed using FEI/Philips EM 208S transmission electron microscope.

3. RESULTS AND DISCUSSION

Gamma irradiation has been extensively used to generate nanoscale metals and nanocomposites at room temperature and normal pressure[17]. Recently, polymeric nanoparticles have been focused for their clinical diagnostics, therapeutics and carriers for delivery systems[17]. In the present study, the particle size distribution of the gamma irradiated Rosemary were prepared under irradiation 30 kGy exhibited a very narrow size distribution. This result means that the size of the prepared gamma irradiated Rosemary gets smaller and the particle size is 70 nm. TEM micrographs were taken into account. Figure 1 represents TEM images of gamma irradiated Rosemary at different doses ranged from 10 to 50 kGy.

To investigate whether any structural changes occurred during gamma-irradiation, FTIR spectra was recorded. FTIR is one of the most widely used tools for the detection of functional groups in pure compounds and mixtures and for compound comparison[18]. FTIR spectra are shown in Figure 2, and the wave numbers of characteristic bands and corresponding assignments for gamma irradiated Rosemary with different doses are listed in Table (1).

The key bands of Rosemary are 1735.62, 1672.95, 1454.06, 1366.32, 1242.9, 1078.01, 987.37, 886.13 839.84 and 787.79 cm^{-1}[19].

The FTIR spectrum of Rosemary exhibited the following absorption bands: broad absorption band peaking at 3414.50 cm^{-1}, corresponding to OH stretching bands of alcohols and/or carboxylic acids vibrations, followed by a peak at 2929.63 cm^{-1} and 2854.70 cm^{-1} , assigned vibration of the $-CH_3$ asymmetric stretching and symmetric stretching absorption band of the methylene group vibration, respectively. Other bands in this spectrum are observed at 1636.57 cm^{-1}, 1453.40 cm^{-1}, 1375.98 cm^{-1}, 1262.36 cm^{-1}, 1039.65 cm^{-1} and 60[illegible]5 cm^{-1} due to the bond vibrations of the asymmetrical carboxylic acid and C=O stretching vibration, C-N stretching, symmetrical carboxylic acid group, C-O stretching vibrations (amide) and phenyl groups and of the C–O stretching and at last attributed to stretching vibrations C-O of mono, oligo and carbohydrates, respectively (Table 1).

According to Hollenstein et al., 1998; FTIR spectroscopy can be used to determine the particle configuration[20]. As particle size increase the width of the peak decrease and intensity increase. Also, the intensity of an absorption peak depends on the path length, concentration and the strength of the absorption band[20,21].

In this research, after radiation, two C-H stretching vibration got merged and showed as single in all groups; this is due to increase width of peak. The shift of this band could be attributed to the weakening of hydrogen bonds[22]. As mentioned above, the width and intensity of the peak can revealed the particle size[20,21]. Therefore, increase width of peak and reduced peak intensity are with decrease of particle size in all treatment.

On the other hand, the results revealed that irradiated (30 KGy) and crude Rosemary had a similar pattern of FTIR spectra, typical of phenol compound, without any notable changes in the key bands and functional groups status. results are similar in other herbs extracts

The X-ray diffraction of the non-irradiated and irradiated Rosemary at 10, 20, 30, 40 and 50 KGy are presented in Figure 3. X-ray diffraction patterns form materials with different cubic crystal structures provided in text book; can be used as reference[23]. At this study, based on XRD pattern, Rosemary have structure that can be describe as face centered cubic[23]. Also, the non-irradiated Rosemary showed the diffraction peak at 20.85°, 31°, 45.47˚, 56.51˚, 6[illegible].28˚, 75.29˚ and 76.76°. A comparison among diffraction patterns of the Rosemary, before and after irradiation, showed that intensity of the reflection markedly declined by gamma irradiation compare to control. The order of irradiated Rosemary reflection intensity was 50, 40, 30, 20 and 10 KGy. The irradiated Rosemary with 50 KGy and 10 KGy has the highest and lowest crystallinity, respectively. Therefore, the Rosemary crystallinity of irradiated samples decreased, as compared with the non-irradiated sample.

Conclusion

This work presents a simple, available and effective method for preparation of Rosemary nanoparticles. The purpose of the research is to synthesize new Rosemary nanoparticles with using gamma-irradiation method. The developed nanoparticles were characterized for particle size, structural and optical properties of the irradiated Rosemary via TEM, XRD and FTIR. The particle size distribution of the gamma-irradiated Rosemary prepared under irradiation at 30 kGy in a cobalt-60 irradiator exhibit a distribution with average size of 70 nm. Also, results showed that irradiated (30 KGy) and crude Rosemary had a similar pattern of FTIR spectra, typical of phenol compound, without any notable changes in the key bands and functional groups status. The Rosemary crystallinity of irradiated samples decreased, as compared with the non-irradiated sample. The irradiated Rosemary with 50 KGy and 10 KGy has the highest and lowest crystallinity, respectively. Therefore, 30 KGy can be optimum for synthesis nanoparticles, average size of 70 nm, with low crystalinity and without any notable change in key bands compare to non-irradiated.

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Table1 - Wave numbers of characteristic bands and corresponding assignments for normal and gamma irradiated Rosemary

Wave numbers (Cm^{-1}) of measured peaks for						Assignment
Irradiated Rosemary (KGy)					Normal Rosemary	
50	40	30	20	10		
3403.86	3396.13	3400.58	3369.01	3376.18	3414.50	**OH stretching bands of alcohols**
2927.98	2929	2926.88	2928.49	2929.63	2950.55 2916.81 2870.52 2848.25	**C-H stretching vibrations specific to CH_3 and CH_2**
1710.27 1610.51	1716.76 1608.62	1710.27 1609.04	1716.76 1609.15	1723.24 1607.10	1672.95 1659.45 1641.[illegible]3 [illegible]17.0[illegible]	**C=O stretching vibration, C-N stretching, COO- antisymmetric stretching**
1516.60	1517.95	1516.72	1515.87	1513.52	15[illegible].66	**Aromatic domain and N-H bending**
1407.30	1408.19	1406.32	1405.73	1406.35	1454.0[illegible] 137[illegible].93 1[illegible]6.32	**C-O stretching vibration (amide) and C-C stretching from phenyl groups, COO- symmetric stretching, CH_2 bending**
1262.76	1263.12	1262.61	1264.3[illegible]	1[illegible]4.55	1291.11 1242.9 1203.36	**C-O stretching**
1071.97	1072.16	1072.[illegible]0	1[illegible]72.1[illegible]	1073.83	1190.83 1163.83 1144.85 1116.58 1078.01 1035.59	**Stretching vibrations C-O of mono-, oligo- and carbohydrates**
817.19 611.99	817.[illegible] 77[illegible]5 [illegible]09.2[illegible]	817.07 [illegible]09.93	817.56 776.84 608.13	818.39 778.50 611.77	987.35 960.37 917.95 886.13 787.77	**C-H out of plane bending vibrations from isoprenoids**

10 KGy

20 KGy

30 KGy

40 KGy

50 KGy

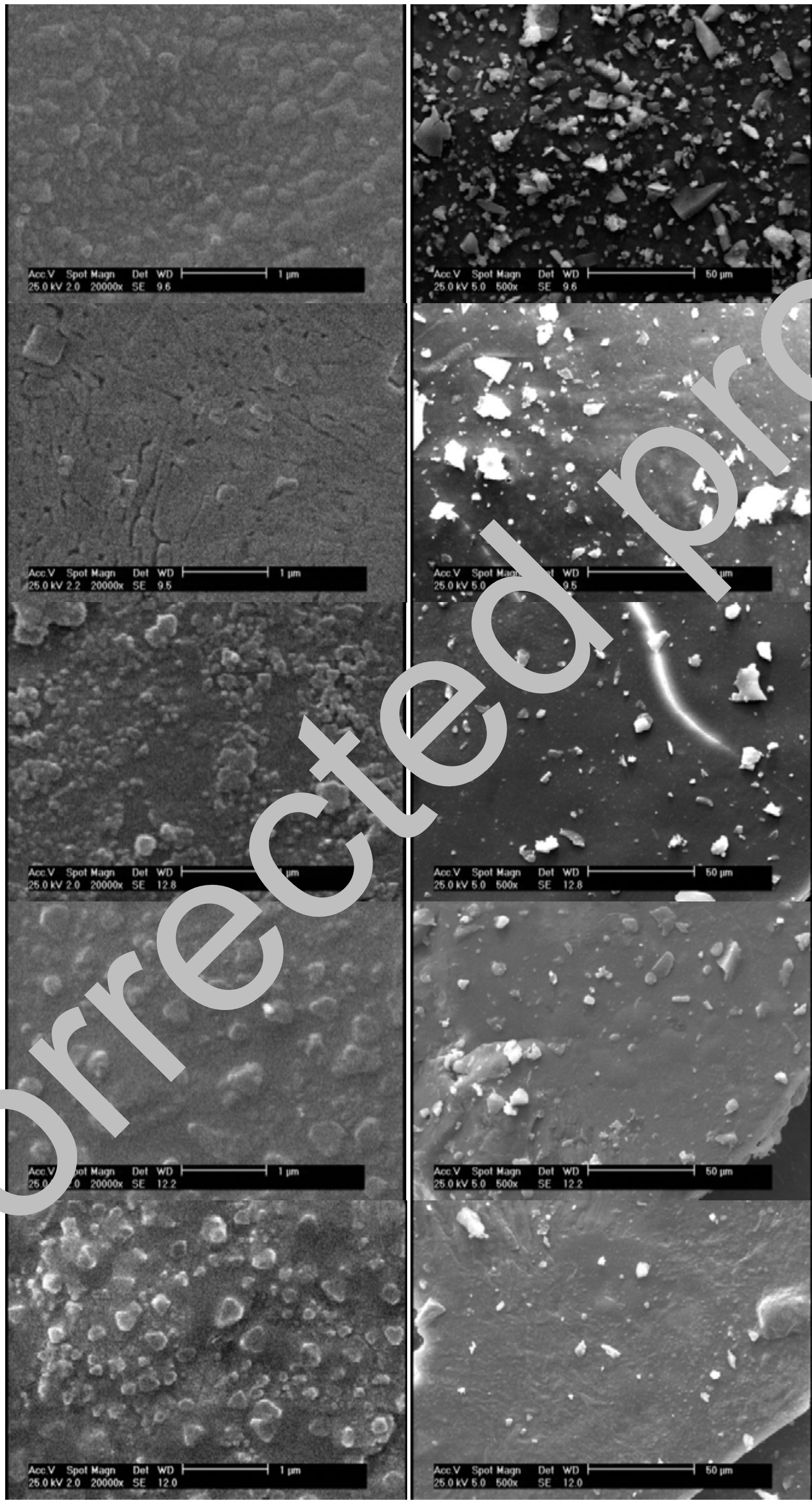

Figure 1- TEM images of gamma irradiated Rosemary at different doses ranged from 10 to 50 kGy

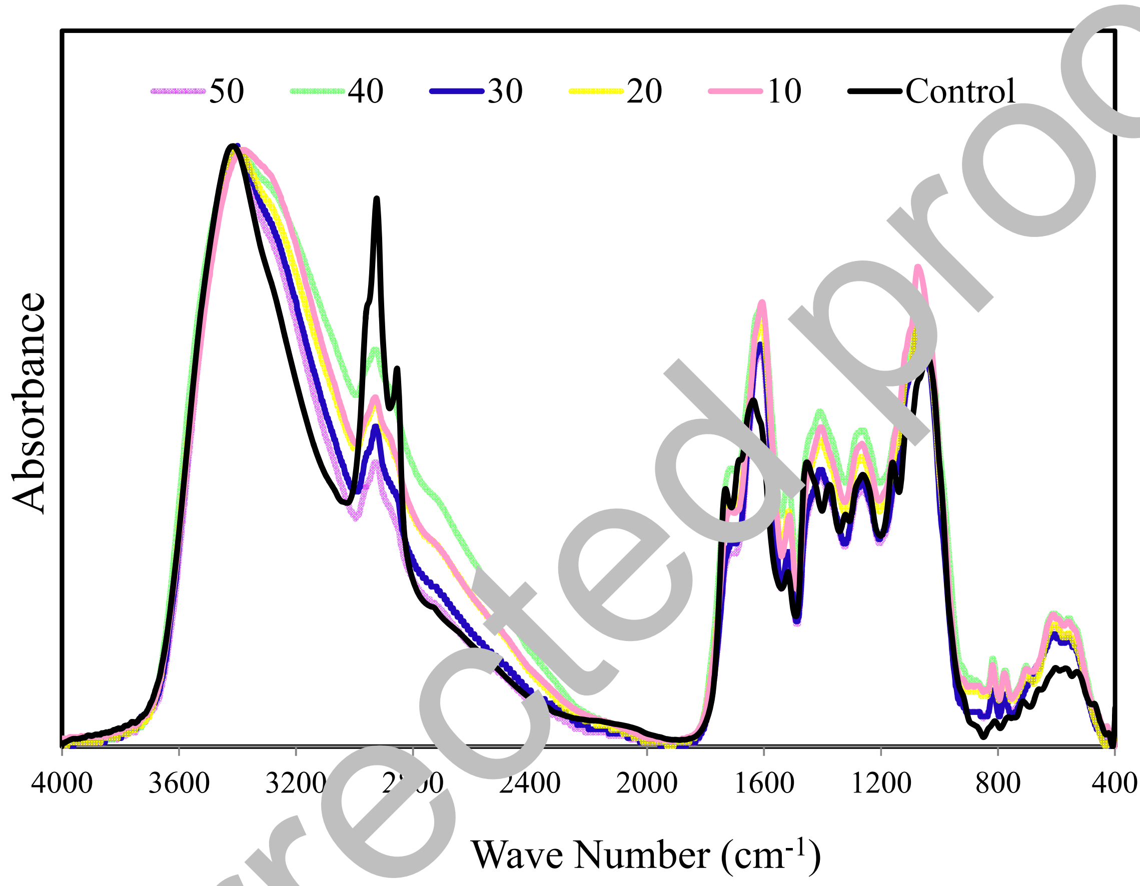

Figure 2. FTIR spectra of gamma irradiated Rosemary with different doses

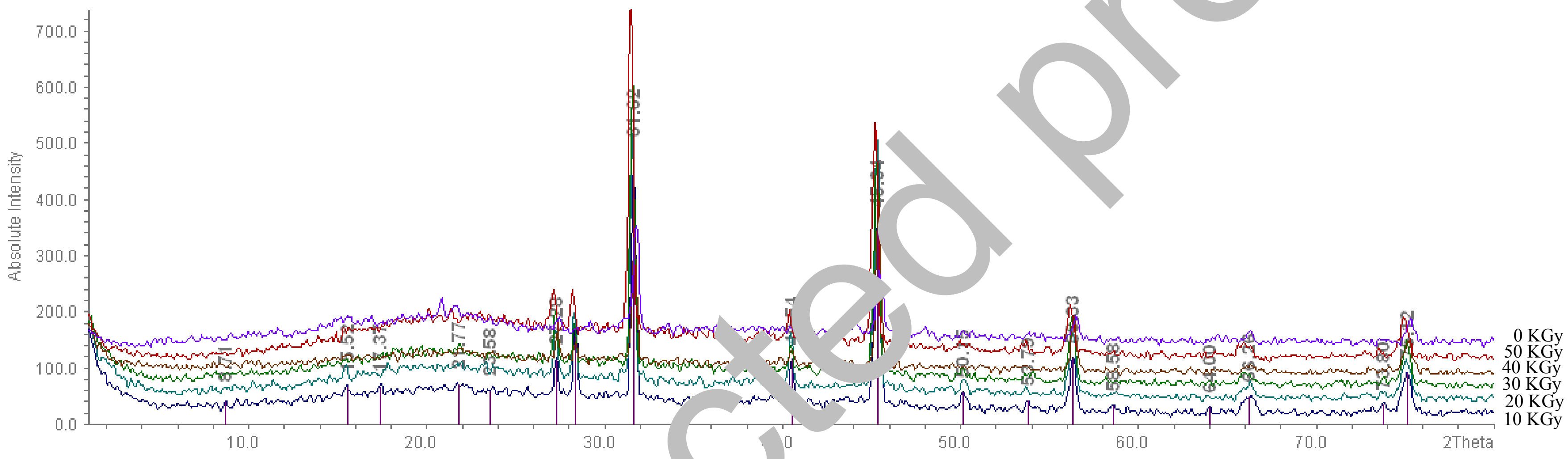

Figure 3 X-ray diffraction of Irradiated and non-irradiated Rosemary