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ORIGINAL ARTICLE

Characterization of Gamma-Irradiated *Rosmarinus officinalis* L. (Rosemary)

Gama Işınlanmış *Rosmarinus officinalis* L. (Biberiye)

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ABSTRACT

Objectives: *Rosmarinus officinalis* L., a member of the family Lamiaceae, is regarded as the spice with the highest antioxidant activity.

Materials and Methods: In this study, the transmission electron microscopy, X-ray diffraction, and fourier transform infrared spectroscopy (FTIR) physicochemical characteristics of the nanostructure of gamma-irradiated rosemary were investigated.

Results: The particle size distribution of the gamma-irradiated rosemary prepared under irradiation at 30 kGy in a Cobalt-60 irradiator exhibited a very narrow size distribution with average size of 70 nm. The results showed that irradiated (30 kGy) and crude rosemary had similar patterns of FTIR spectra, typical of phenol compound, without any notable changes in the key bands and functional groups status. Rosemary irradiated with 50 kGy and 10 kGy showed the highest and lowest crystallinity, respectively. Rosemary crystallinity of irradiated samples was lower compared with the nonirradiated sample.

Conclusion: Therefore, 30 kGy can be optimum for the synthesis of nanoparticles, average size of 70 nm, with low crystallinity and without any notable change in key bands compared to nonirradiated samples.

Key words: Rosemary, gamma irradiation, FTIR, transmission electron microscopy, nanoparticles, X-ray diffraction

ÖZ

Amaç: Lamiaceae familyası bitkisi olan *Rosmarinus officinalis* L., en yüksek antioksidan aktiviteye sahip baharat olarak kabul edilmektedir.

Gereç ve Yöntemler: Bu çalışmada, gama ışınlarıyla ışınlanmış biberiyenin nanoyapılarının fizikokimyasal özellikleri transmisyon elektron mikroskobu, X-ışını difraksiyonu ve fourier dönüşümü kızılötesi spektroskopisi (FTIR) kullanılarak incelenmiştir.

Bulgular: Bir kobalt-60 ışınlayıcıda 30 kGy'de ışınlama altında hazırlanan gama ışınlanmış biberiyenin partikül büyüklüğü dağılımı, ortalama büyüklüğü 70 nm olan çok dar bir boyut dağılımı sergilemiştir. Sonuçlar, ışınlanmış (30 kGy) ve ham biberiyenin, tipik fenol bileşiğinin, fonksiyonel gruplara ilişkin bantlarda kayda değer bir değişiklik göstermeden benzer FTIR spektrum paternine sahip olduğunu göstermiştir. 50 kGy ve 10 kGy ile ışınlanmış biberiye sırasıyla en yüksek ve en düşük kristallenme özelliği gösterdi. Işınlanmış örneklerin kristallenme özelliğinin, ışınlanmamış numuneye kıyasla daha düşük olduğu belirlendi.

Sonuç: Bu nedenle, 30 kGy'nin ortalama boyutu 70 nm olan nanopartiküllerin sentezi için ideal olduğu, ışınlanmamış örnekler ile karşılaştırıldığında düşük kristallenme ve bantlardaki kayda değer olmayan değişikliklere neden olduğu belirlenmiştir.

Anahtar kelimeler: Biberiye, gama radyasyon, FTIR, transmisyon elektron mikroskobu, nanopartikül, X-ışını difraksiyonu

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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 hydroxyanisol (BHA), propyl gallate, and citric acid are commonly used in lipids to prevent oxidation. Recently, these synthetic substances have been shown to cause effects such as enlarged liver size and increased microsomal enzyme activity. Therefore, there is a need for other compounds to use as antioxidants and to render safer food products for mankind.[1-3] Plant originated antioxidants are more suitable as food additives, not only for their free radical scavenging properties, but also because of the belief that natural products are safer than synthetic antioxidants.[4,5] Chang et al.[6] reported the results of investigations of the antioxidative effect of rosemary and sage due to the peroxide value. Naturally occurring compounds in rosemary extracts have been reported to exhibit antioxidant properties greater than BHA and equal to BHT.[7,8] *Rosmarinus officinalis* L. (rosemary), a member of the family Lamiaceae, is an attractive evergreen shrub with pine needle-like leaves that grows wild in most Mediterranean countries. Rosemary has been accepted as the spice 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 plant 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 are namely chemical reduction, interfacial polymerization, solvent evaporation, solvent deposition, nanoprecipitation, emulsification-diffusion, controlled jellification, microwave processing, and irradiation.[11-13] Irradiation 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] Moreover, 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 the nanostructure of gamma-irradiated *R. officinalis* L. (rosemary).

MATERIALS AND METHODS

Plant material

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

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

Ground rosemary powder was suspended in sterile 0.15 M phosphate buffered saline (pH 7.2). A sample was sonicated for 30 min in a water bath sonicator (Jencons, Leighton Buzzard, UK) and centrifuged at 5000 rpm for 15 min.[16] After precipitation in 2.5 volumes of 96% ethanol, the ground rosemary powder sample was dried at 40°C and then milled to the mesh size of 53-125 µm. The remaining powder was packed in a plastic cover and weighed. Irradiation was carried out at a dose rate 10, 20, 30, 40, and 50 kGy with a Cobalt-60 gamma irradiator (PX-30 Issledovapel, Russia) at a dose rate of 0.22 Gy s^{-1}. Furthermore, dosimetry was performed with a Fricke reference standard dosimetry system and after the irradiation process the gamma-irradiated rosemary was stored at 4°C for further experiments.

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

Fourier transform infrared spectroscopy

An amount of irradiated rosemary powder was mixed with KBr powder and, after drying, was compressed to form a disc. The discs were later subjected to FTIR spectroscopy measurement.

These measurements were recorded on a Bruker spectrophotometer (EQUINOX 55, Germany) in transmittance mode with a resolution of 4 cm^{-1} in the wavenumber region of 400 to 4000 cm^{-1}. FTIR measurements were carried out in order to obtain information about the chemical groups present around gamma-irradiated rosemary for their stabilization and to understand the transformation of functional groups due to the reduction process.

X-ray diffraction

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

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

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 on for their use as clinical diagnostics, therapeutics, and carriers for delivery systems.[17] In the present study, the particle size of gamma-irradiated rosemary prepared under irradiation of 30 kGy exhibited a very narrow size distribution. This 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 the gamma-irradiated rosemary at different doses ranging from 10 to 50 kGy.

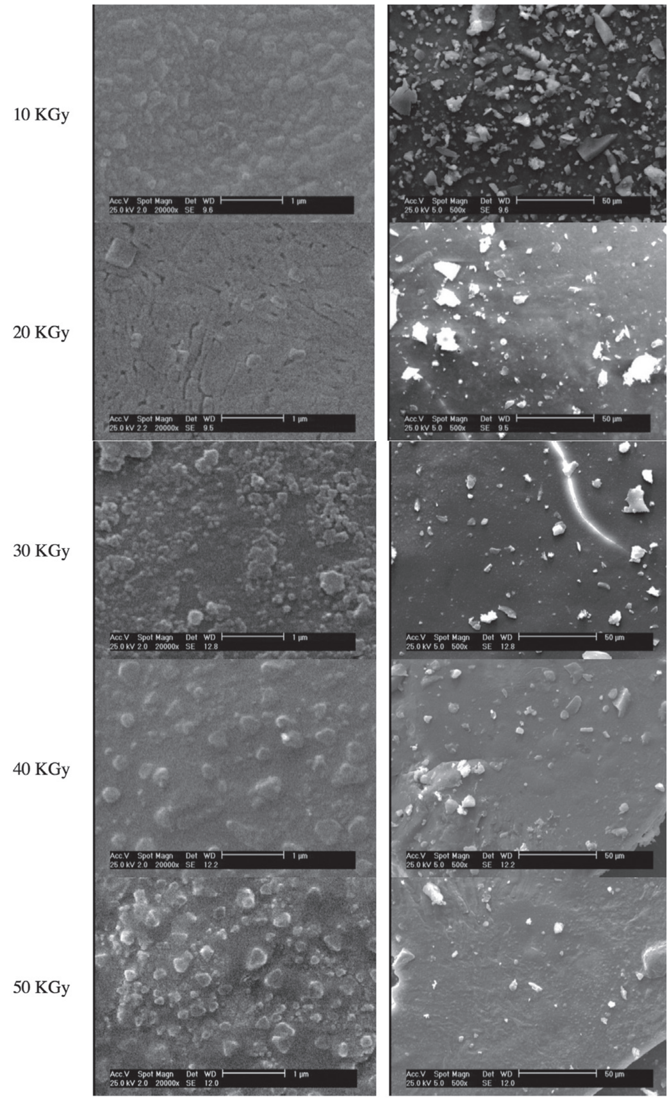

Figure 1. TEM images of gamma-irradiated rosemary at different doses ranging from 10 to 50 kGy

TEM: Transmission electron microscopy

To investigate whether any structural changes occurred during gamma irradiation, FTIR spectra were 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] The FTIR spectra are shown in Figure 2, and the wavenumbers of characteristic bands and corresponding assignments for the gamma-irradiated rosemary with different doses are listed in Table 1.

The key bands of rosemary are at 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 spectra 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 peaks at 2929.63 cm^{-1} and 2854.70 cm^{-1}, assigned to 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 603.35 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 C-O stretching vibrations of mono-, oligo-, and carbohydrates, respectively (Table 1).

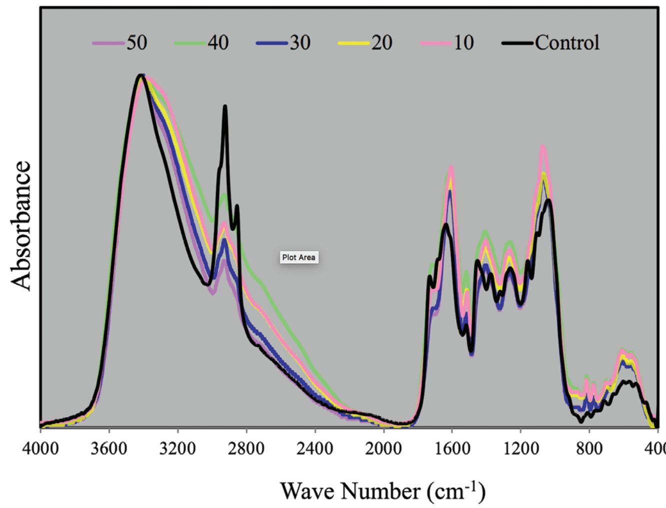

Figure 2. FTIR spectra of rosemary gamma irradiated with different doses

FTIR: Fourier transform infrared spectroscopy

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

In this research, after radiation, two C-H stretching vibrations became merged and appeared as a single vibration in all groups; this was due to the increased peak width. 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 reveal the particle size.[20,21] Therefore, increased peak width and reduced peak intensity accompany decreased particle size in all treatments.

On the other hand, the results revealed that irradiated (30 kGy) and crude rosemary had similar patterns of FTIR spectra, typical of phenol compound, without any notable changes in the key bands or functional groups status. The results are similar in other herb extracts.

The XRD results of the nonirradiated and irradiated rosemary at 10, 20, 30, 40, and 50 kGy are presented in Figure 3. XRD patterns from materials with different cubic crystal structures provided in textbooks can be used as reference.[23] In the present study, based on XRD pattern, rosemary has a structure that can be described as face centered cubic.[23] Moreover, the nonirradiated rosemary showed diffraction peaks at 20.85°, 31°, 45.47°, 56.51°, 66.28°, 75.29°, and 76.76°. A comparison

Table 1. Wavenumbers of characteristic bands and corresponding assignments for normal and gamma-irradiated rosemary

Wavenumbers (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 CH3 and CH2
1710.27 1610.51	1716.76 1608.62	1710.27 1609.04	1716.76 1609.15	1723.24 1607.10	1672.95 1659.45 1641.13 1617.02	C=O stretching vibration, C-N stretching, COO- antisymmetric stretching
1516.60	1517.95	1516.72	1515.87	1513.52	1572.66	Aromatic domain and N-H bending
1407.30	1408.19	1406.32	1405.73	1406.35	1454.06 1376.93 1366.32	C-O stretching vibration (amide) and C-C stretching from phenyl groups, COO- symmetric stretching, CH2 bending
1262.76	1263.12	1262.61	1264.38	1264.55	1291.11 1242.9 1203.36	C-O stretching
1071.97	1072.16	1072.30	1072.12	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	817.25 776.65 609.22	817.07 609.93	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

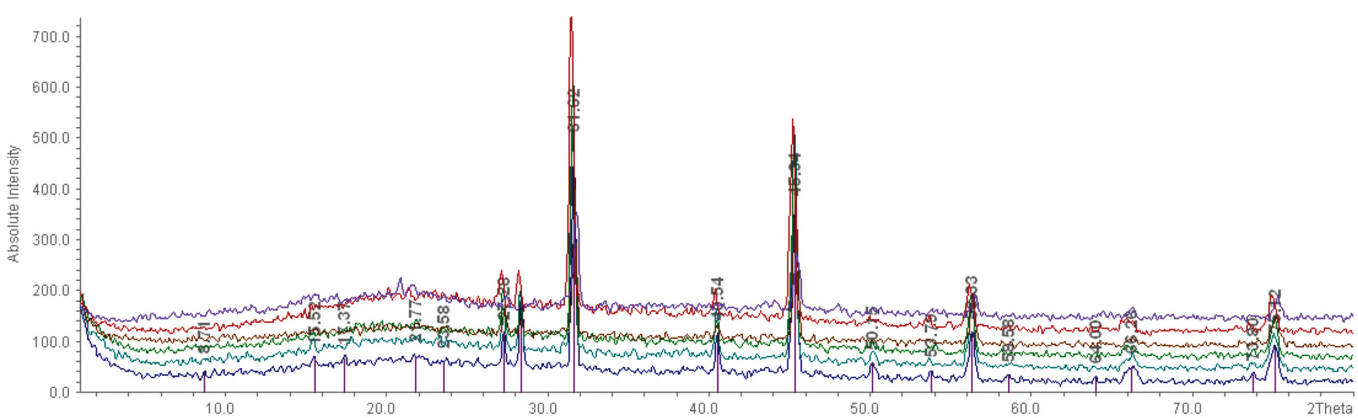

Figure 3. X-ray diffraction of Irradiated and non-irradiated Rosemary

among diffraction patterns of the rosemary, before and after irradiation, showed that the intensity of the reflection markedly declined with gamma irradiation compared to the control. The order of irradiated rosemary reflection intensity was 50, 40, 30, 20, and 10 kGy. The rosemary irradiated with 50 kGy and 10 kGy had the highest and lowest crystallinity, respectively. Therefore, the rosemary crystallinity of irradiated samples was lower as compared with the nonirradiated sample.

CONCLUSIONS

This work presents a simple, available, and effective method for preparation of rosemary nanoparticles. The purpose of the research was to synthesize new rosemary nanoparticles using gamma irradiation. The developed nanoparticles were characterized for particle size and 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 exhibited a distribution with average size 70 nm. In addition, the results showed that irradiated (30 kGy) and crude rosemary had similar patterns of FTIR spectra, typical of phenol compound, without any notable changes in the key bands or functional groups status. The rosemary crystallinity of irradiated samples was lower than that of the nonirradiated sample. The rosemary irradiated with 50 kGy and 10 kGy had the highest and lowest crystallinity, respectively. Therefore, 30 kGy can be optimum for synthesis nanoparticles, average size of 70 nm, with low crystallinity and without any notable change in key bands compared to nonirradiated samples.

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Conflict of Interest: No conflict of interest was declared by the authors.

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